Recent Advances in
Animal Nutrition
2009

P C Garnsworthy
J Wiseman

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P.C. Garnsworthy, PhD

J. Wiseman, PhD

University of Nottingham

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PREFACE

The 43rd University of Nottingham Feed Conference was held at the School of Biosciences, Sutton Bonington Campus, 8th – 10th September 2009. The Conference was divided into themed sessions that grouped papers into areas of topical interest to the animal feed industry covering Ruminants, General Topics, and Non-ruminants.

The main theme of the Ruminant session was reduction of environmental impact of dairy and beef cattle through reducing wastage and improving nutrient efficiency. The first paper provides an update on the influence of fatty acids on reproduction in dairy cows, highlighting the many interactions with other nutrients at different stages of the reproductive cycle. The second paper examines the heifer rearing process and discusses the losses that occur at each stage, together with possible mitigation strategies. The third paper reviews the scope for reducing phosphorus inputs into dairy systems, with a view to reducing excretion of this serious pollutant. The fourth paper provides much-needed information on optimising the efficient use of grazed grass by dairy cows. The fifth paper reviews the current status of feed evaluation for dairy diets in the UK, emphasising that although good nutrition models exist, their development has stagnated due to lack of research funding in recent years.

The session on General Topics contained two papers on animal-environment interactions. The first looks at how livestock production systems might have to be adapted in response to climate change. The second paper summarises legislation affecting animal production systems, particularly that designed to reduce pollution and enhance welfare of pigs. The third paper reviews methods to reduce mycotoxins in animal feeds, thus reducing risks to animal and human health. The fourth paper outlines the complex interactions between nutrition, stress and the immune system, understanding of which could improve animal welfare and performance. The fifth paper provides an update on the current state of GM technology in relation to animal feeds and its potential to help us meet the growing challenges of food security, climate change and healthy diets.

The session on Non-ruminants concentrated mostly on pig nutrition. The first paper provides data on the effects of feed processing technology on pig performance. The second paper looks at the design of piglet starter diets, particularly in relation to changing economic circumstances. The third reviews a series of experiments studying the effects of organic acids in pig diets on animal health and performance. The fourth paper provides a detailed review of amino acid nutrition of piglets with

special emphasis on tryptophan and valine. The final paper discusses how meat quality can be improved, and variation can be reduced, by combining the appropriate genetic, feeding and processing inputs that control meat quality.

We would like to thank all speakers for their presentations and written papers, which have maintained the high standards and international standing of the Nottingham Feed Conference. We are grateful to all members of the Programme Committee (see the List of Participants) for their significant inputs into designing and arranging the conference programme. We would also like to acknowledge the input of those who helped us to chair sessions (Mike Wilkinson, Mike Varley and Andy Salter) and the administrative (managed by Sue Golds, but with excellent deputizing during the event by Emma Hooley), catering and support staff who ensure the smooth running of the conference. Finally we would like to thank the delegates who made valuable contributions both to the discussion sessions and the general atmosphere of the meeting.

It should be noted that due to the increasing number of events that are scheduled for September each year, the Committee has decided to move the conference to June. The next Feed Conference will be held at the University of Nottingham Sutton Bonington Campus during the last week of June 2011.

P.C. Garnsworthy
J. Wiseman

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FATTY ACIDS AND FERTILITY IN DAIRY COWS

KEVIN D SINCLAIR and PHILIP C GARNSWORTHY
Division of Animal Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK

Introduction

Fats are widely used to increase energy concentration of ruminant diets, particularly for lactating dairy cows with the aim to reduce negative energy balance, increase milk yield and improve fertility. Compared to values for forages and cereals, gross energy concentration of fat is approximately double, metabolisable energy is approximately three times and net energy approximately four times (Garnsworthy, 1997). Dietary fats have important effects on rumen fermentation and animal responses. Although moderate levels of added fat can improve energy status of the cow, it can also induce a greater degree of negative energy balance and potentially reduce fertility. This can arise as a consequence of increases in milk yield (Beever, 2006) or reductions in dry matter intake (Allen, 2000). It follows that the response, in terms of improved fertility, to added dietary fat varies considerably between studies with lactating cows (Santos *et al.*, 2008).

The first purpose of the current chapter is to provide a contemporary overview of the effects of dietary fats on reproductive processes and fertility in the modern high-yielding dairy cow, in particular on the direct and indirect effects of fats on ovarian follicular development and steroidogenesis. Dietary fats can also influence inflammatory mechanisms associated with the maternal recognition of pregnancy which are not considered in detail here but are discussed elsewhere (e.g. Mattos *et al.*, 2000; Wathes *et al.*, 2007). Instead, the second purpose of the current chapter is to consider the effects of dietary fats on the follicle enclosed oocyte and pre-implantation embryo and, in so doing, assesses some recent data from non-lactating cattle and sheep which have investigated these effects in detail.

Rumen effects

The fat content of forage and cereal components of ruminant diets is less than 50 g/kg. If added fat increases total fat content of the diet to more than 100 g/kg, digestive problems can occur (Garnsworthy, 1997). Added fat containing polyunsaturated fatty acids (PUFA) is particularly deleterious because PUFA have a detergent effect on bacterial cell walls. Another important consideration is that fats with a melting point at or below rumen temperature (39 °C) can coat feed particles in the rumen, rendering them inaccessible for microbial attack (Devendra and Lewis, 1974). The magnitude of these effects depends on inclusion level and type of fat, but they can result in significant reductions in forage digestion and feed intake. Under normal circumstances, PUFA are partially biohydrogenated by rumen bacteria and fungi to detoxify them (Nam and Garnsworthy, 2007). Thus, fatty acids flowing out of the rumen are mostly saturated.

Various techniques are available to reduce the deleterious effects of fats on rumen fermentation. The products are referred to as "protected fats", "rumen inert fat" or "bypass fat". Rumen protection can be natural, (e.g. whole oilseeds, where the slowly digested seed coat slows the rate of fat release, especially if the oilseeds are heat-treated), chemical (e.g. formation of calcium soaps or encapsulation in formaldehyde-treated casein) or physical (e.g. selection of fatty acids with a high melting point and small particle size). Protected fats offer the potential for increasing absorption of unsaturated fatty acids from the small intestine because they provide some protection against rumen biohydrogenation.

Animal production responses

Production responses to supplementary fat are not always predictable. Responses observed include increased milk yield, increased or decreased milk fat content, decreased milk protein content, increased live-weight gain and decreased live-weight loss. The balance of these responses depends on the quantity of fat, its fatty acid profile and degree of protection, the other components of the diet, overall feeding level, stage of lactation, and genetic merit of the cow. Cows in early lactation partition energy towards milk production at the expense of body fat reserves, so they are usually in negative energy balance for the first 8 to 12 weeks postpartum. Therefore, increased energy intake at this stage of lactation could result in further increases in milk yield or a reduction in the daily amount of body fat mobilised. For example, Garnsworthy and Huggett (1992) found that cows with a high level of body fat reserves responded to high-fat diets by reducing fat mobilisation; cows with a low level of body reserves responded by increasing milk yield. Cows of high genetic merit respond to fat supplementation immediately postpartum by increasing milk yield at the expense of body tissues.

Effects of fatty acids on reproduction

Poor reproductive performance in dairy cows is often attributed to negative energy balance in early lactation (Butler and Smith, 1989). Many studies have shown that rapid mobilisation of body fat reserves is associated with fertility and health problems in dairy cows (see reviews by Garnsworthy and Webb, 1999; Butler, 2003; Chagas *et al*. 2007). Negative energy balance, while not affecting the population of small ovarian follicles, adversely affects the size and ovulatory fate of the dominant follicle and extends the period of anoestrus (Diskin *et al*. 2003). Butler (2003) reported that negative energy balance causes attenuation of LH pulse frequency and low levels of blood glucose, insulin and IGF-I that collectively limit oestrogen production by dominant follicles. Butler (2005) reported that dairy cows losing more than one unit of body condition score (BCS) over the first 30 days post partum ovulated 20 days later than cows losing less than 0.5 units, and that conception rate decreased by 10 % per 0.5 unit BCS loss. As discussed above, supplementary fat is often used to increase energy concentration of diets for high-yielding dairy cows, and has been found to improve fertility in some but not all studies (Mattos *et al*., 2000; Santos *et al*., 2008), possibly due to the aforementioned antagonistic effects of reduced dietary intakes and increased milk yields (Beever, 2006; Allen, 2000).

Fatty acids can affect reproductive processes also in ways that are independent of the energy they supply. Supplementary fat can reduce plasma insulin and glucose, and increase precursors for steroid and eicosanoid secretion, all of which can alter ovarian and uterine function. Supplementary fat can also influence follicle numbers and oocyte quality.

EFFECTS OF FATTY ACIDS ON INSULIN

Dairy cows of high genetic merit typically exhibit prolonged periods of postpartum anoestrus, associated with low plasma concentrations of insulin (Gutierrez *et al*., 2006). We have performed several experiments at Nottingham to investigate dietary effects on insulin in dairy cows and to demonstrate the importance of insulin status for reproductive performance. In a dose-response study with high-yielding dairy cows, CaPFA and sugar beet pulp were substituted for wheat to provide five isoenergetic diets containing 39 , 42, 43, 45, 48 g fat and 231, 183, 159, 135, 87 g starch per kg DM (Garnsworthy *et al*., 2008a). Plasma insulin decreased with increasing dietary fat and decreasing dietary starch concentrations, reaching a plateau break point at 43 g fat, 159 g starch per kg DM. To separate effects of starch and fat, a second study was conducted in which CaPFA were added to a high-starch basal diet to give dietary concentrations of 0, 8, 15, 23, and 30 g CaPFA/kg DM (Garnsworthy *et al*., 2008b). Plasma insulin decreased significantly when dietary concentration of

CaPFA exceeded 15 g/kg DM, equivalent to 0.5 kg/d of added fat. In another study (Gong, *et al*., 2002), a high-fat diet (0.75 kg CaPFA/d) fed for the first 50 days of lactation depressed insulin and delayed first ovulation postpartum compared with an isoenergetic high-starch diet. A significantly smaller proportion of cows ovulated within the first 50 days postpartum on the high fat diet (55%) compared with the high starch (high insulin) diet (90%).

Although dietary fat can depress plasma insulin concentration, which is detrimental for fertility, a high-fat diet fed after a period of insulin stimulation with cereal significantly increased pregnancy rate at 120 days postpartum from 27% to 60% (Garnsworthy, *et al*. 2009). The beneficial effects of fat in this study could be ascribed to influences of fat on progesterone, prostaglandin signalling, or oocyte quality, as discussed in subsequent sections.

EFFECTS OF FATS ON NUMBER AND SIZE OF OVARIAN FOLLICLES

In some studies with lactating dairy cows, supplementary fat in the diet increased the total number of follicles, and stimulated growth and size of the preovulatory follicle (Lucy *et al*. 1991b; Lucy *et al*. 1993). In the insulin study of Garnsworthy *et al*. (2008a), where insulin was altered by concurrent changes in dietary starch and fat concentrations, maximum number of small follicles was decreased by high dietary fatty acid concentrations. In the insulin study of (Garnsworthy *et al*., 2008b), where insulin was altered by CaPFA supplementation of a high-starch diet, fatty acid supplementation stimulated maximum numbers of small (<5 mm) ovarian follicles at all levels tested, compared with the zero level of added fat. Similar results were observed by Lucy *et al*. (1991b), where the average number of small follicles was decreased by fatty acid supplementation over the first 25 days of lactation, but increased during and after progesterone treatment. Discrepancies among studies probably result from differential effects of energy balance, insulin, fatty acids and other factors on recruitment, growth and atresia of small follicles. For example, some studies have reported positive effects of insulin on numbers of small (Gutierrez *et al*., 1997), medium-sized (Oldick *et al*., 1997), or large (Lucy *et al*., 1991a) follicles; others have reported positive effects of fat supplementation (Beam and Butler, 1997; Lucy *et al*., 1991b) on numbers of large follicles. In a review of follicular dynamics in cattle, Lucy *et al*. (1992) concluded that CaPFA themselves, and not the additional energy provided by CaPFA, stimulated the ovary and enhanced movement of follicles from smaller to larger classes. Taken together, these reports suggest that both fatty acid supply and insulin have minimum thresholds, so increasing either factor would stimulate follicle development only when the other is adequate.

A possible explanation for the stimulatory effect of CaPFA supplementation on number of small follicles is that a certain threshold fatty acid supply (between 35

and 41 g/kg DM) is required for normal follicle and corpus luteum development. The mode of action for this effect could be stimulation of circulating progesterone concentrations, which were greater in cows fed supplementary fatty acids (Garnsworthy *et al.*, 2008a,b).

EFFECTS OF FATS ON PROGESTERONE

Inclusion of fat in diets increases plasma concentrations of cholesterol, which is a precursor for progesterone synthesis (Grummer and Carroll 1991) and progesterone concentrations are often observed to increase in lactating dairy cows fed on diets supplemented with fat (Staples and Thatcher, 2005). In a study by Robinson *et al.* (2002), plasma cholesterol concentrations, and diameter of the first dominant follicle, were higher in cows fed a diet supplemented with linoleic acid, and there was an increase in oestradiol during the follicular phase in cows fed a diet supplemented with linolenic acid (Robinson *et al.* 2002). Overall, however, PUFA-supplemented diets reduced plasma progesterone, particularly in the early luteal phase (Robinson *et al.* 2002), suggesting that cholesterol supply is not a factor limiting progesterone synthesis. It is possible that only saturated or monounsaturated fatty acids enhance circulating progesterone concentrations because incubation of dispersed bovine luteal cells with PUFA decreased secretion of progesterone (Hinckley, *et al.* 1996).

Staples and Thatcher (2005) attributed increased progesterone secretion with fat supplementation to increased size of the ovulatory follicle, leading to a larger corpus luteum. In the seven studies they reviewed, the diameter of the dominant follicle was on average 3.3 mm larger (23% increase) in fat-supplemented cows compared with control cows.

Another possible explanation is a slower rate of progesterone clearance in fat-supplemented cows compared with controls, as observed following ovariectomy (Hawkins *et al.* 1995). Wiltbank *et al.* (2007) highlighted the paradox that higher producing dairy cows have a larger volume of luteal tissue, but reduced circulating progesterone (Lopez *et al.*, 2005), and suggested that the increase in liver blood flow associated with greater feed intake in lactating dairy cows leads to elevated metabolism of both oestrogen and progesterone. This would cause a reduction in circulating oestrogen and progesterone concentrations, even with higher production of steroid hormones by the follicle or corpus luteum. Although added dietary fat can depress feed intake (Allen, 2000), there is no information in the literature about the effects of dietary fat on liver blood flow. Furthermore, increased progesterone has been observed when fat supplementation has not altered feed intake (e.g. Garnsworthy *et al.*, 2008a,b). Therefore, the mechanism by which dietary fat influences progesterone remains to be established.

EFFECTS OF FAT ON PROSTAGLANDIN F-2 ALPHA

At the end of the normal oestrous cycle, regression of the corpus luteum is induced in non-pregnant animals by uterine release of prostaglandin F-2 alpha ($PGF_{2\alpha}$). In pregnant animals, interferon-tau, produced by the developing embryo, suppresses $PGF_{2\alpha}$ secretion leading to maintenance of the corpus luteum which is essential for establishment of pregnancy. Feeding dairy cows with omega-3 and omega-6 fatty acids decreases uterine secretion of $PGF_{2\alpha}$ (Thatcher *et al.* 2001), which could reduce embryonic losses caused by ineffective suppression of $PGF_{2\alpha}$ secretion during early pregnancy (Mattos *et al.* 2000).

Fatty acid metabolism of mammalian oocytes and pre-implantation embryos

OOCYTE METABOLISM

Oxygen consumption by the oocyte increases during follicle development, although metabolic rate is greater in oocytes from primary follicles when cellular volume is accounted for (Harris *et al.*, 2009). Oocyte maturation, however, is absolutely dependent on oxygen availability and oxidative phosphorylation. Oocyte maturation is also dependent on glucose, but little glucose is actually taken up and metabolised by the oocyte. Instead, the surrounding cumulus cells metabolise glucose to pyruvate, which is the preferred energy substrate of the oocyte (Sutton *et al.*, 2003). In contrast to the fate of carbohydrates, the contribution of lipids as a metabolic fuel in the oocyte and the early pre-implantation embryo is less well understood. The close apposition of mitochondria to lipid droplets in the mature oocyte, however, suggests a role of triglyceride oxidation in ATP production during maturation and fertilisation (Sinclair *et al.*, 2003). Indeed, lipase specific activity in bovine oocytes is greater than that of surrounding cumulus cells and increases during oocyte maturation (Cetica *et al.*, 2002), hinting at an important role of endogenous lipid reserves in oocyte energy metabolism.

The fatty acid content of oocytes varies greatly between species, and is particularly high in domestic animals such as the pig (Figure 1A). Triglyceride is the major component of intracellular lipid (Figure 1B), thus providing a large potential energy reserve. McEvoy *et al.* (2000) found that the proportion saturated fatty acids (~0.44), MUFA (~0.42) and PUFA (~0.14) did not differ markedly between triglyceride and phospholipid fractions in ruminant oocytes, although cholesterol ester and free fatty acid fractions contained proportionately more saturated fatty acids. The major fatty acids in ruminant oocytes are palmitic (c16:0; 24-27%), oleic (c18:1n-9; 22-38%) and linoleic (c18:2n-6; 6-9%) acids.

A. Fatty acid content of mammalian oocytes
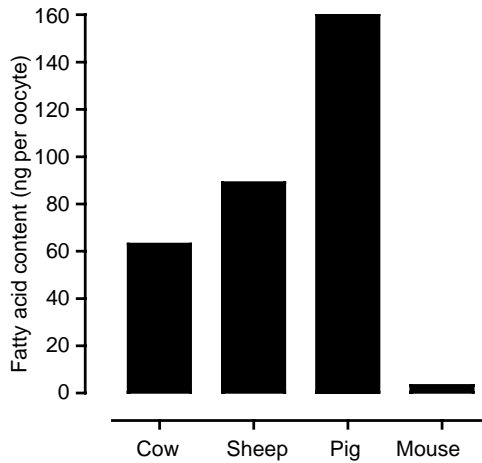
B. Lipid fractions within oocytes
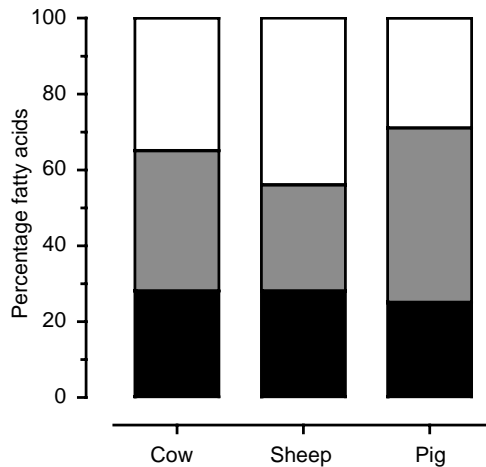
Figure 1. Fatty acid content (A) and lipid composition (B) of mammalian oocytes where black bars represent phospholipids, grey bars triglycerides and open bars cholesterol esters and free fatty acids. Data calculated and/or derived from Loewenstein and Cohen (1964); Ferguson and Leese (1999); McEvoy *et al*. (2000); and Sturmey and Leese (2003).

EMBRYO METABOLISM

Energy metabolism in the pre-implantation embryo is also characterised by a significantly greater reliance on carboxylic acids such as pyruvate and citrate than glucose. The low metabolic activity of the early cleavage-stage embryo, nevertheless, belies its dependence on oxidative phosphorylation for the generation

of > 90% of ATP (Sinclair *et al.*, 2003). Around 40% of glucose uptake by the embryo during this period is metabolised to lactate. The principle function of glucose during this period is thought to be the generation of reducing equivalents (in the form of NADPH) and ribose sugars, both via the pentose phosphate pathway, and as a source of 3-carbon precursors, all for biosynthetic purposes. ATP production on a per embryo basis increases during compaction and blastulation although, on a cellular basis, oxidative and glycolytic metabolism alters little during this period. The embryo, nevertheless, becomes increasingly reliant on glycolysis following compaction, accounting for between 15 and 20% ATP production, possibly reflecting the lower oxygen tension of uterine as opposed to oviductal fluids.

The β-oxidation of fatty acids is thought to generate much of the water and at least some of the energy necessary for blastocoel formation (Wiley, 1987). Fergusson and Leese (1999) reported a 42% reduction in triglyceride content by the 2-cell stage in bovine embryos, but there was no subsequent net change in triglyceride content to the hatched blastocyst stage. However, by maturing oocytes and culturing zygotes in the presence of various concentrations of the mitochondrial carnitine palmitoyltransferase A inhibitor, methyl palmoxirate (MP), these authors were able to demonstrate both independent and additive effects of impaired fatty acid oxidation on early post-fertilisation development (Ferguson and Leese, 2006). By blocking the entry of fatty acids from triglycerides into mitochondria, MP induced a dose dependent reduction in the proportion of oocytes that cleaved following fertilisation and the proportion of zygotes that developed to the blastocyst stage. A retrospective analysis of our own data in sheep (Wonnacott *et al.*, 2010) reveals a similar decrease in total fatty acids during oocyte maturation, with little subsequent change up to the blastocyst stage (Figure 2A). In sheep oocytes around 44% of the triglyceride fraction is composed of MUFA of which oleic acid (c18:1n-9) comprises around 75% (McEvoy *et al.*, 2000; Wonnacott *et al.*, 2010). The reduction in percentage MUFA in MII oocytes depicted in Figure 2B, therefore, probably reflects the extensive oxidation of triglycerides that takes place during oocyte maturation.

During pre-elongation development up to the blastocyst stage there is no net increase in embryo mass, although cell number increases to around 120 (Sinclair *et al.*, 2003). This increase in cell number represents a 60-fold increase in cell surface area that necessitates a significant amount of plasma membrane synthesis. Therefore, the increase in percentage PUFA at the expense of saturated fatty acids in Day 6 sheep blastocysts (Figure 2B) probably reflects the increase in cellular phospholipids at the expense of more saturated cholesterol esters and free fatty acids, although such compositional changes have yet to be properly established. In contrast to the oocyte, little is known about the composition of phospholipids in pre-elongation mammalian embryos, how it might change during development and how

A. Total fatty acids per oocyte/embryo
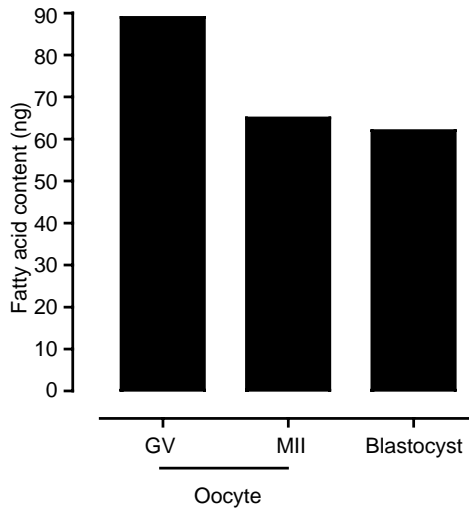
B. Percentage total faty acids

Figure 2. Fatty content and composition (percentage total fatty acids) of immature (germinal vesicle (GV) intact) and mature (metaphase II (MII)) sheep oocytes, and Day 6 embryos (blastocysts) cultured in vitro in the presence of fatty acid free bovine serum albumin. Black bars are saturated, dark grey bars monounsaturated and light grey bars polyunsaturated fatty acids. Data calculated from Wonnacott *et al.* (2010).

it is affected by maternal diet. It is generally recognised, however, that phospholipids are the major structural components of all membranes with negligible pools of free phospholipids in the cell (Rothman and Lenard, 1977). Synthesis of phospholipid

during early pre-implantation development has been described in the mouse embryo using [methyl-^{3}H]-choline as a specific precursor (Pratt, 1980). [Methyl-^{3}H]-choline incorporation into lipid increased 9- to 13-fold up to the morula stage, and increased further up to the blastocyst stage, although the relative extent was difficult to quantify fully in that study. Working with discarded human embryos, Haggarty *et al*. (2006) reported that 6-cell to blastocyst stage embryos had proportionally more PUFA (mostly linoleic acid (C18:2n-6)) than < 6-cell stage embryos. Furthermore, in contrast to isotopically labelled palmitic acid (C16:0), the uptake of labelled linoleic acid increased dramatically beyond the 8-cell stage.

Maternal diet and fatty acid metabolism of oocytes and pre-implantation embryos

Seasonal variations in developmental capacity of bovine oocytes have been related to differences in fatty acid composition of oocytes; embryo development to the blastocyst stage was significantly higher in winter than in summer and phospholipids from follicular fluid, granulosa cells and oocytes contained higher proportions of saturated fatty acids during the summer (Zeron *et al*. 2001). Differences could, however, be due to seasonal variation in ambient temperature and dietary ingredients rather than direct effects of fatty acids.

In a study designed to investigate short-term effects of level of rumen inert fatty acids on developmental competence of oocytes in lactating dairy cows, Fouladi-Nashta *et al*. (2007) compared diets supplying 200 or 800 g CaPFA/d offered for several weeks prior to oocyte recovery and in vitro maturation fertilisation and culture to the blastocyst stage. The high-fat diet significantly improved blastocyst production (38 versus 29% of cleaved embryos; P = 0.017) and the total number of cells within each blastocyst at day 8 post-fertilization (151 versus 133; P = 0.043).

As CaPFA contains mostly saturated (palmitic) and monounsaturated (oleic) fatty acids, we subsequently became interested in investigating the effects of PUFA. However, in contrast to surrounding somatic cells of the ovary, oocytes appear to preferentially accumulate saturated as opposed to unsaturated fatty acids (Figure 3). This observation in sheep is consistent with previous observations in non-lactating cattle at our laboratory (Adamiak *et al*., 2006), and hints at selective uptake mechanisms and/or *de novo* synthesis within the ovarian follicle and oocyte that favours saturated fatty acids. Such compositional differences between cells within the ovary, however, may merely reflect differences in triglyceride, phospholipid and cholesterol ester levels associated with the relative prevalence of cytoplasmic lipid droplets in oocytes and cell membranes in surrounding somatic

cells. The uptake and metabolism of fatty acids by the follicle-enclosed oocyte is certainly an area that requires further study.
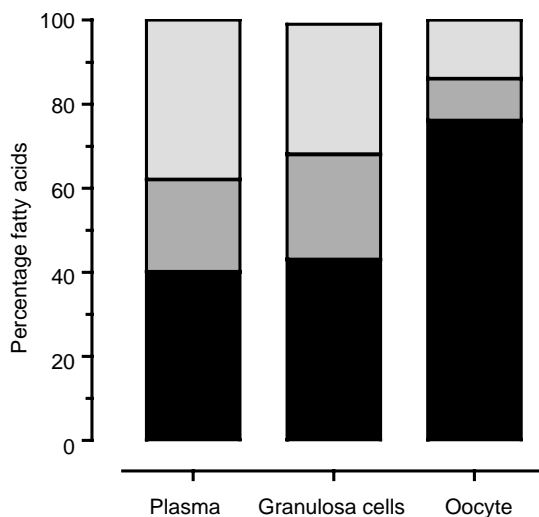
Figure 3. Fatty acid composition of plasma, granulosa cells and oocytes of ewes offered polyunsaturated rich diets. Black bars are saturated, dark grey bars monounsaturated and light grey bars polyunsaturated fatty acids. Data calculted from Wonnacott *et al*. (2010).

In the lactating dairy cow mechanisms within the ovary may be effective in partially nullifying dietary treatment induced differences in plasma saturated and unsaturated fatty acid composition. Fouladi-Nashta *et al*. (2009) offered lactating Holstein cows from Day 42 of lactation one of three isoenergetic diets that contained (i) a rumen inert source of fatty acids rich in plamitic (c16:0) and oleic (c18:1n-9) acids, (ii) full-fat soya (rich in linoleic acid (c18:2n-6) or (iii) linseed, full-fat extruded (rich in α-linolenic acid (c18:3n-3)). Although differences in the fatty acid composition of both plasma and milk reflected that of the three diets, neither the fatty acid composition of granulosa cells nor the post-fertilisation developmental competence of oocytes was altered by diet. The studies of Fouladi-Nashta *et al*. (2007 and 2009), therefore, suggest that level of dietary fat is more important than type of dietary fat in determining oocyte developmental competence in high-yielding dairy cows. This conclusion is supported by the study of Bilby *et al*. (2006), which found no significant difference in blastocyst yield when comparing sunflower oil (high in oleic and n-6 fatty acids), calcium salts of trans fatty acids, calcium salts of vegetable oil (high in n-6 fatty acids) and linseed oil (high in n-3 fatty acids). Similarly, Thangavelu *et al*. (2007) found no difference in the number of transferable embryos recovered from cows fed supplements of saturated fatty acids, whole linseed or sunflower seed.

It is possible that the physiological status of these animals may have contributed to this outcome. Plasma non-esterified fatty acid (NEFA) concentrations are increased during negative energy balance associated with early lactation, and this source of relatively saturated fatty acids (Leroy *et al*., 2005) may more readily be taken up by cells within the ovary, although this remains to be established. In contrast, the fatty acid profile of both granulosa cells and oocytes of non-lactating ewes offered either n-3 or n-6 PUFA enriched diets reflected both plasma and dietary levels of these fatty acids, although once again the proportion of saturated fatty acids (mostly stearic acid (C18:0)) was greater in oocytes than granulosa cells (Wonnacott *et al*., 2010).

The NEFA composition of serum and follicular fluid are broadly similar and NEFA is rich in palmitic (c16:0), stearic (c18:0) and oleic (c18:1n-9) acids (Leroy *et al*., 2005). In that study, the addition of physiological concentrations of either palmitic or stearic acid to bovine oocyte maturation media reduced the proportion of inseminated oocytes that cleaved following insemination, and also reduced the proportion of zygotes that subsequently developed to the blastocyst stage. The inclusion oleic acid was without effect. In contrast, the inclusion of physiological concentrations of α-linolenic acid (c18:3n-3) actually improved bovine oocyte maturation and post-fertilisation development to the blastocyst stage (Marei *et al*., 2009). The mechanism involved in this response was shown to involve enhanced prostaglandin E_2 production by cumulus-oocyte complexes (COC), increased intracellular concentrations of cAMP within COC, and phosphorylation of mitogen-activated protein kinases in oocytes. Collectively, these mechanisms are known to enhance nuclear maturation and the proportion of metaphase II oocytes for fertilisation.

Ewes fed diets supplemented with calcium soaps of fish oil fatty acids (rich in eicosapentaenoic acid (EPA; c20:5n-3) and docosahexaenoic acid (DHA; c22:6n-3)) produced higher "quality" oocytes that were less sensitive to chilling damage (Zeron *et al*., 2002). This was associated with increases in proportions of both EPA and DHA in the phospholipid fraction of cumulus cells but not oocytes; failure to detect PUFA in oocytes was probably due to detection limits of their instruments. In contrast, mice fed a diet enriched in long chain n-3 PUFA produced inferior quality oocytes associated with impaired mitochomdrial function and enhanced production of reactive oxygen species, culminating in reduced post-fertilisation development to the blastocyst stage (Wakefield *et al*., 2008). At present there are insufficient data to reconcile these apparent discrepancies of n-3PUFA in oocytes.

EMBRYO METABOLISM

It is unlikely that the aforementioned discrepancies of n-3PUFA in oocytes are due to species differences in fatty acid metabolism, because Reis *et al*. (2003) observed

similar detrimental effects of n-3 PUFA in sheep embryos. In that study zygotes were cultured for 5 days in Synthetic Oviductal Fluid (SOF) media supplemented with fatty acid-free BSA alone, or with BSA complexed to palmitic acid (C16:0) or DHA (C22:6n-3). In contrast to palmitic acid, which had no effect, culture in the presence of DHA significantly reduced embryo development by inducing peroxidation-mediated growth arrest.

We recently determined the effects of culturing sheep zygotes in SOF media supplemented with high-density lipoproteins (HDL) fractionated from sera of ewes offered n-3 or n-6 PUFA enriched diets (Wonnacott *et al*., 2010). The principal sources of n-3 PUFAs in the experimental diets were salmon oil and linseed, whereas the principal source of n-6 PUFA was sunflower oil. HDL was added to culture media at physiological levels. Control embryos were cultured in SOF with fatty acid-free BSA. Despite the fact that sheep embryos expressed transcripts for the HDL receptor, scavenger receptor class B member 1 (*SCARB1*), fatty acid analysis of Day 6 blastocysts revealed that there was no net uptake of fatty acids from HDL by embryos. The fatty acid composition of Day 6 blastocysts was unaltered by culture treatment. Nevertheless, n-6 PUFA HDL significantly reduced embryo development and transcript expression for genes encoding the LDL receptor and stearoyl-CoA desaturase relative to either fatty acid-free BSA or n-3PUFA HDL treatments. The mechanisms underlying these effects remain to be determined. However, the most abundant fatty acid in Day-6 sheep blastocysts, at 34g/100g total fatty acids, was linoleic acid (C18:2n-6). The mass of this fatty acid in blastocysts was greater than that measured in oocytes and indicates that the sheep embryo, like the human embryo (Haggarty *et al*., 2006), has an absolute requirement for linoleic acid which it acquires, at least in our system, from albumin as opposed to HDL.

Summary and conclusions

The inclusion of fat in the diet of high-yielding dairy cows during lactation has had variable outcomes on fertility which may be due in part to effects of dietary fats on intake, milk yield and, consequently, negative energy balance. Negative energy balance can delay resumption of oestrous cycles and the size and ovulatory fate of the dominant follicle. The picture is further complicated because fatty acids can benefit both ovarian follicle development and steroidogenesis whilst inhibiting insulin, which is known to independently promote ovarian folliculogenesis. Recent studies at Nottingham have demonstrated that the timing of inclusion of fatty acids in the diet of lactating cows is critical; a high-fat diet fed after a period of insulin stimulation with cereals significantly improves pregnancy rates. More detailed studies at our laboratory have shown that cells within the ovarian follicle selectively

assimilate or synthesise saturated as opposed to unsaturated fatty acids. This may also partly account for the variability in response to different forms of PUFA in the diet. NEFA, in contrast, are rich in saturated and mono-unstaturated fatty acids, such as palmitic, stearic and oleic acids; physiological concentrations of which have been shown to impair bovine oocyte maturation in vitro. Omega-3 PUFA have been found to both enhance and reduce oocyte quality and pre-implantation embryo development, whereas omega-6 PUFA are generally inhibitory. The mechanisms underlying these effects remain to be fully elucidated and this will be necessary in order to fully reconcile these apparent discrepancies.

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2

**ENVIRONMENTAL AND GENETIC INFLUENCES ON SUCCESSFUL
HEIFER REARING**

D.C. WATHES, A.M. CLEMPSON, J.S. BRICKELL
*Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts,
AL9 7TA, UK*

Introduction

Dairy heifers represent the future of the herd. The aim of every dairy farmer
should be to rear healthy, well grown individuals with excellent fertility that are
well equipped to join the herd at first calving. Such animals are then able to fulfil
their genetic potential by leading a long and productive life. This in turn reduces
the number of animals lost prematurely due to involuntary culling and so fewer
replacements are needed in the future. This scenario has a positive feedback, as
it makes more time and money available to devote to those animals which are
being reared as replacements.

The pursuit of such a strategy therefore has obvious benefits. Unfortunately
in some cases too great a proportion of the available resources on dairy farms is
devoted to the lactating cows, to the detriment of the replacement heifers. Many
dairy software packages do not include the rearing period at all, so the frequency
and causes of losses during this period, and their associated costs to the business,
are often poorly documented. Overall, on UK farms at the present time around
15% of liveborn heifer calves do not survive until first calving and 12-17% of those
which do are culled or die in their first lactation (Esslemont and Kossaibati, 1997;
Brickell, McGowan, Pfeiffer and Wathes, 2009a). Only about 30-40% of the total
lifetime of the average cow which joins the herd is actually spent producing milk
(Haworth, Tranter, Chuck, Cheng and Wathes, 2008; N. E. Bourne, A. Swali and
D.C. Wathes, unpublished observations). Much of a cow's life therefore occurs
before she ever calves and optimising the management during this rearing phase
is clearly crucial to the financial success of the whole dairy enterprise. This review
examines stages of the heifer rearing process and also considers some recent

developments which may in future enable genetic selection for beneficial traits to be made shortly after birth.

Extent and timing of losses

Heifer rearing covers a number of phases, each with very different demands in terms of management.

PERINATAL

The perinatal period covers the birth of the calf and its' first 24 h of life. Perinatal mortality is defined as calf death before, during or within 24 h of birth following a full term gestation period of at least 272 days. In the majority of cases (>75%) the calf is alive at the start of calving but dies during or immediately after the parturition process (Mee 2008). The current incidence of perinatal mortality in Holstein-Friesian herds in the UK is estimated to be 7.9% (Brickell *et al.* 2009a), a figure almost identical to the 8% reported 10 years earlier by Esslemont and Kossaibati (1996). This is in broad agreement with the incidence of 7-8% reported for American Holsteins (Johanson and Berger 2003; Lombard, Garry, Tomlinson and Garber, 2007) although perinatal mortality is lower in Ireland (4.3%, Mee, 2008). Perinatal mortality is consistently about double in first calving heifers (e.g 13.2 and 6.6%, Meyer, Berger, Koehler, Thompson and Sattler, 2001; 11.1 and 4.6%, Johanson and Berger, 2003; 12.6 and 6.1%, Lombard *et al.* 2007; 12.1% and 5.6%, Brickell *et al.*, 2009a). There are also strong indications for differences between breeds. For example Hansen, Misztal, Lund, Pedersen and Christensen (2004) reported that the stillbirth rate was higher in Holstein-Friesian than in Danish Black and White cows. This suggests that there is likely to be a genetic component to mortality rate. Furthermore, there are suggestions from both the USA and several Scandinavian countries that stillbirth rate is currently increasing, particularly in primiparous cows (Meyer *et al.*, 2001; Mee, 2008).

The traditional main risk factors for perinatal mortality, in addition to dam parity, are dystocia and twinning, with less pronounced influences in some studies of gestation length, calf sex, time of year, herd size and aspects of calving management. Many calves (around 10-40%) of the total born dead following an assisted calving have received traumatic injuries such as fractured ribs and spine during the calving process (Mee, 2008). Twinning rates in Holstein-Friesian cows are generally around 4%, but vary between herds (Esslemont and Kossaibiti, 1996; Silva del Rio, Stewart, Rapnicki, Chang and Fricke, 2007). Twinning is an

unfavourable trait as it also results in underweight calves and freemartins. There is evidence that an increasing proportion of perinatal deaths are associated with smaller, weaker calves born unassisted particularly to first calving heifers (Mee, 2008). These births may be associated with placental dysfunction and the incidence varies according to sire, suggesting a genetic predisposition (Kornmatitsuk, Dahl, Ropstad, Beckers, Gustafsson and Kindahl, 2004).

For calves born alive which die shortly after birth, heat production is a key factor. Most calves experience some drop in temperature at birth, which they need to correct by a rapid increase in thermogenesis, initially using brown fat and then colostral fat for fuel (Quigley and Drewry, 1998). Heat losses from newborn calves will be higher in cold or wet climates and in poorly insulated calving pens and this can rapidly lead to a state of potentially life threatening hypothermia (Diesch, Mellor, Stafford and Ward, 2004). Calves are also born into an environment where they are immediately exposed to a significant pathogen challenge. They therefore need to receive immediate protection against infectious agents from adequate ingestion of colostrum, as considered in more detail below.

Although mean perinatal mortality rate in a recent survey was approximately 8%, the range across the 19 UK farms studied was 3-14% (Brickell *et al.* 2009a). This shows that deaths could be reduced considerably on many farms by following good management practices. This should include better pre-calving management of the dam (considered in the fertility section below), increased supervision around calving and improved training of farm personnel.

NEONATAL PERIOD

The neonatal period covers the first month of life. The study of Brickell *et al.* (2009a) found that 3.4% of female calves alive at 24 h had died or had been euthanized by 28 d. The rate was again variable, with no loss over this period on 8 of the 17 farms, rising to 12% on one farm (Brickell *et al.* 2009a). A further 2% of calves were culled because they were born with a male twin and were presumed to be freemartins. During the neonatal period the main causes of death are gastrointestinal and respiratory diseases (Blowey, 2005). Even if calves are treated and survive an initial infection, animals which have been treated for scouring or pneumonia are 2.5 times more likely to die or to be culled before calving themselves, and age at first calving (AFC) is likely to be delayed (Waltner-Toews, Martin and Meek, 1986). Therefore prevention of disease is clearly better than cure.

Amongst the enteric disorders, *E coli*, salmonella and clostridial infections are of greatest importance in the first 3 days of life followed by rotavirus, coronavirus and cryptosporidia at slightly older ages. A wide range of agents can contribute

to calf pneumonia, with bacterial and mycoplasma infections often developing as secondary to an initial viral disease (Blowey, 2005). Although it is impossible to prevent some exposure to these agents from the dam or the environment, the level of challenge can be reduced significantly by cleanliness in preparation of milk feeds together with good hygiene in the calving pens and calf housing. Bedding needs to be replaced and sheds cleaned regularly. Adequate ventilation is also critical. Keeping calves in matched age groups and away from adults will also reduce degree of exposure. In addition, the ability of calves to deal with pathogenic challenges is critically dependent on adequate colostrum intake. Vaccination can help, for example against Pasteurella, but young calves may die before vaccination is appropriate. Farms with high levels of scouring calves need to obtain a faecal sample analysis to identify the main causative agents and then take active steps to improve hygiene.

CALVES

Calf mortality from 1 to 6 months of age in the UK study was 3.4% (Brickell *et al*. 2009a). Added to the 3.4% found in the first month of life, this produced a figure similar to the 6% death rate found during the first 6 months of life in another recent study based on figures obtained through the UK Cattle Tracing System (Ortiz-Pelaez, Pritchard, Pfeiffer, Jones, Honeyman and Mawdsley, 2008). In contrast, Roy (1990) estimated that calf losses between 1 and 6 months of age were around 1-5%. In the current study, the average figure was again unrepresentative as the majority of farms (11/19) did not lose any calves whereas one had a 29% mortality rate (8 of 28 heifer calves born during the study period died, Brickell *et al.* 2009a). Estimates of calf mortality in the USA are generally slightly higher than for the UK, being in the range 8-10% (Margerison and Downey, 2005).

   Causes of mortality are poorly recorded at farm level, but infectious disease is again the main problem (Blowey 2005; Svensson, Linder and Olsson, 2006). Undersize calves are particularly vulnerable, with low birth weight calves more likely to die young (Swali and Wathes 2007; Waltner-Toews *et al.,* 1986). Small size may reflect some inadequacy of pre-natal development. In addition small calves show retarded behavioural development so are less able to suckle the dam and receive an adequate early colostrum intake (Lawrence, Dwyer, Jarvis and Roberts, 2005).

   Poor early growth is reflected in low circulating concentration of insulin-like growth factor-I (IGF-I). Most IGF-I in the circulation is produced in the liver in response to growth hormone (GH) stimulation (Le Roith, Bondy, Yakar, Liu and Butler, 2001). IGF-I is widely recognized as being a good indicator of metabolic status and has the additional advantage that concentration does not fluctuate

through the day so the time of sampling relative to feeding is not critical (Taylor, Beever, Bryant and Wathes, 2006; Velasquez, Spicer and Wathes, 2008). Low IGF-I at 1 month of age in small calves was a significant risk factor for mortality before 6 months of age (Brickell *et al.* 2009a).

During the first few months of life calves experience major changes in their dietary management, notably at weaning. Housing requirements also change so calves may be re-arranged into different groups. All of these factors affect their growth (discussed below) and survival. Within the USA, the main risk factor for higher calf mortality was failure of passive immunity associated with an inadequate supply of colostrum. Other factors identified were high milk production on the farm (>7710 kg), male staff caring for calves, housing pre-weaned heifers in groups of ≥7, not supplying roughage until > 20d and feeding mastitic milk (NAHMS, 1996).

PRE-BREEDING HEIFERS

Losses due to death or culling during the period from 6-15 months averaged 3.5%, ranging from 0 to 18% across UK farms (Brickell *et al.* 2009a). In both this study and that of Svensson *et al.,* (2006), the main causes of death for heifers of this age were accidents, for example excess barley intake and head entrapment. Such incidents can be difficult to foresee, but risks can be reduced by careful attention to the environment whether indoors or at pasture.

HEIFERS POST INSEMINATION

Dairy heifers are normally bred for the first time at around 14 to 15 months of age to calve at or shortly before 24 months of age. The principal cause of loss during the period after 15 months of age is culling due to poor fertility. For those farms using AI, conception rates to first service are consistently better in heifers than in lactating cows; figures have ranged from 47% to 71% in different studies, but are generally over 60% for heifers (Pryce, Simm and Robinson, 2002; Donovan, Bennett and Springer, 2003; Ettema and Santos, 2004; Kuhn, Hutchinson and Wiggans, 2006; Swali and Wathes 2007; Brickell, Bourne, McGowan and Wathes, 2009b). Nevertheless not all animals conceive readily. In the study of Brickell *et al.* (2009b), 19/450 heifers never conceived at all, of which 2 were belatedly identified as being freemartins. Farms differ in their policy on how long to keep trying to get individual animals in calf, with some heifers receiving multiple inseminations over an extended time period before eventually either conceiving or being culled. Another issue for this age group is late embryonic-early foetal mortality and

abortion. Although the incidence in the study of Brickell *et al.* (2009b) was low (17/431, 3.9%), pregnancy loss occurred after a positive pregnancy diagnosis and was therefore often not noticed until the time of expected calving approached. This led to long delays before animals were re-inseminated and so caused a major delay in age at first calving (AFC) for heifers experiencing late embryonic-early foetal loss (978 ± 44 days, in comparison with 791 ± 6 days for heifers which held their first pregnancy to term).

FIRST LACTATION

Although the focus of this chapter is on rearing heifers, it is pertinent to extend this to consider losses during first lactation, as the causes can often be traced back to the rearing period. Esslemont and Kossaibiti (1997) in a survey of 50 Holstein/ Friesian herds reported that 12% of cows were culled in their first lactation, with reproductive problems by far the main causative factor, accounting for 43.5% of those culled. In a detailed study of one farm, 20/117 (17%) of heifers calving for the first time failed to calve again (N.E. Bourne, A. Swali and D.C. Wathes, unpublished observations). Incidence of culling was strongly related to their AFC and hence reproductive performance as a heifer. All of the 14 animals with an AFC of 22 to 23 months survived in the herd for a second lactation in comparison with 81/94 (86%) with an AFC of 24 to 28 months, but only 2/9 (22%) with an AFC of 32 to 36 months. In addition, those animals which required between 4 and 5 services to conceive as nulliparous heifers took significantly longer to resume oestrous cycles after calving in comparison with those which had conceived to first service as heifers (51 ±12 days, n=9 compared with 19 ± 1 days, n=68, P<0.001) even though they were on average 11 weeks older at calving. In another recent survey of 19 farms, 72 out of 412 heifers (17.5%) were culled during their first lactation. These were divided fairly evenly into animals which were: (i) never served again (n=27); (ii) inseminated at least once but did not conceive (n=28), and (iii) culled following an initial positive pregnancy diagnosis (n=17). Of the 28 animals inseminated, 12 were culled due to poor fertility and were served up to 18 times before a decision was made to cull them. Together these results suggest that heifers with poor initial fertility are less likely to survive in the herd beyond their first lactation.

SUMMARY OF LOSSES

Overall in the survey of Brickell *et al.* (2009a), 8% of calves were born dead and 14.5% of live-born heifers failed to calve for the first time. Only 1 of the 19 farms

studied managed to keep all their heifer calves alive from birth over this entire period. For the others, the overall post natal mortality rate ranged from 3 to 29%. Different farms experienced problems with different age groups; the worst figures for neonatal mortality (4/33, 12%), calf mortality (8/28, 29%), pre-service heifers (5/27, 19%) and post-service heifers (4/19, 21%) each occurred on different farms. These figures suggest that a brief period of inattention during the 2 year rearing period can have major consequences in reducing supply of replacement heifers. However, good results on some establishments also indicate that losses can be minimal if best practice is operated consistently.

Growth and nutritional management

A good nutritional management strategy requires targets to be set at each phase of development to ensure that heifers reach first calving with a large, lean frame having experienced good skeletal growth without becoming too fat. The goal for Holstein-Friesian type heifers should be for insemination at 13 to 15 months of age to calve at 22 to 24 months. At service animals should have achieved about 55 to 60% of their mature body weight and this should have increased to 85 to 90% by first calving (Margerison and Downey, 2005). Calving at 22 to 24 months of age minimises the rearing costs by reducing the period before the animal becomes productive. Furthermore, studies consistently show that well grown animals which calve relatively young go on to perform well in the herd (Wathes, Brickell, Bourne, Swali and Cheng, 2008). Achieving these goals consistently requires that heifers are weighed and/or measured at regular intervals (Figure 1).

Mature weight of Holstein-Friesian cows can vary considerably according to selection policy. To calve at between 550 and 625 kg at 24 months of age, it is necessary to achieve an average growth rate of 0.7 to 0.8 kg/d throughout the entire rearing period. In practice, growth rates are not consistent, and tend to increase from about 0.5 to 0.8 kg/d from birth up to the time of puberty and then gradually diminish towards first calving (Coffey, Hickey and Brotherstone, 2006). Modern Holsteins mature more rapidly than Friesians and attain a greater body size, with mature weight not reached until about 3 years of age (Figure. 2; Coffey *et al*., 2006). Growth is driven primarily by the somatotrophic axis with GH stimulating production of IGF-I by the liver. Circulating IGF-I peaks at around the time of puberty (Velazquez *et al.,* 2008) and is highly correlated with both actual size (whether measured as weight or height) and growth rate (Figure. 2). Advice differs as to whether a steady rate of increase should be maintained throughout the rearing period or whether it is better to vary it. Some studies have suggested that rapid growth (>0.75 kg/d) in the pre-pubertal period increases fat deposition in the udder at the expense of secretory tissue, thus compromising

Figure 1. Measurement of calf (a) weight using a portable weigh platform and (b) height using a height stick.

mammary development and reducing milk yield potential (Serjsen, 2005). Others have found that increasing early calf growth rate from 0.38 to 0.67 kg/d by using a milk replacer with a high protein content improved mammary development (Brown, VandeHaar, Daniels, Liesman, Chapin and Weber Nielsen, 2005). Therefore growth rates of 0.7 kg/d before puberty and 0.8 kg/d after puberty are often recommended (Heinrichs and Hargrove, 1987; Hoffman, 1997; Mourits, Dijkhuizen, Huirne and Galligan, 1997).

(a)

(b)
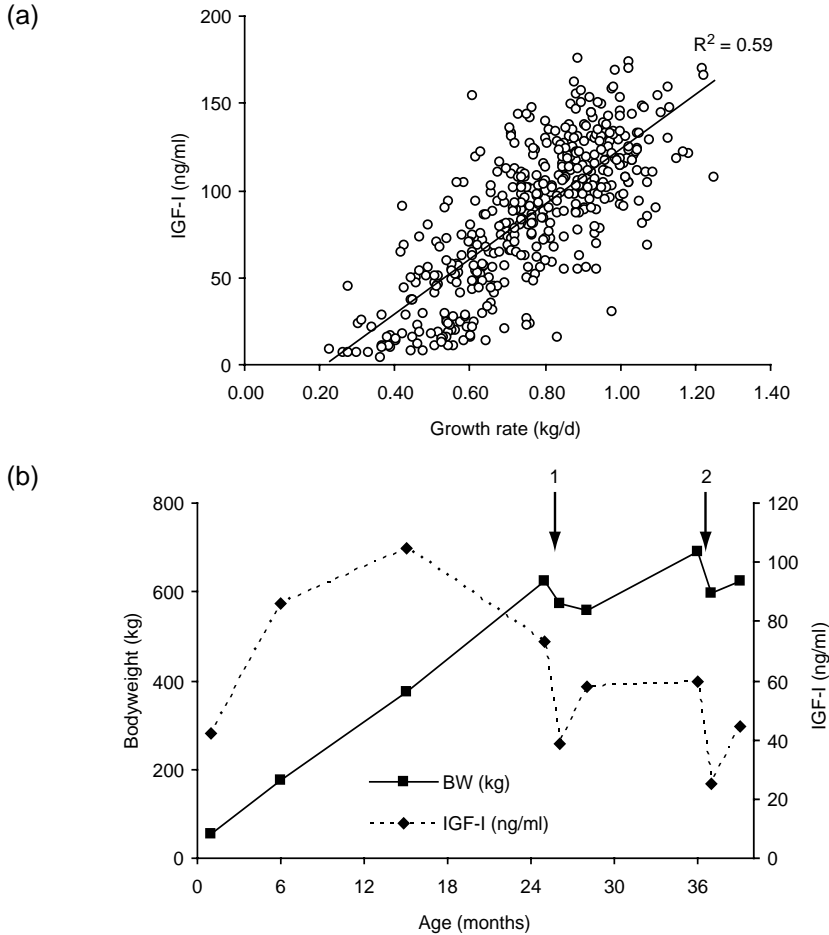
Figure 2. (a) Relationship between heifer growth rate from 1 to 6 months of age and circulating IGF-I concentration measured at 6 months of age in 454 Holstein-Friesian heifers. (b) Changes in body weight (BW) and circulating IGF-I concentration as heifers matured over their first 3 years of age. Animals were sampled at approximately 1, 6 and 15 months of age and at -1, +1 and +8 weeks before and after their first and second calving (arrows 1 and 2). High IGF-1 concentrations at 6 and 15 months of age were accompanied by higher growth rates as weight increased. IGF-I concentrations then declined with a further sharp decrease immediately after each calving. Average weight increased slightly between the first and second calving, but also fell at the start of each lactation.

COLOSTRUM

The first vitally important goal in nutritional management is to ensure that all calves receive an adequate supply of good quality colostrum so that they rapidly acquire passive immunity to pathogens to which they will immediately be exposed. This passive immunity must provide protection for about the first 8 weeks of life until the

calf's own immune system develops. In addition to providing immunity, colostrum contains a variety of antimicrobial agents including lactoferrin, lactoperoxidase, lysozyme and sialic acid which provide immediate protection in the gut against pathogen invasion (Goldman and Smith 1973; van Hooijdonk, Kussendrager and Steijns, 2000). The fat present in colostrum is also essential to provide fuel for thermogenesis (Quigley and Drewry, 1998). The beneficial effects of colostrum are widely known by dairy farmers but in some studies up to 50% of calves tested had serum IgG levels of less than 10 g/l when assessed at 1 to 2 days of age, a level which is indicative of failure of passive transfer (Quigley, Hammer, Russell and Polo, 2005). A more recent American study found a prevalence of 19.2%, suggesting some improvements had been made (Beam, Lombard, Kopral, Garber, Winter, Hicks and Schlater, 2009). Failure to achieve this minimum level of passive transfer can be caused by inadequate volume of intake, provision of poor quality colostrum or feeding it too late. Other risk factors were feeding pooled colostrum (odds ratio (OR) of 2.2), allowing nursing with the dam (OR 2.7) and delaying hand feeding until >4h after birth (OR 2.7) (Beam *et al.*, 2009). Smaller and less vigorous calves have a reduced colostrum intake during their first feed (Vasseur, Rushen and de Passille, 2009). In a dairy which allowed calves to suckle, failure of passive transfer was greater than 50% even from dams with above average IgG concentrations (Besser, Gay and Pritchett, 1991). Absorption of antibodies across the gut diminishes rapidly within 6 h of birth, so it is essential that calves receive colostrum quickly (Quigley *et al.,* 2005). Passive transfer is linear, so the more IgG that is fed, the greater the immunity which the calf will acquire (Quigley and Drewry, 1998).

Colostrum quality is influenced by many factors. The IgG content of colostrum should exceed 50 g/L, but is variable among cows. Pritchett, Gay, Besser and Hancock (1991) reported a range of IgG concentration from <18 to >95 g/l. The average value from 919 calvings was 48 g/l, so the majority of cows had colostrum deemed to be of poor quality. The IgG content of colostrum decreases with time from calving to 73% of original concentration within 10 h (Moore, Tyler, Chigerwe, Dawes and Middleton, 2005). Volume of colostrum obtained at first milking also varies considerably with a range of 2.8 to 26.5 l reported (Pritchett *et al.,* 1991; Moore *et al.,* 2005). Quality is lower in primiparous dams and is also reduced if the dam is ill (Pritchett *et al.,* 1991; Quigley *et al.,* 2005). The minimum amount required ranges from 100 to 150 g IgG for small to large birthweight calves. Suckling should be supervised or, preferably, calves should be fed by hand to ensure that they receive 2 l within 3 h of birth and a total of 4 l within 6 to 12 h (Lorenz, 2008).

On some farms colostrum can be responsible for disease transmission, for example, of Johne's disease (Quigley *et al.,* 2005). Storage of first milkings on farm at room temperature is associated with an exponential growth of bacteria,

another major health risk (Quigley *et al.,* 2005). This, coupled with issues over variability in quality, leads some farmers to feed colostrum supplements or colostrum substitutes. Those on the market are also variable in content and hence quality. For a discussion of this topic see Quigley *et al.,* (2005).

In summary, the costs of failure of passive transfer of immunity are high. In the short term the calf may die, require veterinary treatment and/or experience poor weight gain. In the longer term culling is more likely before first lactation, age at first calving is delayed and unthrifty animals which do lactate have a reduced milk production potential (Faber, Faber, McCauley and Ax, 2005; S.D. Acres, personal communication). Ensuring an adequate colostrum intake is therefore absolutely critical. To achieve this consistently, farmers should use a colostrometer for a quick but approximate assessment of quality, coupled with regular testing of blood IgG levels in some calves. A recent American study reported that farms which did not use routine monitoring of serum proteins in calves as a measure of passive transfer had an OR of 13.8 for failure of passive transfer (Beam *et al.*, 2009).

CALF AND HEIFER NUTRITION

Good early development over the first 12 weeks of life is essential for production of replacement heifers. As a guide, Holstein-Friesian heifers should weigh 56 kg at 4 weeks, 76 kg at 8 weeks and 100 kg at 12 weeks (Berrisford, 2009). Following colostrum feeding, calves require either whole milk or milk replacer. The options have been reviewed previously (van Amburgh and Drackley, 2005; Tanan, 2005) and are not considered in detail here. In brief, growth rates vary according to energy and protein contents of the milk and volume and frequency with which it is supplied. Calves which suckle their dam consume about 20% of their body weight (BW) daily in 6 to 10 meals a day, whereas calf rearing systems typically feed much lower amounts (about 10% BW) in 2 daily feeds to encourage early consumption of solid feed, which is cheaper and easier to supply (Drackley, 2005). This will clearly restrict growth rates considerably below those which are biologically possible. Energy requirements of young calves increase rapidly if the ambient temperature falls below the thermoneutral zone (about 15°C), so it is important to relate supply of milk available to current environmental conditions (NRC, 2001).

Another important aspect to consider is that body composition can be altered by dietary constituents. The crude protein content of whole milk is about 280g/ kg, but most milk replacers contain less than this. For calves on isoenergetic milk replacer diets both stature and body protein deposition increased in a linear fashion as crude protein content increased over the range 75 to 275 g/kg (Drackley, 2005). A recent Irish trial showed that milk replacer was more cost effective than whole

milk. Not only were total feed costs to 56 d lower (£60.90 for replacer compared with £75.90 for whole milk), but calves also achieved a higher weight both before and immediately after weaning, reaching weights of 95.1 kg compared with 86.8 kg by 10 weeks of age (Gleeson, 2009). This study used milk replacer with 270 g protein and 166 g fat per kg dry matter. In summary, use of a good quality milk replacer is a sound investment as it should provide consistent quality, enable good growth rates, and avoid the risk of disease transmission possible with whole milk.

Weaning is a critical period when the calf must transfer from a liquid to a solid diet. Some concentrates should be provided well before weaning to avoid making the transition too abrupt and either hay or good quality straw must be available to the calf to promote rumen development. Fresh water should also be provided from an early stage (Hill, Aldrich and Sclotterbeck, 2005; Lorenz, 2008).

Most dairy heifers will spend at least part of their lives at pasture. The quality of sward available will clearly affect heifer growth rates, as reviewed by Dawson and Carson (2005). Calves are more sensitive to changes in herbage digestibility and are more selective in their grazing habits than older cattle (Leaver, 1974). Herbage intake and hence live-weight gain increases up to an optimum sward height of approximately 9 to 10 cm. Supplementation of concentrates or conserved forage may be necessary if grass supply is insufficient to maintain adequate growth rates. Parasite control is also important; fluke and worm egg counts should be monitored in grazing heifers and used to inform an appropriate worming strategy.

Variability in growth rates

A study of 19 UK dairy farms found that average live weights were 56 kg at 1 month of age, 175 kg at 6 months and 373 kg at 15 months, with average growth rates of 0.77 kg/d up to 6 months (n = 489) and 0.75 kg/d from 6 to 15 months (Brickell, McGowan and Wathes, 2009c). These values were close to recommended rates, but disguised extreme variability between cohorts of calves on different farms, which ranged from 0.49 to 1.02 kg/d between 1 and 6 months of age. At the farm level, early growth rates were enhanced by provision of supplemental colostrum in addition to that received from the dam, and by use of milk replacer as opposed to whole milk. Most farms offered calves ≤5 L of milk per day in two feeds but, when *ad libitum* milk feeding was used, growth rates increased from 0.73 to 0.92 kg/d. The higher rate may, however, be greater than desired. Before weaning, growth rates were higher in smaller groups of ≤ 6 calves, whereas after weaning keeping calves in larger group sizes of >20 was beneficial. Data on optimum group

sizes are not always consistent between studies because associated factors related to housing, such as ventilation and proximity to older animals. influence risks of disease transmission (Heinrichs and Radostits, 2001).

Growth rate was reduced when weaning was phased by giving a period of once a day milk feeding for about a week rather than using an abrupt transition (Brickell *et al*., 2009c). Dehorning animals after weaning also provided a check to growth. A number of factors analysed did not influence growth rate in this on-farm study. These included number of days calves were fed colostrum (from <24 h to >7 d), milk temperature (warm or cold), age at first access to concentrates (from ≤7 d to >21 d) and weaning age (4-6 weeks or >6 weeks). Other studies have, however, suggested that all these factors are influential (Heinrichs and Radostits, 2001). Weaning is clearly a stressful period when calves must not only adjust to a new diet but also to different housing and social groups. Taking measures to minimise these stresses will pay dividends by reducing the check to growth.

Although management factors within farms should have been consistent, variation in the growth rate was observed for animals on the same farm (e.g. from 0.45 to 1.13 kg/d) (Brickell, McGowan and Wathes, 2009c). Two American studies reported similar levels of variation (Sivula, Ames and Marsh, 1996; Donovan, Dohoo, Montgomery and Bennett, 1998). As the study of Brickell *et al*. (2009c) was an on-farm study, the causes of variation at an individual calf level could not be identified because records were not sufficiently detailed. However, it is likely that failure of passive transfer of immunity and subsequent disease would have contributed to the poor growth rates of some animals. Also, when calving is spread out, at less busy times of the year there may be wide variation in age of animals penned together, leading to competition for feed. Although calves with a low growth rate (<0.6 kg/d) in the first 6 months did increase their growth rate later, they were unable to catch-up their peers and so remained significantly smaller at the start of the service period at 15 months of age. The actual body weights recorded at 15 months across the study ranged between 209 and 498 kg (Brickell *et al.,* 2009c). Many heifers were therefore below the target weight of 340 to 360 kg recommended for Holsteins at breeding (Heinrichs and Radostits, 2001).

Animals that experience a check during their growth phase can often make up the deficit by growing more rapidly subsequently but, depending on age at which the growth check occurs, this may result in altered body composition. When growth is restricted, metabolically active organs such as the liver are most affected and fat is utilized as an energy source so animals become leaner. Growth rate increases when an adequate food supply is restored, but animals are less able to compensate for weight losses than for checks to skeletal development (Hornick, Van Eenaeme, Gerard, Dufrasne, and Istasse, 2000; Greenwood and Café, 2007).

Age at first calving (AFC)

HEIFER FERTILITY

It is widely accepted that heifers should calve for the first time at about 2 years of age, although several studies have suggested that AFC can be reduced to 21 or 22 months without adverse consequences (Van Amburgh, Galton, Bauman, Everett, Fox, Chase and Erb, 1998; Simerl, Wilcox, Thatcher and Martin, 1991; Nilforooshan and Edriss, 2004). However actual ages found on farms vary considerably. In the UK, data supplied by National Milk Records showed a spread from 19 to 61 months of age. This was supported by the study of Brickell *et al.* (2009c), where the mean was 810 ± 6 days (27 months) with a range from 21 to 51 months of age. Sub-optimal nutrition during the rearing period delays AFC, so has immediate cost implications. It was recently estimated in a survey by the Kingshay Farming Trust that the cost of rearing a heifer to calve at 24 months was £988 at a growth rate of 0.79 kg/d, but cost increased to £1171 at 0.63 kg/d when first calving was delayed to 30 months (K. Bazeley, personal communication).

Age at calving is a function of age at which the farmer decides to start breeding coupled with the success of heifer fertility. Age at first breeding is affected by growth rate, and will generally be postponed if an individual appears too small. The study of Brickell *et al.* (2009b) found that increased body weight, girth and IGF-I concentration at 1, 6 and 15 months of age and higher rates of skeletal growth from 6 to 15 months were all associated with reduced age at first breeding and at first calving. Most differences among heifers were already apparent by 6 months of age. For example, heifers calving at >775 days of age were on average 23 kg lighter at 6 months of age in comparison with those calving at <775 days of age (160 compared with 183 kg). Excessive growth rate was not, however, beneficial. Heifers with higher growth rate over the entire rearing period up to 15 months of age required more services to conceive and there was also a trend for those animals which failed to conceive at all to have higher growth rate in both weight (0.81 vs 0.76 kg/d, P<0.1) and girth (0.22 vs 0.20 cm/d, P<0.05). This result contrasts with an earlier study on a single farm in which calves which failed to conceive at all had lower IGF-I concentrations at 6 months and were also significantly smaller at 9 months (Swali, Cheng, Bourne and Wathes, 2008). In practice, consistent growth rates of 0.6 to 0.8 kg/d will prevent heifers either remaining too small or becoming too fat and so will provide optimum results.

DYSTOCIA

Dystocia is a well recognized problem for first calving heifers with incidences in the range 40 to 51% reported (Johanson and Berger, 2003; Lombard *et al.,*

2007). In more severe cases dystocia will incur a direct veterinary cost, but the main costs are indirect due to increased calf mortality (Noakes, Parkinson and England, 2001; Johanson and Berger, 2003), periparurient disease or even death of the dam (Erb, Smith, Oltenacu, Guard, Hillman, Powers, Smith and White, 1985), and reduced subsequent fertility (McDougall, 2001) and milk yield (Dematawewa and Berger, 1997). The causes of dystocia have been divided into 3 categories: maternal, mechanical and foetal. The main maternal causes are pelvic constriction, incomplete dilation of the cervix and uterine inertia (Noakes *et al.,* 2001). Pelvic development is more likely to be inadequate in very young or poorly grown heifers, whereas excess fat deposition in overweight animals associated with a high body condition score (>3) is also a significant risk factor. The main foetal cause is a large calf size, which is more likely with a male calf and is also related to choice of bull (Johanson and Berger, 2003; Heringstad, Chang, Svendsen and Gianola, 2007).

A retrospective analysis of data from two studies was conducted to determine whether differences in heifer size and growth rate were related to subsequent incidence of dystocia at first calving. The first study included 106 heifers on a single farm which were measured at birth, 3, 6 and 9 months of age. Of these, 24 animals (23% of the total) required assistance at calving: these heifers had been smaller with respect to weight, girth and crown rump length (CRL) at 9 months of age (Table 1). They also had poorer fertility as heifers, so took longer to conceive, and were on average 68 days older at calving. Despite their initially smaller size, they also had a slightly higher average BCS pre-calving and produced larger calves: the calf weights were 39.4 ± 0.65 (unassisted) and 43.7 ± 1.19 kg (assisted), P<0.05. These results suggest that initially poorly grown heifers had become too fat during their pregnancy. The second data set included information from 204 heifers on 6 farms, which were measured for size and circulating IGF-I at 1 and 6 months of age. In these, 45% received assistance and the only size measure which differed significantly at 6 months between the two groups was CRL, which was lower in heifers needing assistance. Circulating IGF-I was also lower at 6 months, again indicative of poor early development. These studies both suggest that juvenile CRL is a useful predictor of future dystocia. This measures growth of the vertebrae and may reflect development of the pelvic bones more closely than height measurements.

AFC AND SUBSEQUENT PERFORMANCE

Many studies have examined the relationships between age at first calving and subsequent performance, although the follow up period is sometimes restricted to the first lactation only. For example, Ettema and Santos (2004) found that calving

at less than 23 months of age reduced milk production and fertility in the first lactation, but there was no benefit in calving later than 25 months, so the range 23 to 24.5 months of age provided the best economic return. Similarly, Pirlo, Miglior and Speroni (2000) determined the effect of AFC on the difference between milk yield returns and rearing costs in over 1 million Italian Holstein-Friesian cows and determined that the optimum AFC was between 23 and 24 months of age. Carson, Dawson, McCoy, Kilpatrick and Gordon (2002) imposed differing growth rates to calve cows at a similar age (24 to 25 months), but with differing weights and BCS. Animals calving at a greater weight (approximately 600 kg compared with 527 kg) and BCS (approximately 3.5 compared with 2.8) produced more milk in the first lactation but suffered greater weight loss postpartum and had poorer fertility, so over 2 lactations the lighter calving group produced the better return.

Table 1. Measurements in growing calves in relation to subsequent need for assistance at calving.

	Unassisted		*Assisted*		
	n	*Mean ± sem*	*n*	*Mean ± sem*	*P*
Study 1	82		24		
9 month CRL (cm)		154 ± 0.7		150 ± 2.1	0.048
9 month weight (kg)		261 ± 2.6		241 ± 5.6	0.001
9 month girth (cm)		148 ± 0.6		143 ± 1.1	0.001
S/C as heifer		1.5 ± 0.11		2.1 ± 0.25	0.013
Conception to 1st service		65 ± 5%		32 ± 9%	0.004
Age at calving (days)		751 ± 8		820 ± 26	0.008
BCS before calving		2.25 ± 0.07		2.65 ± 0.19	0.018
Study 2	114		94		
6 month CRL (cm)		140 ± 1.0		135 ±1.2	0.005
6 month IGF-I (ng/ml)		107 ± 3.4		94 ± 3.9	0.02

CRL = crown rump length; S/C = services per conception; BCS = body condition score; IGF-I = insulin-like growth factor-I.

In our own work, heifers calving at 22 to 23 months of age not only had good fertility as heifers, but also went on to perform better subsequently. In one herd, such animals survived the longest and produced most milk over 5 years (N.E. Bourne, A. Swali and D.C. Wathes, unpublished observations). Total milk production for the 5 years from birth according to AFC was as follows: 22-23 months, n=14, 25,031 ± 1491 kg; 24-25 months, n=61, 20,395 ± 1127 kg; 26-28 months, n=33, 16,671 ±1335 kg; 32-36 months, n=9, 8,029 ± 2780 kg. In part the higher level of production in the younger calving animals was due to consistently

good fertility, so they progressed through 3 lactations in a 5 year time span. This had additional economic benefit as the proportion of life in the first 5 years spent in milk production (calculated as the days in milk/days alive x 100%) was 48%, 42%, 38% and 18% for the four AFC groups. In summary, growing heifers well so that they can calve for the first time at 22 to 24 months of age will produce the best long term returns.

Possibilities for genetic selection

SELECTION INDICES

Considerable progress has been made in recent years to incorporate health and fertility traits into breeding indexes. For example, £PLI (Profitable Lifetime Index) only gives a 45.2% weighting to £PIN (profit index) which is based on the profit margin for production traits (milk, fat and protein). £PLI has added to this by including lifespan (weighting 21.1%), fertility (18.5%), somatic cell count (5.5%), udder (5.6%) and locomotion (4.1%) traits. Overall £PLI aims to calculate the average economic benefit of these traits over the lifetime of the cow (DairyCo, 2008). Heritability estimates for these additional traits are generally lower than those of production traits (typically about 0.03 to 0.10 compared with approximately 0.50 to 0.70), both because they are more difficult to measure accurately and also because they are influenced more by management and environmental factors. Despite these problems, traits with low heritability can still be improved through selective breeding. The phenotypic measures which go towards calculation of £PLI for bulls are, however, obtained from their offspring only after these animals have joined the milking herd. Data on some of these traits, such as lifespan, do not become available for many years. It would therefore be advantageous to introduce estimates of performance obtained from juvenile heifers which could be used at an earlier age.

MEASUREMENTS OF PHENOTYPIC TRAITS IN JUVENILES

Phenotypic traits such as heifer size (weight, height, girth, crown rump length) at particular ages, growth rate and heifer fertility could be included into traditional breeding indices, as they are all correlated with later performance. Beef cattle evaluations have included basic weight and growth traits for many years, typically assessed at birth, weaning and as a yearling (Crews, 2005). For beef dams, mature live weight and number of calves weaned per lifetime have also been included in some selection programmes (Enns and Nicoll, 2008). An alternative approach

is to use endocrine and metabolic measurements. For example, genetic selection for increased milk yield has resulted in significant changes to the somatotrophic axis. Higher yielding cows experience a greater loss of body condition score in early lactation and this is associated with raised concentrations of GH and reduced levels of IGF-I and insulin. This promotes greater tissue mobilisation and an increased reliance on utilization of fatty acids for energy; glucose levels are reduced, whereas concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) are raised (Wathes, Fenwick, Cheng, Bourne, Llewellyn, Morris, Kenny, Murphy and Fitzpatrick, 2007). Several research groups have postulated that measurements of these metabolic parameters as a juvenile may predict adult performance.

The GH-IGF-I axis is of key importance in growth as well as lactation. Between 1 and 6 months of age there is a strong positive correlation between IGF-I concentration and growth rate in dairy heifers (Brickell *et al.* 2009c; Figure 2). Higher IGF-I secretion is also linked to more rapid pre-pubertal development in dairy bulls (Brito, Barth, Rawlings, Wilde, Crews, Boisclair, Ehrhardt and Kastelic, 2007). Previous studies investigated the predictive use of GH secretagogue challenges in pre-pubertal dairy calves and found that release of GH was related to genetic potential for milk yield (Løvendahl, Angus and Woolliams, 1991), although actual subsequent milk production was not recorded in this study. Endogenous metabolic hormone profiles and the response to a GRF (growth hormone releasing factor) challenge were monitored in 6-month-old dairy calves and related to subsequent fertility and actual milk production over three lactations (Taylor, Beever, Bryant and Wathes, 2006). Plasma IGF-I concentration in pre-pubertal animals was positively correlated with their post-calving IGF-I concentration, whereas glucose concentrations were negatively correlated between these time periods. There was, however, no significant relationships between any measure of pre-pubertal GH secretion, IGF-I, fed or fasted insulin or glucose concentrations and actual milk yield in any of the first three lactations. There was some relationship with fertility; calves which had low IGF-I and high GH concentrations measured at 6 months of age were more likely to experience a delayed interval to ovulation after their first calving (Taylor, Beever, Bryant and Wathes, 2004). In accord with this, Hayhurst, Flint, Løvendahl, Woolliams and Royal (2009) reported that dairy calves with a high basal GH concentration measured at 4 months of age had a longer subsequent calving interval.

GENOMIC SELECTION

Recent developments in genomics offer the promise of basing selection decisions on analysis of DNA of an individual cow or bull. Genetic tests can be performed

on a blood or hair sample obtained shortly after birth. Regions of the genome on particular chromosomes are associated with traits of commercial interest if they contain one or more genes which have a major influence on the trait in question. For example, a polymorphism in *DGAT1*, the gene encoding acyl-CoA:diacylglycerol acyltransferase, the terminal enzyme in triglyceride synthesis, has a major influence on milk production traits, in particular milk fat content (Schennink, Stoop, Visker, Heck, Bovenhuis, van der Poel, van Valenberg and van Arendonk, 2007). Bovine Leukocyte Adhesion Deficiency (BLAD) is a genetic disease of cattle affecting the hematopoietic system which is caused by a single point mutation (Mirck, Von Bannisseht-Wijsmuller, Timmermans-Besselink, Van Luijk, Buntjer and Lenstra, 1995). Homozygote cattle suffer low birth weight and unthriftiness leading to a short life expectancy of < 1 year. Identification of this mutation has enabled breeders of Holstein cattle to avoid crossing heterozygotes, thus preventing birth of affected calves (Nagahata 2004).

The entire bovine genome has now been sequenced and, in common with other species, this has revealed large numbers of minor differences in sequence between individuals. Single nucleotide polymorphisms (SNPs) are variations in the DNA sequence at a single nucleotide. Some of these variations may have a direct effect on phenotype by changing the production of a particular protein. The majority of SNPs do not but, because long lengths of chromosomes are inherited together, such SNPs may be sufficiently close to a causative genetic change to be co-inherited with it. Current research is using panels of >50K SNPs, but technology is advancing so rapidly that within the foreseeable future (5-10 years) it should be possible to sequence the entire genome of selected individual animals for a reasonable cost. Research workers interested in using genomic selection are currently using data on many thousands of SNPs measured throughout the genome as the basis for genomic selection (Meuwissen, Hayes and Goddard, 2001; Hayes, Bowman, Chamberlain and Goddard, 2009). These data are used to estimate genomic breeding values, a strategy which is predicted to significantly increase reliability of estimated breeding values of individual animals and hence to increase the rate of genetic gain possible within dairy cattle populations.

Where the breeding goal is to improve complex traits such as disease resistance and fertility this requires collection of a large body of accurate phenotypic data, preferably under a variety of different management systems. To our knowledge, results of such studies have not yet been directed towards improvements in traits associated with rearing heifers. We have, however, started to analyse data on heifer performance against some individual SNPs. It had previously been reported that several polymorphisms in the leptin gene influenced both milk production and feed intake in lactating cows (Pomp, Zou, Clutter and Barendse, 1997; Liefers, te Pas, Veerkamp, and van der Lende, 2002). Leptin is a hormone which affects many different systems in the body including the placenta and we have found

evidence that the Exon 2FB polymorphism is also associated with the incidence of perinatal mortality in first-calving heifers (Brickell, Pollott, Clempson, Otter and Wathes, 2009).

Designing a management strategy for optimum heifer rearing

In summary, in order to produce well grown, healthy heifers for an acceptable economic outlay it is first necessary for dairy units to draw up a comprehensive management strategy to suit their individual circumstances. Once the requirements and associated costs have been assessed accurately, then consideration can be given as to whether the rearing process is best done on farm. Alternatively all or part of it could be contracted out to a specialist, thus freeing up time and capital but increasing disease risks.

The management strategy must consider facilities and personnel available. Direct costs will include feed, forage, bedding, labour, land costs, semen and veterinary services. Indirect costs will include maintenance and replacement costs for both machinery and buildings, pasture management and fertilizer, water and electricity. As discussed above, the type and amount of feed provided needs to be of sufficient quality and quantity to promote adequate growth rate through each stage of development. Calf housing should be clean and well ventilated and health control measures need to be in place to ensure that adequate hygiene is maintained.

Suitable policies for weaning, vaccination and worming are all required. Breeding strategies should be considered carefully. If AI is to be used then adequate handling facilities are needed. When natural service is the preferred option, then the fertility of the bull is paramount. In both cases services should be followed up with a pregnancy diagnosis and animals should be re-examined at intervals to check that none has lost its pregnancy. Leading up to first calving heifers should be well grown but not allowed to get fat and careful planning is needed to provide them with a smooth and stress free transition into the milking herd. Finally, first calving heifers need close attention and supervision actually at calving.

In addition to drawing up and implementing such a detailed rearing strategy, dairy farms should place increasing emphasis on careful monitoring of heifers throughout this important phase of their lives (Table 2). Good record keeping and benchmarking are key. Accurate records should be kept of timing and reasons for any mortality or culls. Routine health checks should include some measurements of IgG levels in newborn calves and worm counts in animals at pasture. Finally, and importantly, farmers will benefit from assessing growth rates of their animals at regular intervals. We suggest 3 time points as a minimum: shortly after weaning, at about 6 months and again at 15 months of age. Size monitoring can either be

done using a weigh system (which can be portable, see Figure 1) or a weigh band. Height and CRL are also useful measures because they assess skeletal development. This monitoring will reveal whether growth rates are on target in all animals. Feed inputs can then be adjusted up or down to achieve optimum rates. For animals identified with poor early growth careful consideration should be given as to whether it will give a better long term economic return to try to remedy the situation with remedial treatment of that individual (e.g. supplementary feeding) or to cull immediately without incurring further expense. This in turn should avoid the prospect of having some animals join the milking herd with a delayed age at first calving and poor prospects for adult performance.

Table 2. Targets for optimum heifer rearing.

Stage	Target weight	Target mortality rate	Key considerations
Birth	36-40 kg	<5%	Ensure adequate, early intake of good quality colostrum.
1 month	55-60 kg	<3%	Good hygiene for housing and feeding. Feed good quality milk or milk replacer.
3 months	95 -100 kg	<2%	Minimise stress at weaning.
6 months	160 -180 kg	<2%	Monitor growth. If at pasture, check grass quality.
15 months	340 - 375 kg#	<5%	Heifers must be adequately grown. Need good AI facilities and insemination technique, or ensure fertility of bull.
22-24 months	550-625 kg#	<2%	Aim for BCS of 2.5-3.0 at calving. Ensure adequate supervision of first calving heifers.

Target weights need to be established for each farm, based on average weights of mature cows. Heifers should reach 55-60% of mature body weight at first service and 85-90% by first calving.

Data from: Heinrichs and Radostits (2001); Dawson and Carson (2005); Margerison and Downey (2005); Swali and Wathes (2007); Brickell *et al*., (2009a); Brickell *et al*., (2009c).

Acknowledgements

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3

REDUCING DIETARY PHOSPHORUS INPUTS WITHIN DAIRY SYSTEMS

C.P. FERRIS

Agri-Food and Biosciences Institute (AFBI), Large Park, Hillsborough, Co Down BT26 6DR, Northern Ireland

Introduction

The two main inputs of phosphorus (P) to agricultural systems are P in inorganic fertilisers and P in concentrate feedstuffs. Agricultural P is obtained from phosphate rock, with China, the United States of America (USA), Morocco and the Russian Federation currently the world's main producers. However, world reserves of phosphate rock are finite, and at the present rate of use known reserves have a predicted lifespan of approximately 200 years (Richards and Dawson, 2008). Thus there is an increasing need to maximise the efficiency of use of this un-substitutable nutrient, and to minimise losses from agricultural systems.

In many agricultural regions, and at an individual farm level, there is a significant imbalance between P inputs and P outputs. For example, until relatively recently the agricultural P balance sheet for Northern Ireland (NI) (Foy *et al*., 2002) indicated a total P input of 18911 t/annum (9601 t/annum in inorganic fertiliser and 9310 t/annum in feeds), compared to an output (removed in milk, beef, sheep, 'pigs and poultry' and crop) of 6062 t/annum.

Although most surplus P accumulates within the soil, part of this P is at risk of being lost to the environment. In addition to representing a loss of valuable nutrients from agricultural systems, phosphates can cause eutrophication of waterways (enrichment of water by nutrients, especially compounds of nitrogen and P, whereby a body of water changes from a nutrient poor (oligotrophic) to a nutrient rich (eutrophic) state). Eutrophication has a number of adverse effects on water quality, including health risks associated with toxic algae and algal scums in drinking water supplies and recreational waters, damage to habitats leading to loss of species diversity, loss of fisheries, and undesirable aesthetic impacts such

as odours, loss of transparency, and clogging by weeds, the latter reducing amenity value. Phosphorus is often the 'limiting nutrient' within these processes.

In many parts of the world dairy farming is a significant driver of P-induced eutrophication. Problems exist in countries where grassland-based systems dominate, such as New Zealand and Ireland, and in countries where intensive dairy systems are more common, such as the USA and the Netherlands. However, as pressure on water resources continues to grow, all agricultural sectors, dairying included, will be forced to tackle this problem with increasing rigour. Within the European Union (EU), legislation designed to improve water quality is already in place. For example, restoring surface waters to good ecological status by 2015 (including reducing the trophic status) is a target within the Water Framework Directive, and, where it can be related to agricultural activities, action to control nutrient enrichment is also required under the Nitrates Directive**.**

Proportionally 0.58 of P exports to inland waters within NI are of agricultural origin (Smith *et al*., 2005). Although the exact contribution of different agricultural sectors to this problem is unknown, the impact of dairying is likely to be significant due to the importance of the dairy sector within NI, and its relatively intensive nature. This was highlighted in a recent agricultural P balance sheet for NI which indicated that P in dairy feeds represented the second largest P input (3025 t P per annum), exceeded only by P in fertiliser (Foy *et al*., 2002). This feed P input exceeded output of P in milk (1539 t of P per annum), so that even before inputs of P in fertiliser were considered, the average dairy farm was likely to be in P surplus. Analysis of 40 commercial concentrate feedstuffs collected from dairy farms around NI at this time (1999 – 2000) highlighted one of the factors contributing to this situation, namely the high mean P content of these concentrates (7.1 g/kg DM: C.P. Ferris, unpublished data).

Although improved management of P on farms will help to reduce P loss to the environment, control measures must firstly seek to reduce farm-gate P surpluses. For dairy farmers, options by which farm-gate P surpluses can be reduced include reducing the quantity of P brought onto farms in fertiliser and feeds. With regards the latter, this may involve reducing stocking rates, utilising more home-produced concentrate feeds, feeding less concentrates/cow, or feeding concentrates with a lower P concentration. Although reducing P levels in dairy cow diets is recognised as one of the most promising options for reducing P surpluses, it will clearly be unacceptable if animal performance, health, fertility and welfare are compromised.

This chapter reviews findings of 'recent' studies in which dairy cows have been offered diets containing different P concentrations, and examines opportunities for reducing P concentrations in dairy cow diets, together with some of the practical implications of feeding rations containing lower P levels. Where possible, these issues are examined within the context of grassland-based systems.

Phosphorus requirements and uncertainties

Approximately 80% of P within the dairy cow occurs in bones and teeth, principally as apatite salts and calcium phosphate (NRC, 2001). Phosphorus is also involved in acid-base buffer systems of blood and other body fluids, in cell differentiation, in all energy transactions, and as a component of cell walls and cell contents. Dairy cows secrete large quantities of P in milk on a daily basis (approximately 0.9 g/kg of milk), thus greatly increasing their requirements compared to those of growing cattle. Rumen microbes also have a requirement for P (Bryant *et al.*, 1959), and if this is not supplied via the diet, or from P recycled in saliva, microbial activity may be impaired.

The P requirements of dairy cows have been defined in many countries, including the United Kingdom (UK) (AFRC, 1991), USA (NRC, 2001), Germany (GfE, 1993) and the Netherlands (NCMN, 1973). Most P requirement systems adopt a factorial approach which sums the net P required for 'maintenance', growth, pregnancy and lactation, and then divides this by a true absorption coefficient, to obtain a total daily P requirement.

Valk *et al.* (2000) reviewed the calculated P requirements of lactating dairy cows using a number of these systems, and demonstrated that although there was reasonable agreement between systems in net P requirements for milk production, maintenance P requirements and true absorption coefficients varied widely among systems. The differing maintenance requirements are due in part to the different approaches adopted, with maintenance being a factor of cow live weight in some systems, and of dry matter (DM) intake in others. In addition, appropriate data were not always available when these systems were being developed. For example, within the current UK recommendations (AFRC, 1991), maintenance requirements for lactating dairy cows are based on unpublished data obtained from experiments involving sheep. Indeed, recognising the limitations of existing knowledge, and noting that many aspects of P nutrition and metabolism were not well understood, AFRC (1991) included a recommendation that dairy cow P requirements should be validated in long term feeding trials using roughage-based diets.

It is clear that the net P requirements of dairy cows are not known precisely; furthermore, availability of P from many common feedstuffs is not well defined. As a consequence of these uncertainties, together with the perceived benefits, especially in relation to fertility, that farmers associate with feeding diets high in P content, there is evidence that nutritionists and farmers tend to feed P to dairy cows at levels in excess of existing recommendations.

Impact of reducing dietary phosphorus content on cow performance

During the last four decades a number of studies have examined the effect of dietary P on dairy cow performance (see Table 1). However, many of these studies were of a relatively short term nature (less than two years), which is of concern as cows have the ability to deplete phosphorus reserves for milk production over a number of lactations, thus deficiency symptoms may not arise in the short term. In addition, the appropriateness of some of these studies from a UK perspective has been questioned due to the use of monosodium phosphate (a form of P with high availability which is rarely used in UK diets) as a P supplement, and the use of non grass/grass silage-based diets (Hemmingway, 2002). The latter is of concern as the availability of P from different feedstuffs (concentrates, maize silage, alfalfa silage, grazed grass) may vary, as reflected in the different true absorption coefficients adopted within feeding recommendations in different countries.

Although low-P diets have been examined in grassland-based systems (Brodison *et al.*, 1989), this study involved relatively low-yielding dairy cows (5,000 litres), compared to the industry norm at present. However, it was only recently that the recommendation made within AFRC (1991), namely 'validation in long term feeding trials using roughage-based diets', was addressed in a four-year experiment involving a grassland-based system (Ferris *et al.*, 2010a and 2010b).

NUTRIENT UTILISATION AND FOOD INTAKE

Rumen microbes have a requirement for P (Bryant *et al.*, 1959), and if this is not supplied via the diet, or from P recycled in saliva, microbial activity may be impaired, and ration digestibility and food intake reduced. For example, P depletion has been associated with a reduction in microbial protein synthesis and organic matter digestibility in sheep and goats (Breves *et al.*, 1985; Petri *et al.*, 1988). However, in the majority of dairy cow production studies which involved measures of nutrient utilisation, ration digestibility was unaffected when low P diets, including one containing 2.4 g P/kg DM (Valk *et al.*, 2002), were offered. It is therefore likely that diets containing extremely low levels of dietary P are required before ration digestibility is impaired; Satter (2003) suggested that modern dairy cow diets never approach the low dietary P concentrations that can result in impaired microbial growth in the rumen.

Reduced food intakes have been observed with cows offered low-P diets in a number of studies. For example, Call *et al.* (1987) observed a reduction in food intake after a diet with a very low P content (2.4 g P/kg DM) was offered for approximately six weeks. In a separate study (which commenced at week 17 of

Table 1. Details of some of the main studies published during the last 40 years in which dairy cows were offered diets containing different levels of dietary phosphorus

Reference	Main dietary components	Number of cows per treatment	Duration of study	Approximate lactation yield (kg)	Dietary P levels (g/kg DM)
Steevens *et al.* (1971)	Alfalfa hay, concentrates	16	Lactation 1 + 16 weeks of lactation 2	6 100	4.1 and 6.0
Carstairs *et al.* (1981)	Maize silage, concentrates	24	3 months	Unavailable	4.0 and 5.0
Kincaid *et al.* (1981)	Alfalfa hay, grass/alfalfa silage, concentrates	10	10 months	8 500	3.1 and 5.4
Call *et al.* (1987)	Alfalfa hay, corn, molasses, dried beet pulp, soya hulls	8-13	2 months pre-calving until 7-10 months post-calving	7 000	2.4, 3.2 and 4.2
Brodison *et al.* (1989)	Grass silage, concentrates (winter); grazed grass (summer)	35	3 years	5 000	Housed: 3.5 and 4.4, Grazing: 3.5 and 3.5
Brintrup *et al.* (1993)	Grass silage, maize silage, concentrates	26	2 years	7 500	3.3 and 3.9
Dhiman *et al.* (1995)	Alfalfa silage, maize silage, high moisture ear corn, soya bean, barley	23	3 months	Unavailable	3.9 and 6.5
Valk and Šebek (1999)	Grass silage, dried grass, maize silage, wet beet pulp, straw, concentrates	6-9	Week 17 of lactation 1, to the end of the dry period in lactation 2	9 000	2.4, 2.8 and 3.3
Wu *et al.* (2000)	Alfalfa silage, maize silage, high moisture ear corn, soyabean, beet pulp	8-9	1 year	11 000	3.1, 4.0 and 4.9

Table 1.Contd.

Reference	Main dietary components	Number of cows per treatment	Duration of study	Approximate lactation yield (kg)	Dietary P levels (g/kg DM)
Wu and Satter (2000)	Alfalfa silage, maize silage, high moisture ear corn, soya (housed); maize silage, high moisture ear corn, soya, grazed grass (grazing period)	21	2 years	9 500	Housed: 3.8 and 4.8, Grazing: 3.1 and 4.4
Lopez et al. (2004)	Alfalfa silage, maize silage, high moisture ear corn, soyabean	123	5 - 6 months	Unavailable	3.7 and 5.7
Tallam et al. (2005)	Alfalfa hay and silage, corn silage, high moisture ground corn, soyabeans, concentrates	27	10 months	11 000	3.5 and 4.7
Odongo et al. (2007)	Corn silage, alfalfa silage, high moisture ear corn, grain mix	32	2 years	11,000	3.5 and 4.2
Ferris et al. (2010a)	Grass silage, maize silage, concentrates (winter) Grazed grass, concentrates (summer)	50 decreasing to 10	4 years (lactations 1 – 4)	7 500 increasing to 9 000	Housed: 3.6 and 4.9 Grazing: 3.6 and 4.2

lactation) involving a similar diet (2.4 g P/kg DM), Valk and Sĕbek (1999) observed no reduction in food intake until the dry period at the end of lactation 1, with DM intake reduced during year 2 (Table 2) to such an extent that this treatment was discontinued. These studies clearly demonstrate that a dietary P content of 2.4 g/kg DM is inadequate, even during a single lactation. Reductions in food intake have been observed also with diets containing higher P levels, namely 3.1 (Kincaid *et al*., 1981) and 3.5 g P/kg DM (Odongo *et al*., 2007), the latter involving primiparous cows. In a number of other studies in which reductions in food intake were observed (Bortolussi *et al*., 1996; Milton and Ternouth, 1985), ration digestibility was unaffected by dietary P level. Milton and Ternouth (1985) suggested that the reduction in food intake associated with low P diets may be mediated via a metabolic effect at the cellular level. However, intakes were unaffected in studies involving dietary P levels of between 2.8 and 3.3 g P/kg DM (Call *et al*., 1987; Brintrup *et al*., 1993; Valk and Sĕbek, 1999; and Wu *et al*., 2000), and during the winter period of a four-year study (Ferris *et al.,* 2010a) in which cows were offered diets containing approximately 3.6 g P/kg DM (Table 3).

Table 2. Dry matter intake and milk yield of dairy cows offered diets containing three levels of dietary phosphorus (2.4, 2.8 and 3.3 g/kg DM) from week 17 of Year 1 until the end of lactation in Year 2 (Valk and Sĕbek, 1999)

Dietary P concentration (g/kg DM)	2.4	2.8	3.3	2.4	2.8	3.3
	Dry matter intake (kg/day)			*Milk yield (kg/day)*		
Year 1 Weeks 17-27	21.5	20.7	21.1	26.8	25.9	27.5
Weeks 28-37	19.2	19.1	19.2	19.7	22.3	21.4
Dry period	10.5	11.1	11.6			
Year 2 Weeks 2-11	20.8	24.6	25.3	37.9	43.7	44.1
Weeks 12-21	21.9	24.0	24.7	29.9	37.5	37.1
Weeks 22-31	*	21.5	20.8	*	30.5	28.9
Weeks 32-42	*	19.8	19.5	*	24.9	22.1
Dry period	*	10.2	11.5			

* Cows removed from treatment

MILK PRODUCTION

Reductions in feed intake observed by Call *et al*. (1987), Valk and Sĕbek (1999) and Kincaid *et al*. (1981) with diets containing 2.4, 2.4 and 3.1 g P/kg DM, were accompanied by lower milk outputs. However the fall in milk production observed by Valk and Sĕbek (1999) was observed only after the diet was offered during

Table 3. Effect of dietary P concentration over four successive lactations on dairy cow performance and fertility parameters (Ferris *et al*., 2010a and b)

		Mean dietary P concentration	
	Lactation No	*3.6 g/kg DM*	*4.5 g/kg DM*
Intake (kg DM/cow/day)†	1	17.6	17.4
	2	19.9	19.6
	3	20.8	19.8
	4	22.9	22.7
Lactation milk output (kg)	1	7521	7474
	2	8241	8419
	3	9177	9219
	4	9000	8976
Proportion of cows with luteal	1	0.60	0.64
activity pre day 42 post-calving‡	2	0.55	0.64
	3	0.60	0.49
	4	0.65	0.73
Conception to 1st + 2nd	1	0.74	0.67
insemination (proportion basis)	2	0.73	0.56
	3	0.59	0.69
	4	0.31	0.25

† winter period only
‡ based on milk progesterone analysis

a second year (Table 2), highlighting that body P reserves can sustain cows through considerable periods of P inadequacy. In the same study, no reduction in milk production was observed with a dietary P concentration of 2.8 g P/kg DM during either of years 1 or 2, although this study excluded the first 17 weeks of lactation in year 1. In contrast, Call *et al*. (1987) observed a lower persistency of milk yield with diets containing 3.2 g P/kg DM, and Wu *et al*. (2000) observed a reduction in milk yield after cows had been offered a diet containing 3.1 g P/kg DM for 25 weeks, although intake was not affected in either of these studies. Ferris *et al*. (2010a) found milk production to be unaffected when a diet containing 3.6 g P/kg DM was offered over four successive lactations (Table 3), highlighting the long term adequacy of this level of P in sustaining milk yields of up to 9000 kg/lactation. Although it is likely that dietary P affects milk yield through a reduction in food intake, an effect of dietary P on milk synthesis at the cellular level is also possible. Nevertheless, Ferris *et al*. (unpublished data) observed no difference in partial efficiency of lactation (kl) with cows offered diets differing in dietary P levels. In general, there is no evidence of a reduction in milk output in studies involving dietary P levels in excess of 3.3 g/kg DM (Table 1), although all but two of these studies (Brodison *et al*., 1989; Ferris *et al*., 2010a) were of less than two years in duration.

BODY TISSUE RESERVES

When cows were offered diets containing 2.4 g P/kg DM, Call *et al.* (1987) observed a significant reduction in live weight after a 14-week period; a trend towards lower live weight was observed by Valk and Sĕbek (1999). In each of these studies, however, reduction in live weight was associated with other symptoms of P deficiency (reduced intakes and milk outputs). In contrast, Wu *et al.* (2000) observed no significant effect of dietary P level on either body condition score or live weight during a single lactation study, despite a significant reduction in milk yield in late lactation for cows offered a low P diet. More recently, Ferris *et al.* (2010a) observed a significant reduction in body condition score with a diet containing 3.6 g P/kg DM, despite no significant effect of diet on food intake or milk production. These differences became apparent only after cows had been managed on this diet for two full lactations, and the authors concluded that the effect was unlikely to have arisen as a direct consequence of the dietary P levels imposed.

COW HEALTH

Results from a number of studies (Valk and Sĕbek, 1999; Wu and Satter, 2000; Lopez *et al*., 2004; Odongo *et al*., 2007; Ferris *et al*., 2010b) provide no evidence that dietary P level had an effect on incidences of mastitis. Although Wu *et al.* (2000) and Odongo *et al*. (2007) observed a numerically higher incidence of hoof problems in cows offered a low P diet, Wu and Satter (2000) observed the reverse trend. Over four successive lactations, Ferris *et al.* (2010b) observed that dietary P level had no effect on the incidence of lameness. Similarly, in a six-month study involving 247 cows, incidence of foot/leg problems did not differ between cows offered high or low P diets (Lopez *et al*., 2004). More recently, Mullarky *et al*. (2009) observed that neither innate nor cell-mediated immune responses of lactating dairy cows differed when dietary P level was reduced from 5.2 to 3.4 g P/kg DM.

REPRODUCTIVE PERFORMANCE

There is no doubt that the perception of a strong link between dietary P concentration and dairy cow fertility is one of the key reasons why dairy cows are frequently fed diets containing high P concentrations. The origins of this perception have been traced by Ferguson and Sklan (2005), in a review of data published between 1920

and 1960, much of it based on field observations and survey work. However, in the vast majority of 'recent' studies in which the effect of dietary P level on cow fertility is presented (Call *et al*., 1987; Brodison *et al*., 1989; Brintrup *et al*., 1993; Valk and Sĕbek, 1999; Wu and Satter, 2000; Wu *et al*., 2000; Ferris *et al.,* 2010b), reproductive performance was largely unaffected by dietary P concentration. Nevertheless, these studies were designed primarily to examine the impact of dietary P level on cow performance, and not reproductive performance *per se*. Recognising that basic indices of 'fertility success', such as 'conception rate', are affected by a wide range of factors, including human 'intervention', a number of studies have examined the effect of dietary P concentration on physiological changes within the cow, including milk progesterone levels, oestrous behaviour and ovarian activity. For example, Lopez *et al*. (2004) using a radiotelemetric transmitter, observed that dietary P (3.8 and 4.8 g/kg DM) had no significant effect on number of oestrous events recorded, duration of the oestrous cycle, duration of oestrus, number of mounts within each oestrus or total mounting time within each oestrus. Similarly, using ultrasonography, Tallam *et al*. (2005) observed that dietary P (3.5 or 4.7 g P/kg DM) had no effect on days to first post-partum ovulation or diameter of dominant and ovulating follicles, corpus luteum development, or blood progesterone concentration during the voluntary waiting period. Ferris *et al*. (2010b) observed no difference in proportion of cows showing luteal activity prior to day 42 post-calving (Table 3), interval to commencement of luteal activity, and peak progesterone concentration during the first oestrous cycle, with cows offered diets differing in P content.

Replication has been a limiting factor in virtually all studies, with Wu and Satter (2000) observing that approximately 250 cows per treatment would have been required in their study in order to detect a 10% difference in fertility parameters. Realising the limitations of examining fertility data from individual studies in isolation, Satter and Wu (1999) combined data from 13 experiments, involving a total of 785 cows, approximately 392 cows per P level. When reproductive performance was compared across low (3.2-4.0 g P/kg DM) and high (3.9-6.1 g P/kg DM) P diets, diet had no significant effect on days to first oestrus, days to first insemination, days open, number of services per conception, or pregnancy rate. Indeed, reproductive performance was unaffected in studies where dietary P concentrations of 2.4 g/kg DM were examined (Call *et al*., 1987; Valk and Sĕbek, 1999), despite a reduction in cow performance with these diets. This is in agreement with the findings of Ferguson and Sklan (2005), who modelled the relationships between dietary P and conception rate, and between dietary P and pregnancy rate, across a range of studies. These authors concluded that dairy cattle can tolerate dietary P concentrations of between 2.0 and 3.0 g/kg DM, without reproductive performance being affected. In summary, it would appear that because most

modern dairy cow diets contain moderate to high concentrate feed levels, dietary P in isolation is unlikely to be a causal effect of poor reproductive performance.

BLOOD METABOLITES

It is generally accepted that blood P concentration does not provide a good indicator of P deficiency in ruminants (Forar *et al*., 1982). This was highlighted by Wu *et al*. (2000) who observed similar serum P concentrations over a range of diets, despite a significant reduction in milk yield with cows offered a diet low in P. Nevertheless, reduced plasma P concentrations have been observed with cows offered low P diets (Brodison *et al*., 1989; Dhiman *et al*., 1995; Wu *et al*., 2000; Lopez *et al.,* 2004; Ferris *et al*., 2010a). In the study of Ferris *et al*. (2010a), which was conducted over four successive lactations, a significantly lower plasma P concentration was observed with cows offered a diet containing 3.6 g P/kg DM, compared with cows offered a diet containing 4.5 g P/kg DM (Figure 1). The trend for plasma P concentration to be lowest during the post-calving period, and to decline with increasing lactation number, was observed previously (Wu *et al*., 2000; Forar *et al*., 1982). However, it was only during weeks 2-6 of lactation 4 that P concentration fell below the norm (1.44 mmol/l) for samples collected and analysed within NI (M. McCoy, personal communication). With extremely low P diets, however, plasma P concentration may be a useful diagnostic tool; Call *et al*. (1987) observed a significant reduction in plasma P concentration (mean over the lactation of 1.16 mmol/l) with a diet containing 2.4 g P/kg DM.

BONE RESERVES

The extent of P resorption from bone can be significant; Wu *et al*. (2001) suggested that a 600 kg dairy cow could mobilise between 600 and 1000 g P in early lactation. Indeed, there is no doubt that resorbed bone P has an important role to play in meeting P requirements of dairy cows, especially in situations where dietary P is inadequate. AFRC (1991) justified the absence of a safety margin for P requirement by suggesting that the skeleton could be relied upon to provide the necessary 'elasticity' between supply and demand. Similarly, Ekelund *et al.* (2006) suggested that it may be possible to reduce dietary supply of P to dairy cows in early lactation by optimising naturally occurring bone resorption.

A reduction in the P content of rib bones has been observed with cows offered low P diets (Wu *et al*., 2001; Ferris *et al.,* 2010b). However, in the former study bone strength (shear stress or fracture energy) was unaffected at a dietary P content
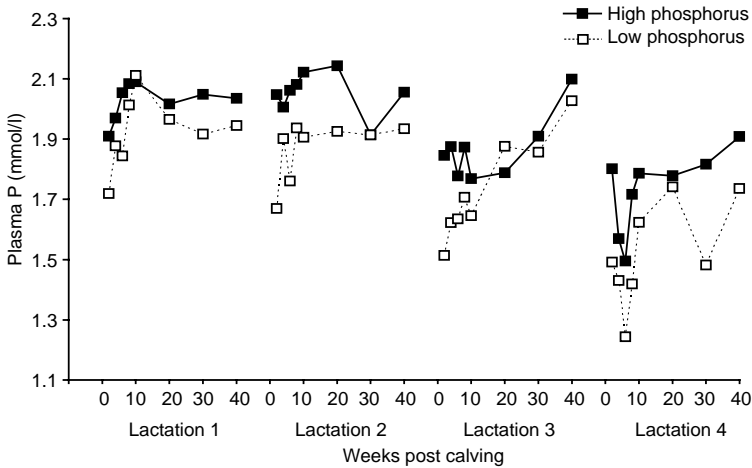
Figure 1. Effect of dietary phosphorus concentration on plasma phosphorus concentration over four successive lactations (Ferris *et al*., 2010a)

of 3.1 g P/kg DM, despite a reduction in milk yield in late lactation. Ferris *et al*. (2010b) observed no evidence of cumulative depletion of bone P reserves across four successive lactations, suggesting that bone P resorbed in early lactation was largely replaced later in lactation, or during the dry period. The findings of this study provide reassurance that the dietary P level examined (3.6 g P/kg DM) was sufficient not only for the needs of the cows in terms of performance, but also for long-term maintenance of bone P reserves.

OVERALL CONCLUSIONS FROM DAIRY COW PRODUCTION STUDIES

Although Valk and Sěbek (1999) suggested that a dietary P content of 2.8 g/kg DM was adequate for cows producing 9000 kg milk per lactation, they recommended a minimum dietary P content of 3.0 g/kg DM in practice. Phosphorus requirements based on this study are currently being recommended within the Netherlands (Valk and Beynen, 2003), although it remains to be seen if these low P levels can actually be achieved in practice through diet formulation, and if they are in fact adequate for dairy cows over the long term. Although no adverse effect on cow performance was observed in a number of studies (including that by Valk and Sěbek) when diets containing 3.0–3.2 g P/kg DM were offered, lower food intakes and milk outputs were observed in other studies involving similar dietary P levels, highlighting that this dietary P level might not be adequate in all situations. These inconsistent findings may reflect a carryover effect of previous P nutrition prior to the start of these studies, or possibly different P availabilities associated with the different diets offered.

For diets with P concentration between 3.3 and 3.5 g/kg DM, available evidence suggests that in most circumstances, although perhaps not in all, these levels will be adequate for lactating dairy cows. However, in the vast majority of studies no adverse effect on performance was observed with cows offered diets containing at least 3.6 g P/kg DM. Thus, based on current knowledge, this would appear to be a safe lower limit for dietary P levels for cows yielding up to approximately 9000 kg milk/year. This was the mean P level in the multi-lactation study undertaken by Ferris *et al*. (2010a), and these authors suggested that this level of dietary P was sufficient for dairy cows with lactation yields of between 7500 and 9000 kg, when managed within a predominantly grassland-based system. With different diets, but cows of a similar yield potential (7500-9000 kg/lactation), Wu and Satter (2000) concluded that a dietary P content of between 3.3 and 3.7 g/kg DM was adequate, although they suggested that 3.8 to 4.0 g/kg DM might be more prudent for high producing cows (>10,000 kg/lactation).

Low input grass-based systems

Although a number of the studies presented in Table 1 were grassland based, none involved the low input spring calving systems that are common throughout Ireland, and that are practiced in some western parts of the UK. Within these systems cows are often turned out to grass immediately post-calving, and minimal (frequently zero) supplementary concentrates are offered during the main part of the grazing season. Total concentrate inputs are normally between 200 and 700 kg/cow/lactation, and the 'concentrates' offered are often low-cost low P by-products, such as citrus pulp (offered to sustain cows through periods of grass shortage), so dietary P intakes from concentrates within these systems can be very low. Few studies have examined the impact of dietary P intake within this type of system, probably due to the practical difficulties of modifying dietary P intake when concentrate supplements are not offered. In addition, oversupply of P within these systems, the key driver of the majority of recent studies, is unlikely to be a problem due to the low concentrate inputs involved. Indeed, interest in P nutrition within these systems is likely to be driven by concern about P deficiency, rather than oversupply.

One study which attempted to address this issue was conducted at Johnstown Castle Research Centre in the Republic of Ireland (Culleton *et al*., 1999). In this study three farmlets were managed to achieve soil P indexes of 1-3 ('Morgan's P index'; where 1 represents a P deficient soil, and 3 represents the target for intensively grazed systems) by applying different levels of inorganic fertiliser P (0, 14 and 28 kg P/ha) over a four-year period. However, it was not until the

fourth year of this study that fertiliser management regime had a significant effect on herbage P concentrations (2.6, 3.0 and 3.3 g P/kg herbage DM, with soil P indexes 1-3, respectively). Although there was no evidence of a treatment effect on milk production or milk composition in any year of the study (cows were re-randomised at the start of each year), cows on the treatment with the lowest soil P index had a significantly lower live weight (46 kg lower) at the end of year 4, compared with either of the other two treatments.

Unfortunately this study was terminated at the end of year 4, leaving the lower live weight of cows on the low soil index treatment unexplained. However, a dietary P content of 2.4 g/kg DM was demonstrated to be inadequate by Valk and Sĕbek (1999), and diets containing 3.0-3.2 g P/kg DM were inadequate in a number of other studies. With a mean herbage P content of 2.6 g/kg DM over the grazing season, it is possible that the cows managed on the low soil P index treatment may have come close to experiencing P deficiency.

To examine this issue further (Table 4), J.P. Murphy (unpublished data) used the Moorepark Dairy Systems Model (Shalloo *et al.*, 2004) to estimated average daily milk yield, concentrate intake, grass silage intake and grazed grass intake (on a monthly basis), for the average Irish dairy cow (4676 kg milk/lactation: National Farm Survey data from the Republic of Ireland: Connolly *et al.*, 2005). Dairy cow P requirements (mean of 46.0 g/cow/day) were then calculated using the French P requirement system (Gueguen *et al.*, 1989), and 'actual' P intakes were calculated for herbage with a P content of either 3.0 or 3.5 g/kg DM (P content of grass silage and concentrates assumed as 3.5 and 5.2 g/kg DM, respectively). With a herbage P content of 3.5 g/kg DM (close to national average for intensively managed farms in Ireland), mean P intake over the year was 46.1 g/day (3.8 g/kg DM), which is close to the calculated P requirement during most months. In addition, total ration P content never fell below 3.6 g/kg DM during any month. With a herbage P content of 3.0 g/kg DM, mean P intake over the year was 42.6 g/day (3.5 g/kg DM), approximately 3.0 g/day lower than the calculated requirement. In addition, total diet P content was less than 3.6 g/kg DM throughout most of the grazing season. If this calculation had been undertaken for herbage with a lower P content, this situation would clearly be much more severe.

Nevertheless, historical evidence from farms where low input systems operate, and from research centres where cows have been managed over multi-lactations with minimal concentrate supplementation during the grazing season (C.P. Ferris, unpublished), suggest that these low input systems are sustainable in terms of P nutrition. Thus it would appear that within systems where the P status of the soil is maintained at a level optimal for herbage production, grazed grass as the sole feed can allow the P requirements of dairy cows to be met. However, with legislation increasingly forcing farmers to reduce P inputs, including inputs of inorganic fertiliser P, problems may arise in the future if the nutrient status of soils is not managed carefully.

Table 4. Calculated phosphorus requirements vs phosphorus intakes within a low input grazing system, with grass of different phosphorus contents (based on J.P.Murphy, unpublished data)

	Milk yield (kg/day)	Dry matter intake (kg/day)				P requirement (Gueguen et al., 1989)		P intake (herbage P, 3.0 g/kg DM)		P intake (herbage P, 3.5 g/kg DM)	
		Concentrate	Silage	Grass	Total	g/day	g/kg DM	g/day	g/kg DM	g/day	g/kg DM
January	1	0.6	11.6	0.0	12.2	33.1	2.7	43.8	3.6	43.8	3.6
February	5.1	1.7	7.0	0.9	9.6	32.6	3.4	35.9	3.8	36.4	3.8
March	11.9	3.2	4.5	3.6	11.2	42.8	3.8	43.0	3.8	44.8	4.0
April	20.0	2.9	1.7	7.9	12.5	54.9	4.4	44.7	3.6	48.7	3.9
May	20.4	1.7	0.0	11.3	12.9	55.5	4.3	42.5	3.3	48.1	3.7
June	20.0	1.7	0.0	11.8	13.4	54.9	4.1	43.8	3.3	49.7	3.7
July	18.0	1.7	0.0	11.7	13.3	52.0	3.9	43.5	3.3	49.4	3.7
August	16.8	1.7	0.0	11.5	13.2	50.2	3.8	43.2	3.3	48.9	3.7
September	15.6	1.7	0.0	11.2	12.9	48.3	3.8	42.3	3.3	47.9	3.7
October	13.2	1.7	1.4	9.8	12.8	44.7	3.5	42.7	3.3	47.6	3.7
November	9.6	1.7	7.1	3.7	12.5	46.0	3.7	44.5	3.6	46.4	3.7
December	3.5	2.0	8.8	0.0	10.8	36.9	3.4	41.2	3.8	41.2	3.8
Mean						**46.0**	**3.7**	**42.6**	**3.5**	**46.1**	**3.8**

The UK P requirement system

Phosphorus requirement systems within the UK have undergone a number of changes during the last forty years (ARC, 1965; ARC, 1980; AFRC, 1991). Within the latter (current recommendations), diet quality has a significant effect on P requirements, with endogenous P loss increasing by a factor of 1.6, and the true absorption coefficient for P being reduced from 0.70 to 0.58, when the metabolisability (q) of the diet, defined as diet metabolisable energy (ME) content / diet gross energy (GE) content, falls below 0.7. For example, according to AFRC (1991), when diet metabolisability is increased from 0.6 to 0.7, the P requirement of a 600 kg cow producing 15 kg milk/day decreased from 45 to 29 g/day (3.8 to 3.1 g P/kg DM, respectively), and for a cow producing 30 kg milk/day, P requirement decrease from 82 to 54 g/day (4.4 to 3.5 g/kg DM, respectively). Although AFRC (1991) recognised that diets with q >0.7 will rarely be attained with forage based ruminant diets in practice, the large increase in calculated P requirement with lower quality diets is difficult to justify, and is largely a function of the low value adopted for the true absorption coefficient of P (0.58). More recent P requirement systems have adopted higher true absorption coefficients for P, namely 0.7 (GfE, 1993; Valk and Beynen, 2003), and 0.64 for forages and 0.70 for concentrates (NRC, 2001).

   Evidence reviewed earlier in this chapter indicates that cows can be managed on diets with P concentrations considerably less than 4.4 g/kg DM without adverse effect, thus suggesting that AFRC (1991) overestimates P requirements of dairy cows offered lower quality diets. This was highlighted in the multi-lactation study by Ferris *et al*. (2010a and 2010b) where P intakes were proportionally 0.79 (winter period) and 0.84 (grazing/late lactation period) of AFRC (1991) P requirements (for diets with a q<0.7). Thus, although the results of this study provide validation 'in long term feeding trials using roughage-based diets' of the 'adequacy' of AFRC (1991) requirements, they also demonstrate the real potential to reduce P intakes below those within AFRC (1991). Indeed actual P intakes in this study were similar to those recommended within NRC (2001), and were slightly higher than those calculated using the new Dutch system proposed by Valk and Beynen (2003). Evidence available suggests a need to review the existing UK recommendations in order to prevent P being overfed, and to bring them into line with more recent recommendations in other countries.

Adoption of reduced P diets in practice

Although evidence reviewed suggests that dietary P levels can be reduced safely to at least 3.6 g P/kg DM without having a negative effect on performance, accurate

ration formulation requires that P content of both the concentrate and forage components of the ration is known. Although P content of concentrates may be declared, or calculated from the P content of individual concentrate ingredients, the P content of forages can be extremely variable, and in practice will not normally be known. For example, the P concentration of 249 NI farm silages ranged from 1.4 to 5.3 g P/kg DM (mean, 3.3 g/kg DM) (R.S. Park, Unpublished data). Although some feed test laboratories routinely analyse silages for P, this normally involves drying and milling the silages prior to analysis, a three step process which incurs additional cost. With Near Infrared Reflectance Spectroscopy (NIRS) having been adopted widely as a low cost methodology to predict both chemical and nutritional characteristics of fresh forages, the use of this technology to predict the mineral content of forages would appear to be a logical development. However, NIRS operates by measuring the Near Infrared radiation absorbed by organic bonds, and will only be able to estimate mineral levels if the minerals are chelated or bound to organic molecules.

This issue was examined by Park *et al.* (unpublished data) who, using 199 grass silage samples, developed NIRS calibrations for P content of fresh silage on both a fresh basis and a DM basis. The actual P content of these silages was determined using an Inductively Coupled Plasma Atomic Emissions Spectrometer (ICP-AES). This calibration was then tested using an independent dataset of 50 fresh silages. Although a reasonable prediction was obtained ($R^2 = 0.74$) for P content of silage on a fresh basis (Figure 2), this was not sufficiently robust to allow prediction of P content of fresh forages on a routine basis. In addition, the relationship was much poorer when predicted on a DM basis ($R^2 = 0.25$).
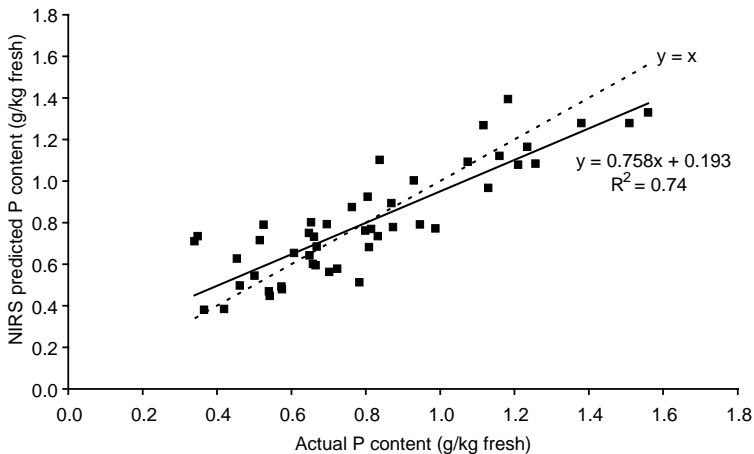
Figure 2. NIRS prediction of phosphorus in fifty fresh grass silage samples, on a fresh basis, compared to actual P content measured using ICP-AES (R.S. Park, Unpublished data)

In addition to forage P content, level of concentrate feeding also influences overall ration P content. Thus when advocating adoption of reduced-P concentrates, it is important that concentrate P levels are adequate for silages of different P contents, over a range of concentrate feed levels. To examine this issue, the relationship between concentrate feed level (4.0, 8.0, 12.0 and 16.0 kg/day) and silage P content (2.0, 3.0 and 4.0 g/kg DM) on total ration P content, was investigated for concentrates containing 4.5, 5.5 and 6.5 g P/kg DM (Table 5). Total DM intakes were modelled for a medium feed value silage from the data of Ferris *et al.* (2001). Values shaded in grey represent rations with a P concentration of less that 3.6 g P/kg DM, which are deemed to be 'potentially inadequate'.

Table 5. Impact of silage and concentrate phosphorus content, and concentrate intake, on total ration P concentration (g/kg DM)*

Silage P content (g/kg DM)	Concentrate P content (g/kg DM)	Concentrate intake (kg/day)			
		4.0	*8.0*	*12.0*	*16.0*
2.0	4.5	2.7	3.0	3.3	3.6
	5.5	2.9	3.4	3.8	4.2
	6.5	3.2	3.8	4.3	4.8
3.0	4.5	3.4	3.6	3.8	3.9
	5.5	3.7	4.0	4.3	4.6
	6.5	3.9	4.4	4.8	5.2
4.0	4.5	4.1	4.2	4.3	4.3
	5.5	4.4	4.6	4.8	4.9
	6.5	4.7	5.0	5.3	5.6

*Shaded section represents rations with P concentrations of less than 3.6 g P/kg DM (P levels assumed as potentially inadequate)

For silages with a high P content (4.0 g/kg DM), total ration P content was adequate under all scenarios. When silage P content was reduced to 3.0 g/kg DM, a dietary P content of less than 3.6 g/kg DM occurred only when a concentrate containing 4.5 g P/kg DM was offered at a low level of supplementation (4.0 kg/day). Thus, at normal concentrate feeding levels ($\geq$8.0 kg/day), and with silages with a P content close to the industry norm, rations are unlikely to be deficient in P even when concentrates containing very low P levels are offered. However, when a silage with a very low dietary P content (2.0 g P/kg DM) was supplemented with 4.0 kg concentrate/cow/day, dietary P contents were below 3.6 g/kg DM with all concentrate P contents. At a concentrate feed level of 8.0 kg/cow/day, dietary P contents were below 3.6 g/kg DM with concentrates containing both 4.5 and 5.5 g P/kg DM. Thus dietary P levels can become critical when silages containing very low P contents are supplemented with low levels of concentrates. Nevertheless, in

most practical feeding scenarios involving grass silage, a concentrate P content of 5.5 g/kg DM will be adequate. However, in situations where a considerable proportion of the diet comprises maize silage or whole crop silages (which normally have lower P concentrations than grass silage), a higher concentrate P content may be necessary to ensure dietary P levels are adequate.

Data in Table 5 do however confirm that there is little justification for dairy cow concentrates to contain in excess of 7.0 g P/kg DM, as was common practice in parts of the UK until relatively recently. A realisation of this fact led to a joint initiative being adopted across the feed compounding sector within NI, whereby agreement was reached that dairy cow concentrates would not contain in excess of 6.7 g P/kg DM (approximately 5.7 g/kg fresh). Although this was certainly a positive move, and one which demonstrates what can be achieved with industry co-operation, nutritionally there is potential for this value to be reduced further, perhaps to 5.5-6.0 g/kg DM.

A limitation to a more significant reduction in the P content of concentrates is that it can actually be more expensive to produce concentrates with low P concentration. This is largely due to the fact that lower cost ingredients, such as maize gluten, which have a low nitrogen:P ratio, must be replaced in part by more expensive ingredients such as soya-bean meal, which have a higher N:P ratio. However, the recent reduction in availability of maize gluten within Europe, a result of difficulties associated with the slow EU approval process for products derived from genetically modified crops, is likely to have resulted in a fall in the P content of many commercial concentrates. In addition, the high P content of forages on many farms adds to the difficulty of reducing overall ration P content. For example, in the study by Ferris *et al*. (2010a) a concentrate with a P content of 4.4 g/kg DM was necessary to achieve a total dietary P content of 3.6 g P/kg DM. This concentrate P content is much lower than would normally be adopted in practice. However, as total P inputs onto dairy farms are reduced, the P content of forages will also decline slowly, making lower P rations more achievable.

Impact of offering reduced P diets on dairy farm P balance

Using actual milk output, concentrate input and stocking rate data, Ferris and Hopps (2006) examined farm-gate P balances for 157 Benchmarked dairy herds in NI (2002–2003) under a number of different scenarios (Figure 3). Scenario 1 was based on 'current' industry practice and assumed a concentrate P content of 7.1 g/kg DM, and an input of P in inorganic fertiliser of 15 kg P/ha. Data points within this scenario clearly highlight the strong relationship between concentrate feed level and farm-gate P balance. Three additional scenarios were also examined, namely; offering concentrates with a P content of 5.5 g/kg DM (Scenario 2);

adoption of a 'zero P' fertiliser policy (Scenario 3); and offering concentrates with a P content of 5.5 g/kg DM, together with a 'zero P' fertiliser policy (Scenario 4). Under Scenario 2, the mean farm-gate P balance was reduced from 23 to 19 kg P/ha/year, the magnitude of the reduction on individual farms increasing with increasing concentrate inputs. With Scenarios 3 and 4 mean farm-gate P balances were reduced to 8.0 and 4.0 kg ha/year, respectively. In these two scenarios, 67% and 93% of farms had a P balance of less than 10.0 kg P/ha, respectively, and a considerable proportion of farms moved from a P surplus to a P deficit situation. Farms with a P balance >10.0 kg/ha/year tended to have concentrate inputs in excess of 2.5–3.0 t/annum.
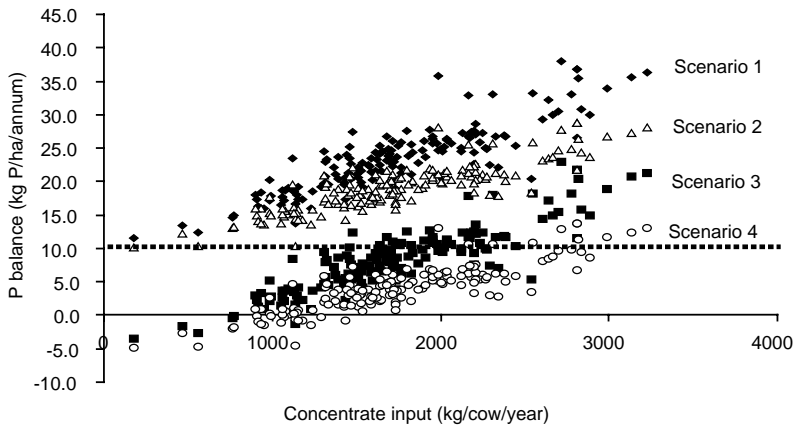
Figure 3. Impact of adopting a number of management strategies on farm P balance (Scenario 1, industry norm: Scenario 2, reduced P concentrates: Scenario 3, Zero P fertiliser policy; Scenario 4, reduced P concentrates and zero P fertiliser policy) (based on Ferris and Hopps, 2006)

The practical implications of this have been highlighted within UK legislation designed to fulfil the requirements of the EU Nitrates Directive, namely 'The Nitrates Action Programme Regulations (Northern Ireland) 2006' and the 'Phosphorus (Use in Agriculture) Regulations (Northern Ireland) 2006'. For example, this legislation prohibits application of inorganic fertiliser P unless a crop requirement can be demonstrated through the results of a soil test. With a recent survey indicating that 73% of grassland within NI (NIEA, 2009) had either high or excessive P levels (Olsen P index 3-5), the decrease in P fertiliser sales since this legislation was introduced is unsurprising. In addition, NI farmers who require a 'derogation' from the EU Nitrates Directive to allow them to operate at a stocking rate of more than 170 kg organic nitrogen/ha, are required to have an annual farm-gate P balance of <10.0 kg/ha. High concentrate feed levels are common on many of these intensively stocked farms, and in these situations, offering concentrates with lower P levels can play an important role in ensuring that farm-gate P surpluses meet the legislation. Indeed,

as concentrate feed levels increase, the opportunity, and sometimes necessity, to reduced concentrate P levels becomes greater.

Although reduction in use of inorganic fertiliser P and adoption of concentrate feeds containing lower P levels may cause few problems in the short term, the longer term implications need to be considered. For example, inorganic fertiliser P levels have a direct impact on soil P status and forage P content, as highlighted in data reviewed by Hemingway (1999). This was demonstrated also in the farmlet study by Culleton *et al.* (1999), where soil P levels in grassland receiving no inorganic fertiliser P become depleted in P over a number of years. As P levels in herbage, both grazed and conserved, decline (as highlighted in Tables 4 and 5), concentrate P content becomes increasingly important in ensuring that overall dietary P levels do not fall below critical levels. This highlights the importance of on-farm nutrient management, including regular soil testing, and the need to develop a low cost system for the rapid determination of P levels in forages.

Impact of reduced P diets on nutrient loss to the environment

In addition to reducing farm-gate P surpluses, reducing the P content of dairy cow diets will reduce P loss to the environment by reducing P excretion in manure. For example, when the P content of dairy cow diets was reduced from 5.2 to 3.7 g/kg DM, P intake was reduced from 103 to 72 g/day, and P excretion was reduced from 75 to 41 g/day (proportionally 0.45) (Ferris *et al.*, 2010b). If this reduction in faecal P excretion is assumed for a 150-day winter feeding period for a farm stocked at 2.5 cows/ha, this represents a reduction in P excretion of 5.1 kg/cow, and 12.7 kg/ha.

In addition to reducing the quantity of P excreted in manure, reducing the P content of a ration will also reduce the solubility of the P fractions excreted. For example, Dou *et al.* (2002) observed an increased proportion of water soluble P in faeces of cows offered high P diets. In addition, Ebeling *et al.* (2002) observed that reducing P in the manure of cows by 45% through dietary manipulation resulted in a 90% reduction in P losses from manure, when applied to arable ground. This can be attributed to the reduction in the soluble orthophosphate fraction in manures from animals offered reduced P diets. More recently, slurry produced by cows offered grass silage-based diets supplemented with concentrates containing different dietary P concentrations, was applied to grassland during the spring, summer and winter (O'Rourke *et al.*, 2007). The P contents of the slurries applied were 13, 10, 9 and 5 g/kg DM. Although the results from this experiment were not as dramatic as those observed by Ebeling *et al.* (2002), the overall trends were similar, with total P measured in run-off generated after a simulated rainfall event decreasing as P content of slurry decreased (Figure 4).
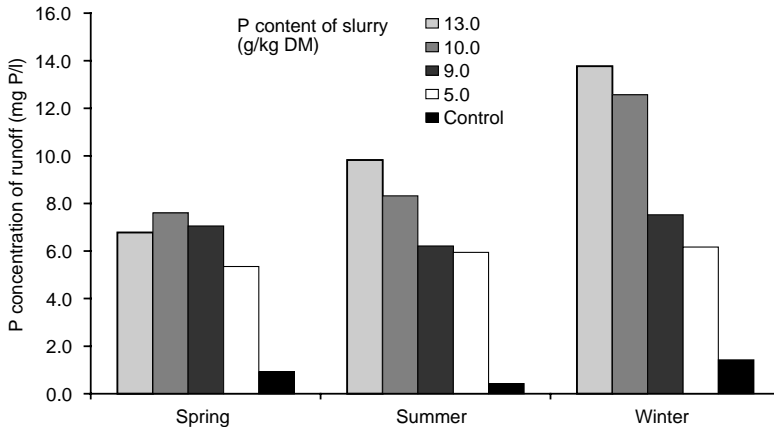
Figure 4. Flow weighted mean concentrations of total phosphorus measured in runoff generated two days after manure application during Spring, Summer and Winter (from O'Rourke *et al.*, 2007)

In addition, P loss to the environment can be reduced by matching nutrient supply from manures to crop requirements, the adoption of 'closed periods' for spreading; limiting spreading to periods when soil and weather conditions are suitable, and adoption of improved spreading techniques. With regards the latter, research is currently underway to examine the impact of different slurry spreading techniques (splash-plate, trailing shoe and shallow injection) on P loss in overland flows.

Conclusions

Phosphorus induced eutrophication continues to reduce water quality in many areas of the world, with agriculture, including dairy farming, contributing to this problem. Reducing the P content of dairy cow diets will both reduce farm-gate P surpluses, and reduce P excretion in manures. There is now a considerable body of evidence to demonstrate that P content of dairy cow diets can be reduced without having a detrimental effect on cow performance, health or fertility. In virtually all studies, a dietary P content of 3.6 g P/kg DM was adequate, and it is suggested that this dietary P content will be adequate in most situations. Nevertheless, improving the accuracy with which P is rationed to dairy cows requires P content of the forage component of the diet to be known. To date the use of NIRS does not appear to be sufficiently robust to allow P content of fresh silage to be predicted with sufficient accuracy. In addition, formulating low-P diets can be difficult if the forages offered contain high concentrations of P, and low P diets can also be more expensive to produce. For lower quality diets, the P recommendations within AFRC (1991) result in dairy cows being over fed P. The UK P recommendations need to be revised, especially in relation to 'maintenance' P requirements, and the true absorption coefficients of P of feedstuffs.

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4

PRACTICAL ASPECTS OF FEEDING GRASS TO DAIRY COWS

P. DILLON

Teagasc, Moorepark Dairy Production Research Centre, Fermoy, Co. Cork, Ireland

Introduction

The recently rejuvenated interest in grazing systems of animal production in many temperate and subtropical regions of the world is a result of lower product prices, the continuing removal of subsidies and tariffs, rising labour, machinery and housing costs, and perceived environment and animal welfare concerns associated with intensive systems. Grassland occupies some 150 million ha in Europe. This is used principally to provide feed for ruminant animals to produce milk, meat and fibre. Over the past 25 years, high product prices in the EU have encouraged systems with high inputs of concentrate feeds, machinery for forage conservation, and inputs of fertilizer. One of the major advantages of grass-based systems of milk production is its low cost per unit of production. It is envisaged that the cost of both concentrate feed and grass silage will continue to increase over the coming years; concentrate feed due to increased world demand and lower supplies, and conserved grass silage due to increases in contractor charges associated with inflation in labour, energy and machinery costs.

Grazing dairy cows is common practice in many European countries, although dairying regions vary dramatically in climatic conditions. Grass grows more regularly from spring to autumn in Western Europe (e.g. UK, Ireland, Normandy in France), whereas in other regions grass does not grow in summer (Pays de Loire and Aquitaine in France) or the grazing season is quite short due to long cold winters (Northern countries). However in recent years, in most European countries there has been a shift away from pasture-based systems to greater use of conserved forage-based systems, especially forage maize. Despite regional differences, grassland should provide the basis of sustainable dairying systems as grazed grass is the cheapest source of nutrients for dairy cows, thus enhancing the competitiveness of pasture-

based systems of production, preserving the rural landscape and promoting a clean, animal welfare friendly image for dairy production.

Grass production and profitability

In the most favourable regions of Europe (UK, Ireland, Normandy in France), a potential grass DM yield of 15,000 kg per ha (Drennan *et al.*, 2005) is achievable and can result in milk output of 1,200 kg of milk fat and protein per ha using a nitrogen input of 250 kg per ha and concentrate supplementation of less than 300 kg DM per cow (Horan *et al.*, 2005a). Such a system has been derived within an EU milk quota scenario (where total farm productivity is capped) to maximise profitability by reducing costs through increased pasture utilization in dairy cow diets. Figure 1 shows the relationship between milk production costs and the proportion of grazed pasture in the dairy cow ration (Dillon *et al.*, 2005). The relationship shows that for every 10% increase in grazed grass in dairy cow ration, milk production costs per litre are reduced by 2.5 euro cents.
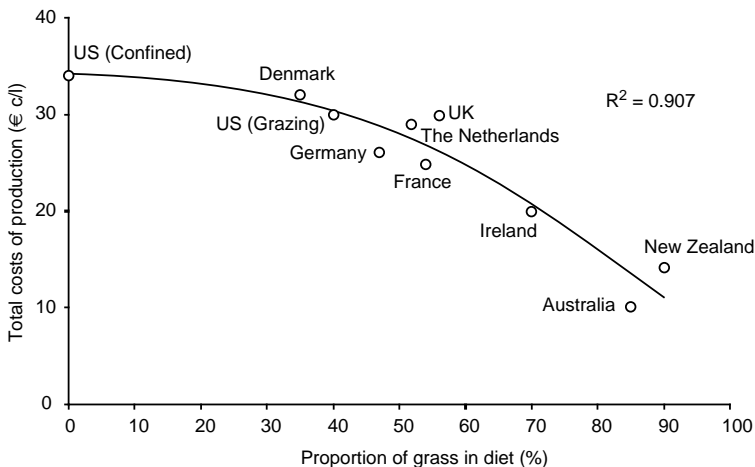
Figure 1. Relationship between total costs of production and proportion of grazed pasture in cows' diet (Dillon *et al.*, 2005).

Stocking rate or grazing intensity is a major determinant of the production per cow and per ha from grass-based system (McMeekan and Walshe, 1963; Le Du *et al.*, 1979; Journet and Demarquilly, 1979). High performance from pasture-based systems is based on high stocking rates accompanied by high herbage utilisation, where individual animal performance is compromised. Figure 2 shows the relationship between pasture harvested per hectare and profitability per hectare within New Zealand pasture-based systems. The analysis showed that pasture

utilised per hectare being the most important factor influencing profitability within a wide variety of milk production systems. Prior to the introduction of milk quotas in Ireland in the mid-1980s the optimum system of milk production was based on spring calving, a stocking rate of 2.5 to 3.0 cows/ha, a concentrate input of 500 to 750 kg/cow and a nitrogen application rate of 270 to 300 kg N/ha (Crosse, 1988). However, with the introduction of milk quotas on dairy farms, the emphasis shifted from maximising performance per hectare to maximising performance per kg of milk quota on the farm. In this scenario where land is not limiting or/and has a low opportunity cost it may be justifiable to allocate the extra land by means of a lower stocking rate to the dairy enterprise rather than to an alternative enterprise. The shift to lower stocking rates was further rewarded through the introduction of Rural Environment Protection Schemes (REPS). However in the future in the absence of EU milk quotas and reduced REPS payment dairy farm profitability will be maximised at relatively high stocking rates (2.7 to 3.0 cows/ha) resulting in a reduction in milk output per cow of approximately 10% and an increase in milk output per hectare of 20% (McMeekan and Walsh,1963).
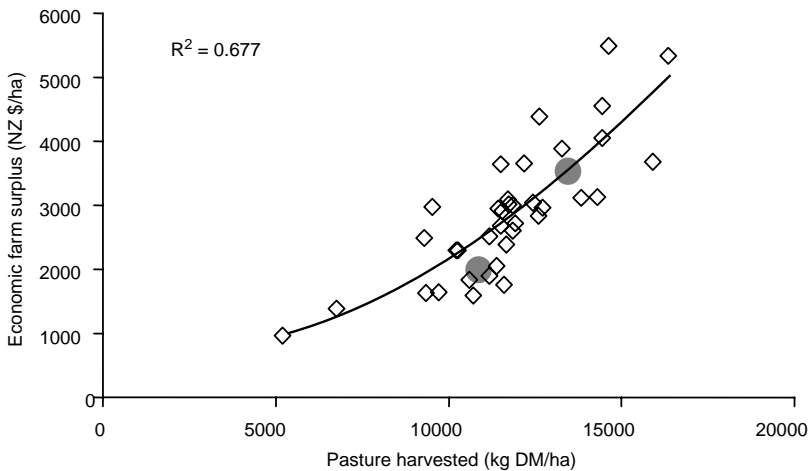
Figure 2. Effect of pasture eaten per hectare on Economic farm surplus per hectare within New Zealand pasture-based systems (Data from Fonterra Dairy Excellence awards 2002)

Plant

SWARD STRUCTURE

Herbage availability can be defined as the relative ease or difficulty with which herbage can be harvested by the grazing animal. Herbage availability is a complex parameter, that takes into account the qualitative and quantitative aspects of the sward and interactions with daily herbage allowance. To maximise intake, animals need to consume plants

that have characteristics that allow rapid consumption and lead to fast rates of passage through the rumen. Rook (2000) defined intake of herbage as the product of bite mass and bite rate, and time spent grazing as the product of meal duration and number of meals per day:

Daily intake = (bite mass x bite rate) x (meal duration x number of meals).

Grazing ruminants vary bite dimensions, bite rate and grazing time in response to changes in sward conditions (Hodgson 1981; Gibb *et al*. 1997). Numerous studies have focused upon the relationship between sward structure and intake per bite, assuming an overriding importance of intake per bite in driving overall herbage intake. Surprisingly, there are few data to quantify the effect of sward structural characteristics known to influence the bite weight upon daily intake of dairy cows. Also, by the nature of the studies they are more relevant to continuous rather than rotational stocking situations. In continuous stocking, herbage intake increases asymptotically with herbage mass or sward height (Le Du, 1980), with maximum intake being achieved at a sward height of 8 to 9 cm. In rotationally grazed pastures, herbage intake is maximised at a sward height of 8 to 10 cm (Stakelum *et al*. 1997). Cows grazing very short swards are unable to eat sufficient quantities of DM, even if the area of pasture offered is very large, whereas on tall, rotationally grazed swards other factors may have a negative effect on daily intake.

On rotationally grazed swards, herbage availability may be determined partly by the proportion of green leaf in the grazed horizon. Wade *et al*. (1989, 1995) first concluded that herbage availability increased with an increasing proportion of green leaf in the bottom of sward when animals cease grazing. This was further demonstrated by Parga *et al*. (2000), comparing two swards differing in the proportion of green leaf material below 15 cm, but with the same proportion above 15 cm. At high herbage allowance, herbage intake was similar for both swards, but when herbage allowance was reduced from 17 to 12 kg OM per day, herbage intake was reduced less in the sward with the higher proportion of green leaf material below 15 cm. Peyraud *et al*. (2004) showed that daily allowance of green leaf was a better predictor of DM intake than daily herbage allowance. This not only takes into account the effect of herbage allowance but also the effect of sward structure for a given allowance. Appropriate grazing management and/ or selection of the appropriate herbage varieties will play an important role in increasing the proportion of green leaf at the bottom of the sward.

SWARD SPECIES COMPOSITION

In general, legumes have characteristics that lead to higher animal performance compared to grasses. Herbage intake and milk production have been shown to be

higher in mixed perennial ryegrass-white clover swards compared to pure perennial ryegrass swards (Wilkins *et al*., 1994 and 1995; Ribeiro-Filho *et al*., 2003). Rogers *et al*. (1982) showed that cows consuming white clover pasture produced more milk and gained more live-weight (85 vs. 80 kg) due to a 30% higher intake. Harris *et al*. (1997) showed that in mixed swards with perennial ryegrass, milk yield was increased by 20% when dairy cows consumed a diet with 55 to 65% clover in the DM, compared to a diet with only 20% clover. No further advantage in animal performance was achieved by offering diets with 80% clover. Clovers contain less structural carbohydrate, leading to more rapid rates of breakdown of OM, nitrogen (N) and cell walls (Beever and Siddons, 1986) and the retention time is shorter compared with ryegrass (Ulyatt, 1981). In Ireland, Humphreys *et al*. (2009) has shown that a white clover/perennial ryegrass pasture is capable of carrying a stocking rate of 2.2 cows/ha with an N fertilizer level of 90 kg/ha in a low input grass-based system of milk production. Despite the clear advantages in intake of white clover over ryegrass, there are issues that need to be considered, such as the cost of increased prevalence of bloat and the additional costs of maintaining swards high in white clover content. Due to increases in fertiliser costs, lower product prices, availability of superior cultivars of white clover, better grazing management and probably benefits in terms of environmental sustainability, it is envisaged that in the future mixtures of white-clover and perennial ryegrass will play an increased role in grassland farming.

RYEGRASS CULTIVARS

In Europe, grass breeders have increased DM yield by 0.5% per year as tested in cutting trials in the Netherlands from 1965 to 1990 (Van Wijk and Reheul, 1991). However, there is little evidence that new grass cultivars have made a significant contribution to increased animal production under grazing. The expense of animal production experiments has often been cited as the reason for using cutting trials in variety evaluations. Gately (1984) compared an early perennial (Cropper) with a late perennial ryegrass (Vigour) for milk production at two stocking rates. At a low stocking rate, the improved digestibility of Vigour gave 8.8% more milk yield than Cropper. However, at high stocking rate, Cropper gave 6.6% more milk than Vigour, because of the greater pasture production in early spring at the time of peak milk yield. Hageman *et al*. (1993) obtained higher performance from tetraploid compared to diploid cultivars of perennial ryegrass with grazing dairy cows. Gowen *et al*. (2003) obtained higher DM intake and milk production from late heading compared to early heading perennial ryegrass cultivars when cows were stocked to allow adequate feed allowance. The higher performance with the late heading perennial ryegrass cultivars was associated with a higher proportion

of green leaf in the grazed horizon. Tas *et al*. (2005) found no difference in DM intake and milk production when comparing eight diploid perennial ryegrass cultivars differing in water-soluble carbohydrates content, and with inconsistent differences in crude protein and NDF content.

IMPROVED GRAZING EFFICIENCY THROUGH GRASS BREEDING

Animal production from grazed pasture could be improved through increased use of herbage species or varieties with increased intake and digestibility potential. Traditionally, plant breeding objectives were mostly focused on increasing DM yield and pest and disease resistance, with little emphasis on factors that affect animal performance and the characteristics of animal produce. Digestibility is a heritable characteristic and some improvement has resulted from conventional breeding, with further increases likely to result from biotechnological modification.

Wales *et al*. (2005) suggested that the use of techniques to genetically modify plants will enable development of plants with elevated concentration of ruminal undegradable dietary protein and high-energy yielding compounds, such as starch or triacylglycerides. Grazing studies have shown that animals have a strong preference for herbage with high concentrations of soluble carbohydrates (Ciavarella *et al*., 2000). In zero grazing studies, dairy cows offered pasture with high water soluble carbohydrate concentrations consumed more DM and produced more milk than cows fed grasses with lower concentrations (Moorby *et al*., 2001). However Tas *et al*. (2005) found no difference in intake, milk production or milk composition in cultivars of perennial ryegrass differing in water soluble carbohydrate. Another major objective of grass breeding should be to increase the length of grass growing season. Collection of ryegrasses from the Swiss uplands provides evidence that early spring growth and winter hardiness could evolve together (Tyler, 1988). This has been exploited in present day conventional breeding programmes, with new varieties such as Navan, having early spring growth some 10% higher than Portstewart, the previous leading late-flowering diploid perennial ryegrass. In the future it maybe possible to exploit this to a greater extent due higher winter temperatures as a result of climate change.

Animal factors

The influence of animal genotype on DM intake occurs not only through the animals' ability to consume greater quantities of herbage, but also through the capacity of the animal to calve each year at a time that facilitates the maximum amount of herbage to be incorporated in that animals diet. Worldwide, dairy cattle

are managed under a wide range of environments and production systems. Even within temperate conditions, these can range from grazing on lush temperate pastures with very low levels of supplementation to totally non-grazing or confinement systems feeding concentrates and conserved roughages. Only about 10% of the world's milk comes from grazing systems (World Animal Review, 1995), consequently the majority of dairy cattle have not been selected under grazing. Cattle on grazing systems must be able to graze effectively, survive fluctuations in feed supply and to walk long distances, abilities that are not required in confinement systems, plus conceive and calve at the right time every year.

There is now strong evidence to show that cattle which are genetically best suited to non-grazing systems are not best suited to grazing systems; an interaction between genotype and feeding system (Dillon *et al.*, 2006). Successful grazing systems require dairy cows that are capable of achieving large intakes of forage relative to their genetic potential for milk production, so that they are able to meet their requirements almost entirely from grazing. Until recently, in the world of dairy cattle breeding, the term "high genetic merit" was synonymous with high milk production potential. Now it is acknowledged that the complete index for high genetic merit should reflect as many characteristics as are required to reflect total economic profitability. In particular, due to the decline in reproductive efficiency within the Holstein, many countries have diversified their breeding goals to include measures of survivability or functionality (Philipsson *et al.,* 1994; Veerkamp *et al.,* 2002).

This should also increase the likelihood of survival in seasonal grazing systems, for which maintenance of a 365-day calving interval, and good fertility are essential to optimise financial performance (Lopez-Villalobos *et al.,* 2000). This limit to intake when grazing also suggests that cows most suited to grazing environments are likely to have lower genetic potentials for milk production and live weight, than cows best suited to more intensive diets. DM intake estimates differed by only 0.4 kg DM/day between the high production North American Holstein-Friesian selected solely on milk production and New Zealand Holstein-Friesian selected from a seasonal pasture-based system (17.9 v. 17.5 kg DM) on a grass only diet grazed to a post-grazing height 6 to 7 cm (Horan *et al.*, 2006) despite a large differential in milk production potential and live weight. A greater differential in total DM intake (1.9 kg/day) was observed when both genotypes were offered a daily allowance of 3.7 kg concentrate DM (20.8 v. 18.9 kg DM/day) while grazing. This is in agreement with the results of Kolver *et al.* (2002), who reported values for DM intake of 16.6 and 20.4 kg/day for grazed pasture and 17.3 and 24.0 kg/day on TMR, for New Zealand Holstein-Friesian cows or North American Holstein Friesian cows, respectively. For both strains, intakes were lower on pasture than on TMR, but on TMR the North American Holstein-Friesian cows showed a much bigger increase in intake (3.6 kg/day) than the New Zealand

Holstein-Friesian cows (0.7 kg/day). The higher grass-concentrate substitution rate (resulting in a low response to concentrate supplementation) with the New Zealand Holstein-Friesian cows suggests that they achieve a greater proportion of their potential milk production on grass alone than the high production potential North American Holstein-Friesian cows. Linnane *et al.* (2004) concluded that the grazing appetite of the New Zealand Holstein-Friesian is compromised by the provision of supplementary food. Horan *et al.* (2005b) found that the lighter New Zealand Holstein-Friesian had a higher grass DM intake per kg live weight.

   Additionally in grass-based systems, crossing the Holstein-Friesian with an alternative dairy breed (e.g. Jersey) can provide farmers with an alternative to increase overall animal performance by increasing herd health, fertility and milk value through hybrid vigour. The Jersey and Norwegian Red (crossbreds) have proven to be most compatible in an Irish seasonal grass-based system (Buckley and Berry, 2009). A large on-farm study, involving 46 commercial dairy herds over 3 years, has confirmed the advantages of crossbreeding with Norwegian Red. This study has demonstrated substantial improvements in reproductive efficiency (e.g. 6 week in-calf rate +15%) and udder health (-25,000 SCC/ml) with Norwegian Red×Holstein-Friesian compared to pure Holstein-Friesian, without compromising milk production potential. Crossbreeding with Jersey gave a significant improvement in milk composition (+0.7% fat and +0.3% protein), annual milk solids output (+13kg) and feed/production efficiency (+10%). Reproductive efficiency is also markedly superior with the Jersey crossbred cows (e.g. 6 week in-calf rate +16%).

Management factors

Numerous management factors exist which are conducive to achievement of high herbage intakes and have the potential to greatly enhance efficiency of pasture-based systems. In practice, management factors interact with the environmental, plant and animal factors discussed previously. Management factors, such as farm infrastructure (farm roadways, paddock access, water points) are also critical in achieving high grass DM intake under various climatic conditions. The management factors considered here are those with the greatest influence on grazing dairy cows' ability to achieve high DM intakes.

HERBAGE ALLOWANCE

On rotationally grazed pastures, grass allocation is commonly described in terms of daily herbage allowance, which is the weight of herbage cut above a sampling

height (i.e. kg of herbage DM per cow per day) (Greenhalgh *et al.*, 1966). Daily herbage allowance is more often estimated to ground level or at a cutting height of 4 or 5 cm, assuming that the material below the cutting height is not available for grazing. Herbage allowance is one of the primary factors influencing herbage intake. A number of studies have shown a curvilinear relationship between herbage allowance and herbage intake (Greenhalgh *et al.*, 1966; Peyraud *et al.*, 1996; Maher *et al.*, 2003). On vegetative perennial ryegrass swards, Peyraud and González-Rodríguez (2000) showed that herbage intake increased by 0.25 kg OM per day, per kg increase in herbage allowance ranging between 11 and 16 kg OM per day (above 4 to 5 cm). When herbage allowance increased above 20 kg OM per day, a much smaller increase of 0.05 kg of OM intake was achieved. Delagarde and O'Donovan (2005), comparing seven published relationships between herbage allowance to ground level and herbage intake of grazing dairy cows, showed an average increase of 0.20, 0.15 and 0.11 kg DM per kg DM increase in herbage allowance in the ranges 20 to 30, 30 to 40 and 40 to 50 kg DM herbage allowance to ground level, respectively. Intake predictions are similar between models for medium herbage allowances, but predicted intake differences are greatest at low (< 30 kg DM/day) and high (> 50 kg DM/day) herbage allowances. Table 1 shows the results from an Irish study comparing three different DM allowances for dairy cows in early to mid lactation (Maher *et al.*, 2003). Daily herbage allowances of 15.9, 19.8 and 24 kg DM per cow per day (> 3.5 mm) resulted in post grazing sward surface heights of 45, 55 and 66 mm, respectively, with corresponding daily milk productions of 20.8, 22.3 and 23.0 kg per cow. Increasing daily herbage allowance from 15.9 to 19.8 kg DM per cow increased herbage DM intake by 0.33, while increasing daily allowance to 24 kg DM only increased DM intake by 0.12 kg per day. The small increase in grass DM intake with increased daily herbage allowance above 20 kg DM (> 35 mm) indicates only a limited opportunity to increase DM intake from grass for cows yielding 23 to 25 kg per day.

Table 1. Effect of daily herbage allowance on performance of spring-calving dairy cows (Maher *et al.*, 2003)

	Herbage allowance (kg DM/ cow)†		
	15.9	*19.8*	*24.0*
Daily milk yield (kg)	20.8	22.3	23.0
Daily grass DM intake (kg) †	14.7	16.0	16.5
Postgrazing height (mm)	45	55	66
Herbage utilisation†† (%)	87	82	78

† Intakes are based on n-alkane measurements
†† Herbage allowance and utilisation are based on pre- and post-sward measurements above a sward height of 35 mm.

GRAZING INTENSITY

Grass growth rates in temperate pastures are highly seasonal, with little or no growth in winter and very high growth in May and June. Late spring/early summer pasture growth rate are generally about twice the daily cow requirement at recommended stocking rates (Dillon *et al.,* 1995). This surplus pasture can be harvested as silage or hay, or can remain as surplus herbage for summer grazing. Low rates of pasture utilisation will result in wastage and may also reduce animal production in summer. Thomson (1985) has shown that grazing at a low intensity at one point may reduce animal production at a later stage, through a decline in feed quality. Low grazing intensity in spring has resulted in reduced milk production of dairy cows (Holmes and Hoogendoorn, 1983; Hoogendoorn *et al.,* 1985) in the following summer. Stakelum and Dillon (1990) showed that pastures with high grazing pressure in spring/early summer produced swards of lower herbage mass, lower post-grazing height, higher proportion of green leaf and lower proportion of grass stem and dead material compared to swards with low grazing pressure. Increasing post grazing sward surface height above 5 to 6 cm has been shown to result in a deterioration of sward quality in mid and late grazing season (Mayne *et al.*, 1987; Stakelum and Dillon, 1990).

   Milk production results showed that pastures grazed to a post-grazing sward surface height in the May to June period of 5.5 to 6.5 cm compared to 8 to 8.5 cm achieved a higher DM intake (+0.8 kg per day) and higher milk production (+1.2 kg per day) in the July to September period (Stakelum and Dillon 1990). Additionally, in the May to June period, there was no difference in milk production per cow from the two swards, with the lower post-grazing swards achieving greater grass utilisation through higher stocking rates.

   Alternative strategies to achieve high DM intakes while maintaining low post-grazing residuals may include mechanically topping pastures post-grazing (Stakelum and Dillon, 1990), adopting a leader-follower grazing system with lower producing after high producing animals (Mayne *et al.*, 1986), provision of supplementary concentrate with intensive grazing, or, in the longer term, development of swards that would allow higher DM intakes while at the same time allow the sward to be grazed to a low residual height (Peyraud and Gonzalez-Rodrigez, 2000).

SUPPLEMENTARY FEEDING

Concentrate supplementation. Concentrate supplements are offered to grazing dairy cows either because of a shortfall in grass supply, or to increase overall intake and milk production. Efficiency of a supplement is expressed by the increase (kg)

in milk output per kg increase in concentrate DM intake. Substitution rate is the reduction in herbage DM intake per kg increase in concentrate DM intake. The interaction between level of concentrate supplementation and herbage allowance can have a substantial influence on milk production. Substitution rate increases with increasing pasture availability, from 0 for high grazing pressure to 0.6 - 0.8 for low grazing pressure (Stakelum *et al.*, 1988; Stockdale, 2000; Peyraud and Delaby, 2001). Efficiency, and thereby substitution rate, is influenced by a range of factors, such as herbage allowance, herbage composition, concentrate feeding level, concentrate composition and milk yield potential (genotype) of the cows being evaluated (Bargo *et al.*, 2002). Delaby and Peyraud (1997) estimated that milk production response reached a plateau at 4 kg/d of concentrate when herbage allowance was high; whereas when herbage was restricted there was a linear response up to 6 kg/d of concentrate.

From a review of literature published up until the early 1990s, average substitution rate was around 0.6, resulting in an efficiency of approximately of 0.4 to 0.6 kg of milk per kg of concentrate DM (Journet and Demarquilly, 1979; Meijs 1981; Leaver 1985; Stakelum *et al.*, 1988). However, most of these studies were carried out with low to moderate yielding cows in the region of 15 to 25 kg per cow per day. Since the late 1990s, lower substitution rates and higher efficiencies have been observed. From nine studies published, the average substitution rate was 0.40, resulting in an efficiency of 0.92 kg of milk per kg of concentrate. The higher response to concentrate supplementation with cows of higher genetic merit may be attributed to greater nutrient partition to milk production than with cows of lower genetic merit (Dillon *et al.*, 2006).

Faverdin *et al.* (1991) showed that substitution rate is lower with high yielding cows when energy requirements are not being met. Delagarde *et al.* (unpublished) demonstrated that substitution rate is directly related to the net energy balance of the unsupplemented cows. In situations where grass alone was far from being sufficient to meet energy requirements, substitution rates were low (0.1) and energy balances were strongly negative (-21 MJ per day), whereas cows in good grazing conditions had higher substitution rates (0.6) and higher energy balance (+28 MJ per day). In such situations, concentrate supplementation only reduces herbage intake slightly and increases animal performance appreciably. Horan *et al.* (2006) showed a strong relationship between substitution rate and milk production efficiency (Figure 3). At substitution rates of 0.6 kg, milk production efficiencies were 0.4, whereas at substitution rates of 0.2 kg, milk production efficiencies were 1.1.

Concentrate supplement energy source (starch or fibre) has been shown to have only small effects on intake or milk production, especially when moderate levels are offered (1 to 6 kg per cow per day). On pasture, herbage intake was shown to be about 1 kg higher when cows were supplemented with 5 kg of a high fibre

concentrate compared with a 5 kg high starch concentrate (Kibon and Holmes, 1987). However, the effect of energy source becomes much more important at higher levels of supplementation (Sayers *et al*., 2003).
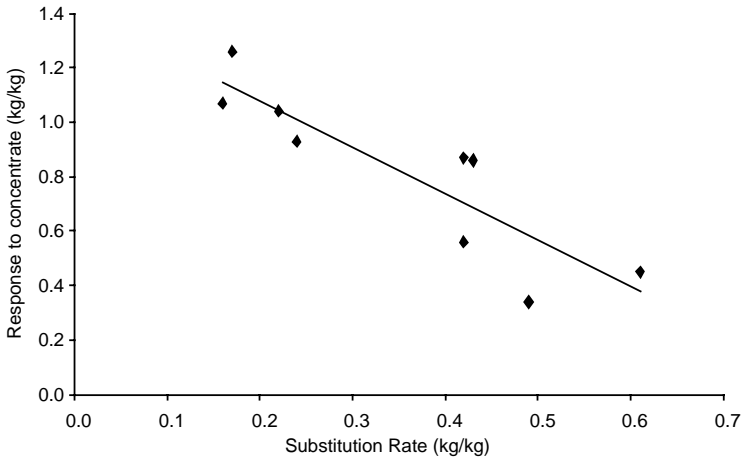
Figure 3. Relationship between milk production response to concentrate supplementation and substitution rate of pasture for concentrate (Horan *et al*., 2006).

Forage supplementation. In a review of the use of conserved forages as a supplement during the grazing season, Phillips (1988) concluded that under situations where ample herbage was available, supplementation with grass silage reduced both milk yield and protein yield with variable effects on fat yield. Supplementation with grass silage under these conditions resulted in a large reduction in herbage intake (substitution rates of 0.84 to 1.02 kg OM/kg supplement OM intake. The large substitution effects obtained with the forage supplement appear to result from reduction in grazing time of approximately 43 minutes/day for each kg of silage DM consumed. In these situations, supplementary forage feeding could result in under-utilisation of the grazed grass area and consequent deterioration in sward structure and composition. In a grass shortage situation, supplementary forage feeding generally results in increases in DM intake and milk production. Maize silage supplementation had a positive effect on milk production when the amount of pasture offered was low (Stockdale, 1994). However, where pasture allowance was adequate, supplementation with maize silage reduced pasture DM intake and resulted in similar total DM intake and similar milk production (Holden *et al*., 1995). Substitution rates are generally higher for forage supplementation than for concentrate supplementation due to higher fill value of forage.

On/off grazing

Two of the obstacles to achieving a greater number of days at grass, especially in early spring, are poor soil conditions and periods of high rainfall. Allowing animals access to pasture for two three-hour periods per day (on/off grazing) has been shown to maintain milk production and milk protein concentration when compared to supplementing with a grass silage diet (Kennedy and O'Donovan, 2009). It also enables access to grazing in marginal grazing conditions. When on/off grazing is practiced cows become more efficient grazers, grazing for 98% of their time at pasture. During this time cows consume 95% of the intake that cows grazing fulltime achieve. On/off grazing protects swards from poaching, which is detrimental to pasture re-growth.

FEED BUDGETING

It was not until the 1970s that a relationship between milk yield and pasture allowance was identified (Hodgson, 1975). To be useful to farmers this required simple and accurate methods of estimating short term rationing at the paddock level. Sward height, especially after grazing can be used for this purpose (Stakelum, 1993; Mayne *et al.*, 1987). O'Donovan (2000) established that including daily herbage allowance as well as post-grazing sward surface height, greatly improved grass DM intake at farm level. Low post grazing height will indicate an insufficient feed supply and imply that the average intake of grass by the herd was lower than it should be. High pre-grazing sward heights (>12 cm) will be difficult to graze down and grass DM intake will be reduced.

Clark and Jans (1995) referred to the concept of feed profiling, feed budgeting and grazing plan and to the development of decision support models for pasture management in New Zealand. Feed budgeting will be required at farm level for short term rationing at paddock level, for medium term budgeting on a weekly/ biweekly basis and long term on a yearly basis; which introduced the concept of farm grass cover (Stakelum, 1993). O'Donovan (2000) developed targets for average pasture grass cover, expressed as either on per hectare or per cow basis. Pasture cover is important in a short-term basis, to allocate sufficient daily herbage allowance. For medium term budgeting, grass growth can be highly variable, even under standard management conditions. For example, in Southern Ireland in a grass growth study managed to a strict and consistent protocol at one site, mean growth rate over 23 years of simulated grazing for the month of May was 95 kg DM/ha/ day, but the range was from 72 to 123 kg DM/ha/day. If this level of variation can be expected under 'standard' management conditions, clearly variation of sward

growth for a given time of year on-farm is likely to be even more pronounced, as sward age, and grazing and fertiliser management vary. This variability in sward growth rate is one of the factors that results in poor or variable utilisation of herbage produced on-farm, as farmers are unable to manage grazing with precision. By increasing predictability of grass growth and animal requirement, feed budgets can be drawn up with confidence. Taking this further, decision support systems can be designed, based on plant growth models and including the interaction between the herbage produced and the animals' intake, to be a grazing management aid. Long term feed budgeting will entail a yearly feed budget, taking cognisance of total herd feed demand and the grass production potential of the farm, and also the quantity of fertilizer and concentrate required to be purchased.

In recent years there has been substantial development of decision support tools to help dairy farmers to increase animal performance from grazed grass. The grazing season is divided into three periods: spring, mid-season and autumn. In spring the Spring Rotation Planner is used from turnout until grass growth equates herd demand (late January until early April). The Spring Rotation Planner is a tool which provides clear guidance at this time. The planner works off simple parameters; turnout date, weekly calving pattern, grazing area and finally the targeted finish date of the first rotation. For the plan to be successful, the following is required:

*   Stick to the target area allocated by the planner, do not graze more or less per day
*   Graze to a post-grazing plate meter height of 4cm to ensure high quality grass in the next rotation
*   If, after allocating the correct proportion of the farm, post grazing height is greater than 4 cm, then feed allocation is too high and supplementation should be phased out; if post grazing height is less than 4 cm, then feed allocation is too low and supplementation is required.

The Pasture Wedge is used during the main grazing season (early-April until end of August) to control grass supply talking into account herd demand, rotation length and post-grazing residual. The Pasture Wedge is a simple method used to interpret data obtained from taking a farm grass cover measurement. A profile of the paddocks pasture cover (kg DM/ha) from highest to lowest is set out on a graph. The pasture wedge visually illustrates the breakdown of the pre-grazing herbage mass distribution on the farm. A line is superimposed onto the graph calculated from the intended herd demand, rotation length and grazing residual.

The objective of autumn grazing management is to maximise the amount of grass utilized while at the same time finish the grazing season in late-November/early-December with the desired farm grass cover so at to set up the farm for an

adequate amount of grass for the following spring. There are two key periods in autumn, (i) the period of autumn grass build up and (ii) managing the final grazing rotation. Generally rotation length should be increased from early/mid August. The focus of this period is to gradually build pre-grazing herbage cover to 2000-2500 kg DM/ha (>4cm). Pre-gazing covers >2500 kg DM/ha are difficult to utilise and should be harvested as surplus silage. Removing paddocks after the first week of September should be avoided if possible as it will reduce grass for autumn grazing. The following are guidelines:

- Extend grazing rotation length from 28 days in early August to 35 days by mid-September
- Close first paddock for grazing the following spring by early October
- Have at least 60% of the farm rested by the end of the first week of November for grazing the following spring
- All paddocks should be grazed to a post-grazing height 3.5 to 4cm during the last rotation to encourage winter tillering

Conclusions

Due to economic, environmental and animal welfare constraints, it can be envisaged in the future that a larger proportion of milk produced in temperate regions will be produced from grazed pasture. However, increased use of pasture-based systems poses many research and technology transfer challenges. Internationally, the balance of research resources is nevertheless strongly in favour of controlled indoor feeding of dairy cattle. There is considerable scope to improve animal performance from grass-based systems given recent developments in our understanding of management factors that influence grass intake. Efficient exploitation of grass by grazing will require development of grazing systems designed to maximise daily herbage intake per cow, while maintaining a large quantity of high quality pasture over the grazing season. Grazing systems will not be limited by peak DM production during the peak two to three months of the grazing season, as high animal performance from pasture will supersede high animal performance per hectare. Daily grass intake will be maximised by adhering to important sward characteristics such as maintaining a high proportion of green leaf within the grazing horizon and allocating an adequate daily herbage allowance. The challenge for the future will be to develop swards through management and grass breeding that will maintain high DM intake while at the same time result in low residual sward height. Likewise, in the future cow genotype must be compatible with the system of milk production, and prediction of the phenotypic performance of dairy cattle must be based on knowledge of the cow's genotype as well as the

environment in which they are managed. Development of reliable, easy to use decision support tools that facilitate increased reliance on grazed grass, to be used by farmers and extension services will contribute to optimising grazed grass based systems of milk production.

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5

PRACTICAL CONSIDERATIONS OF FEED EVALUATION SYSTEMS FOR DAIRY COWS

JONATHAN BLAKE

DietCheck Ltd. and Forage Analytical Assurance Group, Highfield Office, Andover, Hampshire SP11 6JE

Introduction

Most practical rations for UK dairy cows are based on feeding models. Initially animals were fed on the basis of chemical analysis of feeds, but over time nutritionists found that feeds also needed to be described in terms of what they did within the animal. The first of these was the energy that an animal could extract from the diet, as energy intake is probably the most important factor defining animal performance. Descriptive terms for protein followed in the 1970s and 1980s, and by the 1990s more complex models, such as the Cornell Net Carbohydrate and Protein System (CNCPS), had been published quantifying the effects of rumen microbes and identifying factors important in maintaining a stable rumen environment (Fox *et al*., 1990).

Describing feeds in the 21st century has become a combination of chemical and biological terms. Dairy rations in the UK are described using both historical and new terms required for the Feed into Milk system (FiM, 2004). Many of these terms are predicted by near infrared reflectance spectroscopy (NIRS), providing values quickly and cheaply. Inevitably there are inaccuracies within any prediction system, not only from requirement equations, but also from variation in feed analysis due to sampling, wet chemistry or prediction equation error. The importance of considering feed variability has been reviewed previously (Green *et al*., 2007). This paper reviews the current status of feed evaluation for dairy diets in the UK.

Why is there a need for nutrient values?

Perhaps a better question is why is there a need for animal models? Clearly most animals survive, grow and reproduce without a mathematical model to assist in their feeding. For productive animals, however, pressures of modern animal production systems do not allow such a relaxed attitude. Every day, farmers and their advisors need reliable models to predict performance so that they can feed economically and efficiently while minimising waste with its associated environmental effects. The seemingly simple question of what are the animal requirements to achieve a defined performance level and product yield continues to challenge nutritional science. In dairy cows, feed evaluation systems have become complex as models are expected not only to predict energy and protein requirements, but also to include factors such as rumen stability and effects of diet change on milk composition.

The perfect feeding model would be based on nutrients and would not rely on all-encompassing terms such as Metabolisable Energy (ME). Although an improvement on gross energy, ME can only be considered as a total term because it describes neither the form of the energy within a feed (starch, protein, fibre, etc.), nor the fate of the energy within an animal. The Holy Grail for animal feeding systems is the mechanistic model where biochemistry defines yields of metabolites, and yield of metabolites defines animal performance. This approach has been described by a number of groups (Baldwin *et al*, 1977; France *et al*, 1982; Murphy *et al*., 1982; Murphy, 1984; Dijkstra *et al*., 1992; Van Laar *et al.,* 2004) but, despite considerable philosophical and scientific efforts, there is no published system that predicts successfully even the first stage of a mechanistic model, i.e. production of microbial fermentation acids. Without a successful rumen model, further development is impossible, because rumen metabolism defines supply of the majority of carbohydrate and protein substrates to the cow. Outline plans for such a model in the UK have been suggested (TCORN, 1998), and that report concluded that such an approach was technically feasible. However, the reduction in funding of nutrition research, and the resultant loss of scientific expertise, means that such a project is now impossible in the UK. Despite the huge potential benefits to nutrient efficiency, the expense and effort would require a joint European or perhaps global approach, and such an initiative is unlikely.

Without these fundamental relationships, nutritionists rely on broader descriptive terms to predict overall animal performance. Generally, dairy cow models calculate amounts of nutrients and energy required for a specified level of performance. This 'factorial' approach determines net requirements for energy, protein, minerals and vitamins, and applies efficiency factors for their conversion from dietary components. To supply these requirements in the diet, models throughout the world use fundamentally similar approaches, although the level of complexity varies.

Feed characterisation and measurement

As animal models developed, so did the number of feed characterisation terms. Each new animal model required new methods to analyse and describe feeds. The FiM model specifies 11 animal and 32 feed descriptors (Table 1). Of the 32 feed terms, 22 are chemical and 10 biological.

Table 1. Terms required by the Feed into Milk model

	Descriptors
Energy requirement	Weight, weight change, days pregnant, milk yield, milk fat
Energy supply	Metabolisable Energy (ME)
Energy quality	ME, Gross Energy
Protein requirement	Weight, weight change, days pregnant, milk yield, milk protein, DM intake
Protein supply	Metabolisable Protein (MP)
Protein quality	Crude protein (CP), sN, aN, bN, cN, sDM, aDM, bDM, cDM, Acid Detergent Insoluble Nitrogen (ADIN), protein equivalent from urea, lactic acid, volatile fatty acids
Estimate of Dry Matter Intake (DMI)	Silage Intake Potential (SIP), condition score, concentrate DMI, milk energy output, forage starch, week of lactation, concentrate protein content
Decision Support Systems	
Rumen stability	Requirement – milk yield, milk fat, meal frequency, lactation number
	Supply – Neutral Detergent Fibre (NDF), Potential Acid Load (PAL)
(ii) Milk composition change	Milk yield, ME intake, week of lactation, CP, NDF, oil, starch, sugar, saturated fat, mono-unsaturated fat, poly-unsaturated fat and long chain ($\geq$C20) poly-unsaturated fat
(ii) Metabolisable amino acids	Lysine, methionine, cysteine, histidine, leucine and threonine

CHEMICAL TERMS AND MEASUREMENT

Chemical terms include components such as dry matter (DM), crude protein (CP), starch, sugar, Neutral Detergent Fibre (NDF) and amino acids. These parameters should be the simplest and easiest to measure, because methods have been available for many years. Although nutritionists allocate simple descriptive

names to the results of these chemical procedures, values obtained are a function of the method. For example, material that was not extracted by neutral detergent and after the removal of the ash was defined by Van Soest *et al.*, (1991) as fibre. It is also referred to as total fibre or plant cell wall, but does the method measure all fibre? Summation of the expected nutrients in some feeds reveals missing dry matter (Table 2).

Table 2. Nutrient contents (g/kg DM) of wheat and molassed sugar beet pulp (SBP)

Feed	CP	Starch	Sugar	Oil (AH*)	NDF	Ash	Total
Wheat	110	748	27	23	92	18	1018
SBP	118	0	243	12	325	101	799

*Acid Hydrolysis method

Although the nutrients in wheat account for all the dry matter, over 200 g is 'missing' from SBP. The secretive culprit may be pectins, which are dissolved by neutral detergent and therefore do not appear as part of the NDF fraction. Pectins are a diverse group of compounds and therefore difficult to measure chemically. There are few published values for pectin concentration in feeds, but grasses contain 20 to 50 g/kg, legumes 60 to 140 g/kg, SBP 250 to 300 g/kg, citrus pulp 360 g/kg, and soyabean meal (480 g CP/kg) 140 g/kg (Hall *et al.*, 1999). Pectins are part of the cell wall where they seem to act as glue between individual cells in plant fibre bundles and are resistant to mammalian enzymes. They should, therefore, be included as part of 'fibre' component of the diet. The FiM model overcomes problems of incomplete feed analysis in prediction of rumen microbial growth by considering dry matter degradability rather than attempting to calculate degradability of each nutrient.

Chemical methods are often considered as the 'gold standard' and there is a tendency to forget that chemical methods are subject to sampling, method and laboratory variability. For some of the more difficult techniques, such as NDF and starch determination, variation can be large, particularly in heterogeneous feeds such as forages. For example, starch determination requires forage material to be pulverised to a fine powder by ball milling to ensure efficient release of sugars by amyloglucosidase (Kirton *et al.*, 2005). (Figure 1 and Table 3.)

Table 3. Starch determination by enzymic incubation in whole-crop cereal silage

Pre-treatment	Starch (g/kg DM)
Standard mill	24.9[a]
Ball mill – 2 mins	27.5[b]
Ball mill – 4 mins	27.3[b]
Ball mill – 10 mins	27.9[c]
P value	<0.001

Figure 1. Results of ball milling of a whole crop cereal silage

Even for simple feeds, starch measurement is variable. For commercial laboratories, the standard derivation of starch in pig rations is 19 g/kg DM. Outliers are highlighted only when they are outside twice the standard derivation, so the range is ± 38 g/kg DM i.e. 74 g/kg DM (S. Shelley - Personal communication).

Published UK ruminant feed values have not changed since the last major database review by the FiM group in 2001. There may be analyses carried out by feed suppliers and manufacturers, but these are used commercially and are not widely available. The lack of a dynamic feed database encourages over and under feeding of nutrients leading to unexpected performance, inefficient use of resources and increased environmental pollution. In addition, there is no public domain system to evaluate new feeds, such as biofuels co-products, leaving the nutritionist either to rely on the supplier or make an educated guess of the likely nutrient content. Although considered unexciting, it is an essential area of science which perhaps needs European commitment.

In the UK the only freely available and routine feed analysis is for forage and particularly silage. Forage is the most variable feed component and generally represents a high proportion of the diet, so an accurate analysis is essential for meaningful ration formulation. Drying forages may change nutritional quality and combined with the requirement for rapid turnaround and low cost, has led to the widespread use of NIRS for wet forages. First introduced during the late 1980s and developed independently by commercial laboratories, it became clear that repeatability between silage laboratories was unacceptable. The need to reduce variation between laboratories and to provide methods to measure the new feed terms for the FiM model, led to the formation in 2000 of the Forage Analytical

Assurance (FAA) group. The aim was to introduce industry agreed quality control procedures ensuring that forage results were accurate, reliable and repeatable. The quality control protocols developed by the group identified that wet NIRS scanning requires more technical expertise than dry scanning due to interference of the spectra by the large water peak. Individual instruments were standardised with the master laboratory machine and protocols introduced specifying the use of internal standards, cell packing and scanning techniques, sample temperatures and the use of agreed prediction equations and software. Regular large scale ring tests were introduced for each forage annually with routine monthly bias checks during the silage season. The levels of equation accuracy and FAA group prediction for grass silage (December 2008 ring test) are shown in Tables 4 and 5.

Table 4. Mean results of the FAA group December 2008 grass silage ring test – master laboratory

Parameter	Chemistry	NIRS	R^2
DM (g/kg)	281	281	0.96
N (g/kg)	6.0	5.8	0.91
NDF (g/kg)	114	119	0.77
Lactic acid (g/kg)	24	19	0.84
pH	4.1	4.2	0.76

Table 5. Digestible organic matter (DOMD) NIRS predictions of the FAA group December 2008 grass silage ring test – all laboratories

Laboratory	DOMD (g/kg DM) NIRS	Agreement with master lab (R^2)
Master	67	-
A	67	0.87
B	69	0.97
C	66	0.87
D	70	0.93
E	66	0.90
F	67	0.97
G	65	0.93
H	65	0.94

No published feeding model currently incorporates variation of either feed measurement or animal prediction equations so, although feed databases and animal models often give values to 1 or 2 decimal places, the precision of the models does not justify this level of accuracy. Perhaps future systems will incorporate variation, but before attempting to improve the accuracy of any single parameter, sensitivity analysis should be completed to identify which parameters

are important. For example, although starch prediction for maize silage is poor, this is less important to the FiM model because starch content is only part of the Decision Support Systems for DM intake and milk composition. Far more important is the energy content of maize silage.

BIOLOGICAL TERMS

As for chemical parameters, most biological feed values have been fixed since the publication of the FiM database from data collated in 2001. Some further work has been completed by the FAA group to improve prediction of DOMD for high DM grass silages and to develop NIRS prediction equations for whole crop silages. There has been no further animal work on PAL (used in the rumen stability model) or degradability characteristics of silages. Currently there is no agreed energy prediction method for maize silage, and no PAL or degradability NIRS predictions for maize and whole crop silages.

   The NIRS prediction of the N and DM degradability terms for silages as required by the FiM model has proved difficult. The terms are based on the *in situ* polyester bag method (Ørskov and Mehrez, 1997) providing data on the proportional degradation of the feed in the form

$$\text{Degradability} = a + b\ \{1 - e^{(-ct)}\}$$

   where a = immediately soluble component (proportion)
   b = potentially degradable component other than 'a' (proportion)
         c = fractional rate of degradation of the 'b' component (h^{-1})
         t = time in the rumen (h)

Early work within the FiM project highlighted that this approach did not differentiate between the immediately soluble pool and fine particles that where lost through the bag pores. This was overcome by introduction of a solubility measurement ('s') which allowed estimation of soluble, small and large particle pools within the rumen. Each pool was calculated using appropriate outflow (to estimate rumen retention) and degradation rates, with outflow rates predicted from ME intake and proportion of forage in the diet.

   Clearly the *in situ* technique cannot be used for routine analysis of silages and therefore NIRS equations were developed by Hillsborough in Northern Ireland from 136 silages with polyester bag degradability values and wet NIRS spectra. However, direct prediction of the degradability terms was unsuccessful and led to the Baglim method which involved NIRS prediction of DM and N losses at

0, 6, 12, 24, 48 and 72 hours followed by curve fitting to generate the a, b and c values. This method was used until it became clear that although accuracy of prediction of the individual time points was acceptable, the errors combined to give unacceptably inaccurate predictions (Figure 2 and Table 6).
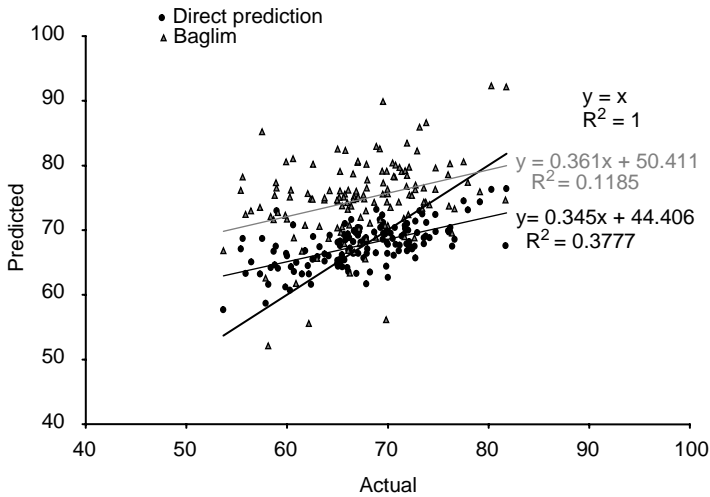
Figure 2. Comparison of NIRS predictions of N 'b' term by direct NIRS equation or the Baglim method (FAA group – personal communication)

Table 6. Prediction of DM and N degradability characteristics of grass silages by direct NIRS equations or the Baglim method (FAA group – personal communication)

	Direct NIRS prediction	Baglim prediction
	R^2	R^2
DM – 'a'	0.56	0.51
DM – 'b'	0.16	0.07
DM – 'c'	0.22	0.20
N – 'a'	0.38	0.12
N – 'b'	0.27	0.11
N – 'c'	0.20	0.00

Further work by the FAA led to a new approach which predicted effective degradability of N (EDN) and the microbial DM yield (MDM). Values were calculated for the 136 Hillsborough grass silages assuming standard outflow rates of 0.075 (h^{-1}) for liquid and small particles and 0.045 (h^{-1}) for large particles. Mean results for the dataset were 0.71 (proportion) (min = 0.52; max = 0.92) for EDN and 107 (g/kg silage DM) (min=51: max=166) for MDM, with the NIRS prediction equations R^2 values of 0.52 ad 0.64. Despite the improvement, prediction was insufficiently accurate for individual silages and their use as direct

feed terms would require alteration of the published FiM system. To overcome these problems, silages were grouped into four bands defined by the calibration statistics and the predicted MDM and EDN values used to allocate an individual silage to a band (Tables 7 and 8).

Table 7. MDM groups for DM degradability characteristics of grass silages (FAA group, 2006)

Range			Degradability Values (SD)			
MDM min	*MDM max*	*no*	*sDM*	*aDM*	*bDM*	*cDM*
	<85	17	0.16 (0.05)	0.32 (0.07)	0.43 (0.11)	0.034 (0.008)
>=85	<119	66	0.22 (0.05)	0.32 (0.06)	0.51 (0.01)	0.036 (0.008)
>=119	<153	25	0.27 (0.05)	0.34 (0.07)	0.57 (0.11)	0.036 (0.006)
>=153		2	0.31 (0.08)	0.31 (0.08)	0.69 (0.01)	0.038 (0.004)
P value			<0.001	0.56	<0.001	0.77

Table 8. EDN groups for N degradability characteristics of grass silages (FAA group, 2006)

Range			Degradability Values (SD)			
EDN min	*EDN max*	*no*	*sN*	*aN*	*bN*	*cN*
	<0.6	3	0.36 (0.09)	0.64 (0.05)	0.24 (0.06)	0.065 (0.011)
>=0.6	<0.68	18	0.51 (0.04)	0.65 (0.07)	0.24 (0.05)	0.066 (0.016)
>=0.68	<0.76	70	0.58 (0.04)	0.68 (0.05)	0.23 (0.05)	0.079 (0.018)
>=0.76		17	0.66 (0.08)	0.69 (0.05)	0.26 (0.05)	0.084 (0.017)
P value			<0.001	0.08	0.39	<0.01

The system was introduced by FAA laboratories for the 2007 silage analysis season. Although not ideal, the approach is based on good science and better predicts degradability values of practical silages.

Rumen stability and fibre terms

Fibre supply and its description is a complex area of ruminant nutrition. It is particularly important in high performance animals where diets with a high energy content can challenge the animal's ability to maintain a stable rumen environment. As a simplification, fibre has two major roles

As a supplier of energy for rumen microbial growth and, as a by-product, a supplier of fermentation acids that can be absorbed and oxidised by the animal as energy sources.

As a major influence on rumen stability. This is partly because fibre is fermented more slowly than non structural carbohydrates such as starch, and partly because

its structural functionality promotes rumination with the associated introduction of buffers via the salvia.

Models have accounted for the energy supply role by measuring or calculating organic matter digestibility (e.g. by DOMD or Total Digestible Nutrients). Similarly, microbial protein production from fibre sources is estimated in the MP system.

Fibre and its role in rumen stability are less easy to define, and this has led to a number of different terms to estimate its rumen stability potential. These include chemical, physical and combinations of chemical and physical (Table 9).

These parameters are rarely used in isolation and recommended feeding levels depend on level and type of non structural carbohydrates and also on the feeding system. Nutritionists use both a range of recommendations which they have found to be helpful in practice, and complex models such as CNCPS or FiM. The CNCPS model moderates microbial protein yield and predicts rumen pH on the basis of eNDF; the Cornell, Penn, Miner system relies on peNDF.

The FiM rumen stability system uses the principle that rumen pH is regulated by salivation, which is stimulated by chewing, set against the effect of feed ingredients to lower rumen pH.

Table 9. Common fibre terms in dairy nutrition

Term	Description	Recommendation
NDF	'Total' fibre (cellulose, hemicellulose and lignin)	>25 - 32% DM
Forage NDF		>21% DM
eNDF	'Effective' NDF (based on particle size)	>21% DM
peEDF	'Physical Effective' NDF (refined version of eNDF)	>21% DM
ADF	Cellulose and lignin	>19 - 21% DM
ADF:NDF	Measure of fibre quality	0.7 – 0.8
Lignin		>4% DM
RSV	Rumen stability value	FiM
Particle Size	Penn State separator	TMR – 6-10%, 30-50% and 40-60% in upper, middle and bottom sieves respectively

From:- Fox *et al*. (1990), Mertens (1997), FiM (2004), Heinrichs and Kononoff (2009)

The Structural Value System (SVS) of de Brabander *et al* (1996) defines the ability of feeds to buffer rumen pH by chewing, but it does not make allowance for acids in feeds or their intrinsic buffering capacity. The FiM system adapted the SVS system to include these factors and created the Rumen Stability Value (RSV). The

system provides the minimum RSV requirements needed in diets to avoid rumen acidosis as indicated by depression of milk fat concentration (Figure 3).
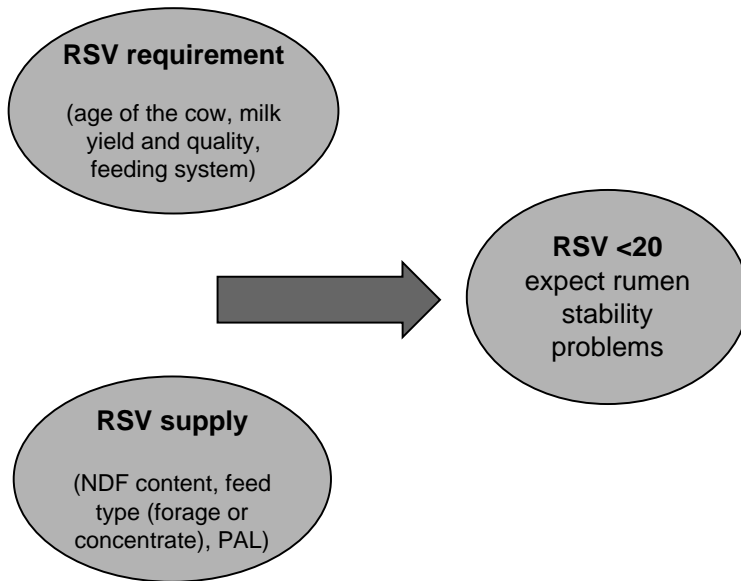
Figure 3. The FiM Rumen Stability Value system

where PAL = Potential Acid Load in the rumen which accounts for:

- the amount of acid already in the feed (e.g. silages)

- the amount of acid that will be produced by the rumen fermentation of the feed

- the inherent buffering capacity of the feed (which will reduce the requirement of buffering from saliva)

Although no specific particle size term is included in this system, RSV supply predictions differ for forages and concentrates. A feed with the same NDF content and PAL value has a RSV of 250 meq/kg DM if defined as a forage, but only 34 meq/kg DM as a concentrate. Therefore the system predicts that forage buffers the rumen more effectively than concentrates. Such approaches are difficult to validate, but industry feedback indicates that the FiM RSV system reflects animal performance and, as importantly, appears to correct problem diets.

One situation where the FiM RSV system appears incorrect is when high quality forages such as spring grass or grass silage produced from spring grass are fed. For these feeds, the RSV system fails to account for reduced 'effectiveness' of the NDF because NDF degradability can be extremely high (Figure 4), preventing the formation of a rumen mat and reducing chewing activity.

Feed name	DM (g)	ME (MJ)	Protein (g)	NDF (g)	NDF degrad (prop)
Grazing - early spring	180.00	12.00	230.00	380.00	0.89
Grazing -good	200.00	11.75	230.00	475.00	0.81
Grazing -medium	220.00	11.50	200.00	550.00	0.71

Figure 4. Grass NDF degradabilities. MAFF (1990), FiM (2004), DietCheck Ltd

In practical situations these high quality forages often result in watery and loose dung and lower milk fat concentration, both indicative of low rumen pH. To better reflect practical observations, DietCheck (a feeding software package based on the FiM model) describes the 'effective' rumination quality of forage by taking account of its NDF degradability to moderate RSV supply. For example, the FiM system predicts a difference between RSV requirement and RSV supply of 86 when spring grass is fed as the sole fed (Figure 5). As the RSV trigger point for low rumen pH is 20, the model predicts a stable rumen with no milk fat reduction.

	Amount fed (kg DM/d)	Nutrient name	Daily intake	% DM	Requirement	Shortfall/Excess
Feeds		DM (kg)	17.0		15.1	1.9
Grazing - early spring	17.000	Forage as fed (kg)	94.4			
		ME (FiM) (MJ)	204.0	12.00	203.0	1.0
		NDF %DM	38.0			
		RSV	188.0		102.0	86.0

Figure 5. FiM RSV system for a Holstein cow in mid lactation yielding 25 kg milk/d

The DietCheck system modifies the 'effectiveness' of forage fibre to reduce RSV supply (Figure 6) which reduces the RSV difference to 21. This is close to the RSV trigger point and better reflects farm observations and the need for diet change.

	Amount fed (kg DM/d)	Nutrient name	Daily intake	% DM	Requirement	Shortfall/Excess
Feeds		DM (kg)	17.0		15.1	1.9
Grazing - early spring	17.000	Forage as fed (kg)	94.4			
		ME (MJ)	204.0	12.00	203.0	1.0
		NDF %DM	38.0			
		NDF from Forage %DM	38.0			
		Forage NDF deg (%ForNDF)	89.0			
		RSV	123.0		102.0	21.0

Figure 6. DietCheck modified RSV system for a Holstein cow in mid lactation yielding 25 kg milk/d

Silage sampling

On farm sampling of forages is a major source of concern for silage laboratories. Advisors are quick to criticise results of forage analysis as inaccurate without always considering variations that exist within any large volume of biological material. Poor silage clamp sampling that does not collect a representative sub

sample is worthless and confusing. Studies completed as part of the Whole Crop NIRS LINK project highlighted variation across the clamp face and calculated the number of samples needed to obtain a useful result (Table 10).

Table 10. Number of samples required per clamp for fermented whole crop cereal silage (95% confidence)

	Mean	*Acceptable range*	*No. samples*	*Acceptable range*	*No. samples*
DM (g/kg)	490	470-510	14	460-520	6
pH	4.2	4.1-4.3	1	4.1-4.3	1
CP (g/kg DM)	95	88-102	21	80-110	5
NDF (g/kg DM)	425	405-445	16	395-455	7

The current FAA group protocol for collection of an adequate representative sample of a silage states that between 9 and 15 samples should be collected from 15 to 20 cm behind the face along a 'W' shaped pattern across the clamp. The bulk sample should be mixed well and a sub sample of approximately 0.5 kg to be sent to the laboratory should be prepared using the method of quartering. Silage results from samples collected using this method are more likely to give the predicted performance.

Conclusions

Dairy models have developed over time and contain a mixture of chemical and biological terms. Ideally models should be based on biochemical and mechanistic approaches, but such models seem out of reach in the current economic environment. Although these types of model hold the promise of considerable benefits, their generation would require substantial research input.

For dairy feeding systems to be used successfully, both nutrient requirements and supply must be based on reliable science. Animal inputs need to be measured carefully, and feeds (particularly forages) must be correctly sub sampled for analysis. The UK ruminant feed database has stagnated since the last review in 2001. There is no public domain system to evaluate existing or new feeds, leaving nutritionists to make educated guesses of nutrient content. The lack of a dynamic feed database encourages over or under feeding leading to unexpected performance, inefficient use of resources and increased environmental pollution. Silages are the only feeds analysed routinely. Nutrient measurement is largely based on wet NIRS techniques with the FAA group ensuring reliable and reproducible analysis in the UK. However, NIRS predictions are based on chemical procedures which are themselves variable.

Estimation of silage degradability characteristics is difficult and further animal work would be required to improve predictions. Description of fibre within the FIM system for predicting rumen stability works well in practice, apart from forages containing highly degradable fibre. Inclusion of a measure of effectiveness of the fibre better reflects farm observations when these forages form a major part of the diet.

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6

ADAPTING LIVESTOCK PRODUCTION SYSTEMS TO CLIMATE CHANGE

CJ STOKES[1], SM HOWDEN[2], AJ ASH[3]

[1]CSIRO Sustainable Ecosystems, CSIRO Climate Adaptation Flagship, PMB PO Aitkenvale, Qld 4814; [2]CSIRO Sustainable Ecosystems, CSIRO Climate Adaptation Flagship, GPO Box 284, Canberra, ACT 2601; [3]CSIRO Climate Adaptation Flagship, 306 Carmody Rd, St Lucia, Qld 4067

Introduction

Livestock production is the world's dominant land use, with 'open' (non-forested) grazing lands covering about 45% of the earth's land surface (Asner *et al*., 2004). Although grazing land includes productive pastures in mesic environments, often livestock production is the land use of last resort when land is not suitable for more productive uses such as cropping. As such livestock production occurs in some of the harshest and most variable climates in the world, and coping with these challenges has been an important part of developing sustainable production systems (e.g. McKeon *et al*., 2004). The risks of climate change are now adding to these challenges and there is growing evidence that humans are contributing significantly to these changes through increased emissions of greenhouse gases (GHG) (IPCC, 2007).

Even if global actions are taken to reduce GHG emissions, past emissions have already committed the planet to several more decades of warming. The harsh environments in many rangelands are already marginal for livestock production and, with few alternative livelihood options, these areas will be particularly vulnerable to climate change (Stafford Smith *et al*., 2007). Climate change could affect the amount and quality of produce, reliability of production and the natural resource base on which agriculture depends. In some areas, new opportunities are likely to emerge, but realising these potential benefits will require proactive changes in management practices. These challenges require high levels of adaptive responses (Howden *et al*., 2007a). In order to continue to thrive in the future, livestock industries need to anticipate these changes, to be prepared for uncertainty, and to develop adaptation strategies now. There is a clear imperative

for a greater, coordinated effort to tackle the challenges a changing climate will pose. For agriculture in particular, it is becoming increasingly urgent to develop options and capacity to adapt to climate change (Howden *et al.*, 2007a). This chapter considers some of the specific impacts and opportunities that climate change may bring to livestock industries and some of the options available for adapting to them.

Effects of climate change

Although climate change will have some direct effects on livestock, the dominant influences will be through changes in plant growth and the timing, quantity and quality of forage availability. Climate change will involve a complex mix of responses to (1) rising atmospheric carbon dioxide (CO_2) levels, (2) rising temperatures, (3) changes in rainfall and other weather factors, and (4) broader issues related to how people collectively and individually respond to these changes (Table 1).

Table 1. Summary of climate change effects on livestock production systems

Plants & Natural Resources	*Livestock*
CO_2	
Enhanced plant resource use efficiency (water, N, light) and pasture growth	No direct effects
Reduced forage quality (protein and digestibility)	
Increased non-structural carbohydrates in temperate pastures could improve forage quality	
Prolonged moisture availability (and growth) at end of wet season from water savings	
Species-specific CO_2 responses cause shifts in vegetation composition (e.g., favour nitrogen-fixers and deep-rooted plants)	
Temperature	
Reduced water use efficiency and increased evaporation	Increased effects of heat stress and water requirements
Decreased forage quality (digestibility)	Livestock concentrate more around water points
Earlier start to spring growth in cooler climates	
Poleward expansion of pests, weeds, native species and suitable ranges of planted pasture species (e.g., C4 species)	Poleward expansion of tropical pests and diseases
Rainfall, sea level rise and other changes in climate	
Changes in forage production magnify percentage changes in rainfall	Changes affect availability of feed and water for livestock

Table 1. Contd.

Plants & natural resources	*Livestock*
Changes in seasonal rainfall affect seasonality of forage availability (e.g., declining spring and/or autumn rainfall would reduce the length of growing seasons)	
Increased rainfall intensity and interannual variability create greater challenges for managing forage supplies and limiting soil erosion	
In coastal areas, rising sea levels could cause saltwater intrusion and increase risks of flooding, and increases in cyclones or storms could increase wind and water damage	

Broader context and other issues
Potential shifts in land use and competition between land uses (e.g., expansion of cropping into pasture areas, less productive cropping lands converting to pasture, loss of land for carbon sequestration and renewable energy generation)
Uncertainty over climate change impacts and adaptation options could create reluctance and delays in taking pre-emptive action, exacerbating impacts
Changes in regional and international competition from geographic variation in effects of climate change (magnitude of impacts/benefits and adaptability of producers)
Changing demand for livestock products (meat and wool) as a result of climate change and consumer attitudes to GHG-efficiency of food products
Cost-price squeeze from GHG reduction and climate adaptation measures that increase input and processing costs (indirect: e.g. cost and availability of feed supplements)
Direct costs of reducing GHG emissions from livestock production systems (especially methane)
Conflicts and synergies with other public and private policies and initiatives (especially drought, water, natural resource and GHG emission policies)

RISING ATMOSPHERIC CO_2

The most certain aspect of the changing environment for future livestock production is rising level of CO_2 in the atmosphere. Because CO_2 emissions spread and mix rapidly in the atmosphere to influence plant growth within years, this will also be the most globally uniform and immediate influence. Atmospheric levels of CO_2 have risen almost exponentially from pre-industrial levels of about 280 ppm to current levels of about 390 ppm (a 37% increase). In the lowest emissions trajectory, of a set of possible scenarios developed by the IPCC (Nakicenovic and Swart, 2000), CO_2 could stabilise at about double pre-industrial levels; the high-end scenarios, however, indicate CO_2 levels could exceed 900 ppm by the

end of the century. Currently, emissions are growing rapidly (3.5% per year) and are exceeding the worst case scenario (Canadell *et al.*, 2007).

Plants are already growing in CO_2-enriched environments and rising levels of CO_2 will further enhance the 'fertilisation' effect, stimulating plant growth through increases in efficiency with which plants use limiting water, light and nutrient resources (Gifford, 1979; Morison and Gifford, 1984; Drake *et al.*, 1997; Campbell and Sage, 2002). At the leaf level, elevated CO_2 increases light use efficiency by reducing photorespiration, an effect that is largely associated with 'cool season'/'temperate' C_3 grasses and is weaker in 'warm season'/'tropical' C_4 grasses (which have an internal CO_2-concentrating mechanism). At the field level, light use efficiency can be increased also by increasing diurnal or seasonal duration of photosynthesis, or simply by increases in canopy leaf area. Elevated CO_2 increases water use efficiency by increasing the rate at which CO_2 diffuses into a leaf through stomatal pores relative to the rate at which water vapour escapes. Depending on physiological responses of plants in regulating stomatal pores, this benefit may be expressed as: (1) an increase in carbon fixation while transpiration rates stay the same; (2) reduced transpiration while carbon fixation remains the same; or (3) an intermediate combination of increased carbon fixation and reduced water use. Under field conditions over whole growing seasons, overall use of soil water remains largely unaltered and most of the water savings translate ultimately into increased plant growth for both C_3 and C_4 species (e.g. Morison and Gifford, 1984; Volk *et al.*, 2000; Stokes *et al.*, 2008). At field scales there may be further increases in water use efficiency from increases in leaf canopy area that increase transpiration (CO_2-sensitive) relative to evaporative losses of soil water. Nitrogen use efficiency can increase either passively, when greater carbon fixation under elevated CO_2 is not matched by increases in nitrogen uptake, or through active physiological down-regulation, where Rubisco levels are preferentially reduced in compensation for increased efficiency of this carbon-fixing enzyme.

Evidence from field-based CO_2 experiments has shown that elevated levels of CO_2 stimulate production of both C_3 and C_4 forage plants, and that this effect is related largely to altered patterns of water use over whole growing seasons (Owensby *et al.*, 1993; Niklaus *et al.*, 1998; Volk *et al.*, 2000; Morgan *et al.*, 2004). The benefits of CO_2 in stimulating pasture growth may be greatest in pastures receiving intermediate amounts of rainfall (about 500 – 1000 mm/yr, depending on latitude) where water is limiting during most periods of active plant growth (Nowak *et al.*, 2004; Stokes and Ash, 2006). However, this increased productivity is associated with a decline in forage quality from reduced protein levels and lower digestibility. There are also likely to be changes in pasture composition, with shifts towards species that benefit the most from higher CO_2 levels. In grazing lands with mixed grasses and woody plants, increased drainage from water savings under elevated CO_2 may favour deeper rooted woody species. In pastures with mixed

legume species (either woody or herbaceous), increased carbon fixation and greater N demand may favour N-fixing symbioses, but overall changes to the N cycle related to changes in other climate factors and soil microbial processes are likely to be more important than changes to N fixation (Thomas *et al.*, 2007).

There are strong interactions between pasture responses to CO_2 and other variables, such as temperature, soil moisture and soil nutrient availability, especially nitrogen (Fischer *et al.*, 1997; Suter *et al.*, 2002). Consequently, the influence of CO_2 on plant growth under field conditions, although present in both moist temperate (Campbell *et al.*, 1997) and arid (Smith *et al.*, 2000) conditions, can be quite variable (Nowak *et al.*, 2004), and could become more variable as vegetation composition and species interactions change over time (Smith *et al.*, 2000; Edwards *et al.*, 2001). Although CO_2 will have a largely positive effect on pasture growth, these benefits will taper off at higher CO_2 levels because much of the benefit is already being experienced from increases in CO_2 that have occurred already. In contrast, many of the negative effects that rising GHG levels will have on climate and agricultural systems lag at least several decades behind the rises in GHG levels that cause them, and impacts become more negative as GHG levels rise.

TEMPERATURE CHANGE

The next most certain aspect of climate change is rising global temperatures, which is the primary effect of increasing GHG levels on the climate. Past GHG emissions alone are estimated to have committed the planet to a warming of about 0.2°C per decade for the next several decades and global average temperatures are projected to increase by 1.1 to 6.4°C by the end of this century (IPCC, 2007). At temperate locations, each 1°C increase in temperature will cause a shift in climate that would be roughly similar to moving about 145 km (or about 2° in latitude) closer to the equator. However, changes in temperature are likely to vary geographically. For example, oceans are likely to moderate temperature increases in coastal areas and the interiors of continents are likely to warm the most (IPCC, 2007). Northern temperate regions are projected to warm more than tropical regions, and there may be further effects of local topography and changes in ocean and atmospheric circulation patterns. Any change in temperature will affect livestock production both through effects on forage production and through direct effects on livestock (Table 1).

In cool temperate climates, where low temperatures limit pasture growth during winter, rising temperatures could extend the length of the growing season and reduce frost damage. However, in a field experiment with perennial subterranean clover, it was found that continuous warming of the atmosphere by 3.5°C did not

increase winter growth, and for the whole year decreased herbage growth by almost 30%, offsetting a positive response to concurrent elevated CO_2 concentration (Lilley *et al*., 2001). In addition, increased plant growth in the cooler months could deplete soil moisture at the expense of subsequent pasture growth in the spring, such that the net effect is highly situation-dependent. Temperature also affects diet quality; warmer conditions can significantly decrease non-structural carbohydrate concentrations (and digestibility in tropical species) and also slightly reduce leaf protein content (Wilson, 1982).

Rising temperatures will also alter water use in pastures by increasing the vapour pressure deficits (VPD: the 'dryness' of the air), provided increases in day-time and night-time temperature are similar. Higher VPDs adversely affect plant growth through the compounding effects of increases in evaporation and lower water use efficiency. Reduced stomatal apertures at higher CO_2 levels could have an additional effect on VPD and water use efficiency by reducing evaporative cooling of the leaf from transpiration, increasing the temperature differential between the leaf boundary layer and the air and thus increasing effective VPD. Reduced transpiration (with reduced evaporative cooling) would create additional daytime warming of plant leaves above and beyond the leaf temperature increases associated with warming of the lower atmosphere. In warm, dry climates in particular, increased heat stress, and possibly increased evaporative demand, would probably have negative effects on pastures.

Interactions between elevated CO_2 concentration and temperature are complex. Although there seemed to be solid theoretical reasons why the magnitude of the CO_2 growth response of C_3 species would increase with temperature (Gifford, 1992), synthesis of experimental evidence from the literature indicated no trend of increased CO_2 sensitivity with increasing temperature (Morison and Lawlor, 1999). Hence, it cannot be assumed that plant growth will necessarily become more responsive to CO_2 with global warming. The effects of changing temperature on pastures will therefore be complex, involving interdependent effects on photosynthesis, respiration, transpiration, nutrition, and plant development.

Warmer more humid conditions will substantially increase frequency of heat stress days for livestock. Although this issue will be greatest in tropical and subtropical environments, it will also affect cooler climates. A significant increase ($\approx 60\%$) in incidence of stress days has occurred already over the last 40 years in some parts of Australia (Howden and Turnpenny, 1997). This will reduce livestock productivity, decrease reproductive rates and increase concerns about animal welfare in locations where grazing populations are concentrated, such as feedlots (Howden and Turnpenny, 1997; Howden *et al*., 1999; Mader and Davis, 2004; Amundson *et al*., 2006). As conditions in temperate regions become harsher, hardier livestock breeds (and crosses) will have to be used, and these hardier traits are associated with lower productivity, fecundity and meat quality. Livestock water

requirements will also increase as temperatures increase. For example, 2.7ºC of warming would increase water demand by approximately 13%, increasing non-linearly with further warming (Howden and Turnpenny, 1997). This will also mean that livestock will be unable to travel far from watering points in rangelands, concentrating grazing pressure and risks of soil degradation near watering points while areas further from water are left under-utilized.

Warmer temperatures are also likely to facilitate spread of tropical grasses, weeds, pests and diseases to higher latitudes, which could pose major risks for livestock industries (Sutherst, 2001). For example, projections in Australia indicate a southward expansion in distribution of the insect vector of blue-tongue disease, *Culicoides wadia* (Sutherst, 2001), and the spread of cattle ticks (*Boophilus microplus*) could cause reduction in live-weight gain of 21,600 tonnes/yr by 2100, compared to a present estimated reduction of 6,600 tonnes/yr (White *et al*., 2003). Changes in plant species are likely to alter pasture composition (of both native and invasive species) and to alter suitability of forage species in planted pastures. For example, warmer conditions may favour an increase in C_4 grasses (Cullen *et al*., 2008), which generally provide less nutritious forage than C_3 species. The 'desirability' and management of species range changes will need to be viewed and re-evaluated in the context of the potential species that are most suitably adapted to the emerging climate (rather than trying to maintain familiar species that used to grow in particular locations). It may be more productive to recognise, facilitate and direct climate-induced changes in species distributions rather than trying to completely prevent them.

RAINFALL AND OTHER CHANGES

Changes in rainfall are likely to have the greatest effect on livestock production systems (Hall *et al*., 1998; Crimp *et al*., 2002), but they are likely to be one of the most geographically variable aspects of climate change. At a global scale, higher temperatures are expected to intensify the hydrological cycle but, at regional and enterprise scales, local factors such as topography and changes in circulation patterns are likely to affect rainfall changes strongly. Even subtle changes in wind patterns and storm tracks can redistribute rainfall between regions. Because of the complexity associated with these fine-scale processes, confidence in climate change projections declines in moving from global to regional and local scales and much of this uncertainty is likely to remain, even as climate models improve. An essential element in adapting to climate change will be to accept the inherent uncertainty in future climate change, including uncertainties in the geographic variation in projected changes.

Some generalisations on geographic patterns of climate change can be made. For example, there are likely to be latitudinal shifts in rainfall linked to changes in the Hadley Circulation (hot moist air rising near the tropics and descending at about 30° N and S). This circulation is projected to expand poleward and weaken, accompanied by an increase in sea level pressure at mid latitudes and a poleward shift in storm tracks. Rainfall is projected to increase in the tropics, particularly in the tropical Pacific, where monsoonal rains may intensify. In the subtropics, rainfall is projected to decline, but at higher latitudes, rainfall is projected to increase. Coastal regions may also be exposed to more frequent and severe storms and tropical cyclones. There are also likely to be changes in rainfall intensity, seasonal distribution of rainfall, and year-to-year variability. Detailed global projections of geographic patterns of climate change have been developed and these have been analysed for many regions around the world (e.g., CSIRO and Australian Bureau of Meteorology, 2007 for Australia).

Modelling studies suggest that pasture growth responses will amplify changes in rainfall, so that the magnitude of change in forage production will exceed the percentage change in rainfall (McKeon *et al.*, 2009). For example, in a simulation study of the sensitivity of pasture growth to changes in rainfall across Australian rangelands, a 10% decline in rainfall reduced pasture growth by 11 to 15% (depending on soil type), and a 10% increase in rainfall produced a 12 to 18% increase in pasture growth (Crimp *et al.*, 2002). This amplification was greatest in arid and semi-arid areas, and was least in tropical pastures. Several other studies have demonstrated the sensitivity of pasture production to small changes in climate (Scanlan *et al.*, 1994; Johnston *et al.*, 1996) and non-linear responses to rainfall (Hall *et al.*, 1998). Sensitivity analyses that include effects of temperature and CO_2 in simulations suggest that rising temperatures amplify the effects of declining rainfall, although rising CO_2 may have some ameliorating effect, emphasising the importance of being able to correctly estimate the impact of these opposing effects (McKeon *et al.*, 2009). In addition, changes in river flow regimes and beneficial flooding may alter production of locally important ephemeral pastures on floodplains (White, 2001) and these changes too are likely to magnify any changes in rainfall. For example, in arid regions a 10% change in rainfall can lead to a 30 to 40% change in runoff (Chiew *et al.*, 1995).

Changes in seasonal distribution of rainfall will modify seasonal patterns of variation in forage quality and availability. Declines in spring and autumn rainfall would tend to shorten growing seasons. In contrast, as discussed previously, growth seasons could be extended either by an earlier start to spring in temperate areas (Cobon and Toombs, 2007) or by prolonged availability of soil moisture (and delayed grass senescence) at the end of the growing season as a result of CO_2-induced conservation of water by grasses. Altered seasonal patterns of variation in forage protein and utilisable energy will have consequences for ruminant

nutrition (Beever, 1993), but the combined effects of these interacting climate change influences has yet to be determined. It will be difficult to generalise how the nutritive quality of forage will change in any one area because in some situations the increase in digestible energy content will dominate but, in other situations the effect of decreased protein content will dominate (Howden *et al.*, 2008).

Increases in rainfall intensity are likely to increase soil erosion by increasing runoff, particularly where drying climates reduce protective vegetation cover (McKeon *et al.*, 2004). Erosion risks will probably be exacerbated further by increased year-to-year variability in rainfall, creating a greater chance of erosion events where a wet year (high runoff) follows a drought (when plant cover is low and soils become highly susceptible to erosion). Soil erosion may become an increasingly challenging management consideration. As climate and vegetation changes, it will be essential to maintain perennial grasses and shrubs to provide dry season and drought feed, fuel for fires where appropriate, and surface cover to protect soils. Recent drought episodes in Australia have demonstrated the potential of climate extremes to cause widespread plant mortality, even for long-lived trees (Fensham, 1998). It is not known how most existing pasture species will respond to unprecedented extremes of temperature and desiccation, so changes in vegetation will have to be monitored in order to adapt appropriately.

BROADER CONTEXT

Livestock production systems will be affected also by a broad set of societal responses related to climate change, including economic and policy considerations. Policies related to GHG emissions will have particularly important implications. Costs associated with reducing emissions targets are likely to affect both input costs and the costs of processing meat and wool. In addition, government and consumer concerns over methane emissions from ruminant livestock could see the imposition of financial disincentives and reduced demand for (ruminant) meat.

Other changes in markets could also influence livestock producers. For example, meat prices are influenced strongly by meat production in other countries (influenced in turn by world grain production) and hence global variation in climate change impacts will also influence the financial performance of local grazing enterprises (White, 1972; Herne, 1998). Availability of grain for livestock production may also become restricted by growing demand from the emerging biofuel industry. The strong trend to increased consumption of livestock products such as meat and dairy in Asia (Dalton and Keogh, 2007) seems unlikely to be affected by climate change concerns (except perhaps for issues related to greenhouse gas emissions, discussed above). In contrast, climate changes seem likely to have a negative impact on both wool demand and wool supply in that

warming climates may reduce demand for woollen clothes and countries like China may have improved conditions for grazing (Keeling *et al.*, 1996; Myneni *et al.*, 1997; Harle *et al.*, 2007).

Climate change will also influence patterns of land use and competition between different land uses. In some cases production on marginal land could cease to be viable, and in other cases changes in climate could make alternate uses, such as cropping, more profitable. Grazing lands could also be converted to agroforestry and other carbon sequestration uses.

Adapting livestock production to climate change

The ultimate impacts of climate change are likely to be modified (potentially for both better and worse) by the way in which people, individually and collectively, respond to these challenges. Vulnerability to climate change can be reduced by preparing, evaluating and implementing adaptation strategies that limit the risks of negative impacts and take advantage of new opportunities. It will be crucial, therefore, to develop the capacity to adapt to climate change in all aspects of society, but most particularly for climate-sensitive activities such as livestock production. Building adaptive capacity will require (1) availability of effective adaptation options, (2) capability of enterprise managers to implement these options and (3) a policy and institutional environment that promotes development, evaluation and adoption of effective adaptation strategies (Marshall *et al.*, 2010).

DEVELOPING ADAPTATION OPTIONS

Livestock production systems vary substantially from country-to-country and region-to-region because of diversity in pastures, climate, social and economic characteristics. Overlaying this diversity, the impacts of climate change are likely to vary between regions. There is therefore a wide variety of options for dealing with the impacts of climate change, and their suitability will depend on local conditions (Table 2). In the short-term, many adaptation options are likely to correspond strongly with efforts to promote existing 'best practices' that are both economically and environmentally sustainable. This would include practices such as managing diet quality (using fertilizer, legumes, choice of planted pasture species and diet supplements), matching stocking rates to pasture production, adjusting herd management to altered seasonal patterns of forage production, using fire to control woody thickening, and monitoring the spread of pests, weeds and diseases. Enhancing such practices should be an initial priority because they provide an immediate benefit, irrespective of whether climate change occurs, and should therefore have greater appeal in communities where

scepticism or uncertainty over climate change might otherwise delay action. As part of this approach, existing best-practice recommendations should be evaluated under future climate scenarios to determine which practices will continue to be appropriate and which will require modification.

Table 2. Options for adapting to climate-change in the livestock industry (modified from Stokes *et al.*, 2010)

Adaptation option
Broad-scale adaptation
'Mainstream' climate change considerations into existing government policies and initiatives (particularly those relating to drought, GHG emissions and natural resource management)
Encourage uptake of 'best practice' in livestock enterprises (and evaluate current recommendations to ensure benefits will continue under future climate scenarios)
Work with the livestock industry to evaluate potential adaptive responses to the system-wide impacts of a range of plausible climate change scenarios
Continuously monitor climate change impacts and adaptation responses adjusting actions to support and ensure effective and appropriate adaptation
Grazing and pasture management
Introduce stocking rate strategies that are responsive to seasonal climate forecasts and longer term climate change trends
Accept climate-induced changes in vegetation and modify management accordingly
Improve on-property water management, particularly for pasture irrigation
Improve nutrient management using sown legumes and phosphate fertilisation where appropriate
Develop 'climate-ready' forage species that will be better suited to future projected climate conditions
Develop software to assist proactive decision making at the on-farm scale
Expand routine record keeping of weather, pests and diseases, weed invasions, inputs and outputs
Diversify on-farm production and consider alternate land uses
Managing pests, diseases and weeds
Improve predictive tools and indicators to monitor, model and control pests
Increase the use of biological controls (with caution)
Incorporate greater use of fire and alternative chemical and mechanical methods for controlling weeds and woody thickening
Livestock management
Select animal lines that are resistant to higher temperatures but maintain production
Modify timing of mating, weaning and supplementation based on seasonal conditions
Provide extra shade using trees and constructed shelters

A wider variety of options is likely to be viable in high-input, productive managed pastures than for more extensively managed rangelands. In more arid environments, where coping with climate variability is already a management priority, it will

be useful to build climate change considerations into existing strategies that track climate variation by pre-emptively adjusting stocking rates in response to seasonal climate forecasts (McKeon and Hall, 2000; Stafford Smith *et al.*, 2000; Ash *et al.*, 2007). Building capacity to cope with climate variability can serve as a strong foundation in preparing for climate change because (1) in the short term, land managers are more likely to be concerned about the immediate challenge of year-to-year variation, as climate change effects will be largely within the bounds of existing climate variability; (2) adjusting management for seasonal differences from year to year builds in automatic tracking of longer-term climate trends; (3) these approaches encourage a shift from less flexible management styles to ones where managers adaptively monitor and respond to changes over time, and (4) managing for extreme events is already important in agriculture and will become more so as extreme events become more frequent under climate change (Stokes and Howden, 2010).

Over the longer term, it will be necessary to develop new management options that are better suited to emerging novel climate conditions. This would include research that develops new climate-ready varieties of pasture plants and hardier livestock breeds, and other innovations that could diversify livelihood options or modify existing practices. However, developing practical solutions to climate challenges is not simply a technical task and strategies will not be effective unless they are adopted by end users. Policy makers and enterprise managers are more likely to value and implement adaptation options if they are included in the development processes through closer engagement with researchers. This will require research approaches that (Marshall *et al.*, 2010) (1) apply existing knowledge in more effective and innovative ways including greater collaboration with decision-makers; (2) broaden the array of research approaches used to identify practical solutions, and (3) continue basic research that fills fundamental knowledge gaps, tests the validity of key assumptions, and evaluates the effectiveness of proposed adaptation options.

ENABLING CHANGE

As indicated above, to effectively deal with climate change it will be necessary to create conditions that encourage enterprise managers and policy makers to incorporate adaptation strategies and options into decision-making. Creating these conditions will require: effective communication of climate science to provide confidence that climate change projections are real, consistent with historic observations and distinguishable from high background year-to-year variability; acceptance that these changes will have practical impacts on livestock production

enterprises; demonstrating the practical benefits of adaptation options in reducing potential impacts; protecting early adopters against establishment failure in implementing adaptation strategies; altered transport and market infrastructure that supports adapted livestock production systems; and evaluation and monitoring systems that track changes in climate and management practices to allow iterative learning and improvement of adaptation strategies over time (McKeon *et al*., 1993; Howden *et al*., 2007b). However, there are also likely to be situations where incremental adjustment to existing livestock production systems may be insufficient to cope with impacts of climate change, or where beneficial changes could make alternative land uses more suitable and profitable. Such transformative changes will have wide-ranging impacts on regional communities, so it will be important to identify these situations in advance and to provide the necessary policy and institutional support during these risky and disruptive transitions.

To create a policy environment that is conducive to adaptation, climate change needs to become a routine consideration in policy development so that potential conflicts and perverse incentives can be identified and avoided, while capitalising on synergies with policies that have complementary objectives. The policy areas that are most likely to require attention are those relating to drought (climate variability and extreme events), greenhouse gas (GHG) emissions, and natural resource management. In particular, there is currently active policy development in many countries on the dual challenges of climate change mitigation (reducing GHG emissions to limit climate change) and adaptation (dealing with the residual change that does occur), and there is no reason to expect that measures in these two areas will always complement each other. Measures aimed at reducing GHG emissions could potentially increase vulnerability to the impacts of climate change, although some adaptation options may increase greenhouse gas emissions. It will be important to check for such conflicts at an early stage in identifying and developing mitigation and adaptation options.

A potential barrier to implementing adaptation strategies is the desire to wait until more reliable information is available (Sarewitz and Pielke, 2007). Although it may be tempting to delay action until there is greater certainty about climate change projections and the effectiveness of adaption strategies, some uncertainty will always remain. If timely, pre-emptive adaptation is to occur, planning needs to start now, accepting that decisions will have to be made with uncertain and imperfect information. This will require focusing on adaptation options that are robust enough to cope with a range of plausible climate change scenarios (Dessai *et al*., 2009) and implementing pre-identified options in a flexible manner that is responsive to changes as they occur, rather than relying too heavily on tailoring location-specific strategies to 'average' or 'best-bet' projections. An inevitable aspect of this approach will be to accept that decisions made on the best available,

imperfect knowledge may, in retrospect, turn out to be suboptimal. It will therefore be equally important collectively to learn from such experiences and to incorporate this and other new knowledge in iteratively improving adaptive responses over time.

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7

LEGISLATION AFFECTING ANIMAL PRODUCTION SYSTEMS

JOHN CHAMBERS[1] AND MIKE BRADE[2]
[1]JC Consulting, Carmel Cottage, Parsonage Lane, Chilcompton, Radstock BA3 4JZ, UK
[2]Newquip Ltd, NQ House, Coneygarth Way, Leeming Bar, Northallerton DL7 9EE, UK

For many years legislation has been a factor in the care of animals whether for food or leisure and it is recognised that the majority of the 'rules' laid down over the years have been beneficial for the welfare of animals. Recently there has been a steady increase in the legislation to prevent the pollution caused by animal wastes and manures. However as the pressure builds on governments to reduce emissions and prevent pollution, new legislation is deemed necessary and, as pig and poultry units become bigger, they have been brought into the category of 'industrial' rather than 'agricultural', leading to a new approach to pollution prevention and control set out in European Directive 2008/1/EC, known as the Integrated Pollution Prevention and Control (IPPC) directive, which applies to any business with more than 750 sows or 2000 pig places for 'production' pigs over 30kg or 40000 birds.

The current chapter intends to examine some possible future developments in welfare legislation. For example the IPPC raises the issue of the suitability of fully slatted floors for pig production which is also a discussion point in animal welfare legislation. The topic of the farrowing crate , that up to now is exempt from the ban on keeping sows in individual 'crates or stalls', will also be considered.

IPPC

In the UK, permit applications for existing farms had to be submitted by the end of January 2007. Permits were issued throughout the year and the inspection regime started. In 2008 the England and Wales Pollution Prevention and Control Regulations were incorporated into the Environmental Permitting Regulations. Although the detail remains unchanged, the EA have changed their forms and

guidance, the most significant being that the application form is now common across a range of regulatory regimes including waste.

There can rarely have been a piece of legislation that has generated so much paperwork both in terms of guidance material and that which has been required of the applicant. Much of this has merely documented what producers have been doing on pig units for many years. Farmers benefited from an application process simplified by the EA and industry working in partnership to ensure that the information supplied was precise and clearly laid out so making it easier for both compiler and processor.

Since permits were issued, farmers and regulators alike have been coming to terms with the new regime, its rules and obligations. Permit holders have had to produce further reports and keep records of activities, wastes produced, training etc. that is bureaucratic and time consuming. Reviews of housing and drainage have been compiled and agreed with the EA, stating what improvements are required and time scales. Failure to keep these records or to comply with plans without prior agreement may result in non-conformity reports being issued that can result penalty points counting towards higher annual permit subsistence charges.

One of the most challenging aspects of IPPC is that it had been known from the outset that the rules will change over time. It is possible that the rules will be extended to smaller units in the longer term, although the decision has been made recently to maintain current thresholds for the time being. A recent review of the EU directive sought to reduce the threshold on turkeys and laying hens for example, and introduce combined thresholds for pigs and poultry. These proposals have not been adopted, but will be reviewed at a future date, so the threat remains.

Other Directives impinge onto IPPC, for example the Habitats Directive places further demands on farms close to protected Habitats sites, for example Sites of Special Scientific Interest (SSSIs). There is considerable pressure from the Environment Agency and Natural England on some producers operating close to sensitive sites or sensitive receptors such as housing where odour nuisance for example is a cause for concern.

The scope of the legislation is wide. It covers: containment of polluting effluent, emissions of ammonia, dust and odour. It also covers noise and production of waste etc and there are further elements, for example the improvement of energy efficiency with consequent effects of reducing the need for power generation nationally.

The legislation tries to take a comprehensive approach to each aspect it covers – hence the word "integrated" in its title. If ammonia emissions are examined as an example – one of the main target areas for the pig sector - the principles would be:-

- minimising the emissions from the pigs – match protein levels in feed to the class of stock and keep the pigs clean
- house in "low emission" buildings
- remove slurry from the building as soon as possible
- store slurry in a covered tank
- spread onto land at prescribed rates using "low emission" equipment
- spread in conditions unlikely to cause run-off
- incorporate the manure quickly after spreading

There was significant consultation with the industry. Producers tended to concentrate on policy areas that affect day-to-day decisions on the farm – how the pig unit is managed and investment priorities to include those that are obligatory. IPPC introduces the concept of Best Available Technique – BAT – to the production process. The rather complex explanation of BAT can be found in the Guidance Document " How to Comply" and a simple interpretation is "the most effective means to achieve the objectives of IPPC at an affordable cost" – bearing in mind that affordability changes with time as does what is deemed "best practice". BAT applies both to the design of facilities of the unit and the management process. Once producers' businesses are exposed to scrutiny, there appears, in the longer term, to be little control over what they could be asked to achieve as environmental demands change. Instances where producers have been asked to go beyond BAT have already been recorded.

An example of a change some producers have to consider is the position of fans, moving them to the ridge when there is a need to better disperse ammonia and odour to reduce local environmental impacts.   .

The Environment Agency has set a target of 2020 for pig businesses to be fully BAT Compliant. A building put up in 2006 would only be 14 years old – relatively new in pig industry terms – and producers generally would be concerned if asked to make significant changes to facilities at that stage.

Producers have so far barely been able to get beyond the application process and the follow-up paperwork; however a few are now experiencing new challenges as they wish to reinvest in their facilities. To replace a building or add a new one for example requires for a permit to be formally "varied", this involves supplying additional information to the EA, a process which can be as complex as a full new permit application. Most producers would not be too averse to taking on board minor changes in management practices to achieve improvements but would be very concerned where they are asked to make significant financial investment. Producers need to be prepared to argue their point if they feel they are being asked to go beyond what they feel is justified.

Pig housing

An important aspect in the future management of permits will be in the choice of new housing. When producers make an investment in buildings they are looking for facilities to maximise performance in terms of efficiency of production, ease of management and labour use and minimising running costs. They are generally looking to achieve this at the least initial cost.

A significant problem for producers is that perhaps the most common type of specialist housing developed over the last 20 - 30 years (Fully Slatted floors with several months' slurry storage below slat) is the base system that European legislators have decreed needs to be improved.

Operating under an IPPC permit, producers will be guided down specific routes when it comes to choice of new pig buildings. The European "Bref" (BAT Reference document) produced a range of housing designs which have been shown to produce lower ammonia emissions than a conventional Fully Slatted house. Most of these designs have been developed and tested in the Netherlands and Denmark.

When examining the Environment Agency's "How to comply" manual, details of these housing options for different classes of stock may be found. The design principles are based largely on two principles:-

i.   Reducing the exposed surface area of slurry. The larger the slurry surface the greater the emissions of ammonia. The designs here are largely based on part-slatted systems, some using channels with sloping sides to further reduce the slurry surface.
ii. Removing slurry frequently, thus reducing the time for material to break down and release ammonia. A conventional fully slatted floor could be used but with slurry removal at frequent intervals e.g. every 2 – 3 weeks. Flushing systems, not unusual in the dairy industry, can also be used below slats. Flushing systems vary from the simple – flushing with the liquid fraction from separated slurry – to more complex systems aerating the liquid fraction. Different systems use various methods of below-slat handling.

One problem associated with rapid removal of slurry from buildings is the consequent need for additional (covered) slurry storage outside. The problem is not simply additional cost. A planning application for example for a new finishing house might need to also include a new slurry store with additional planning concerns.

Typically new slurry storage may cost in the region of £40 - £60 per m^3 bearing in mind that it would need to be covered. When constructing slurry storage under slats, the additional cost of making the channel deeper is relatively low.

Two other techniques can be considered which have some bearing relating to outside slurry storage. These methods reduce emissions from housing directly either at source or by removing ammonia from the exhaust air. They will enable significant ammonia reductions whilst retaining the ability to store slurry below slat level.

The first is "Surface Cooling". This uses a system of floating heat exchangers cooled with pumped groundwater or chilled recirculated water where the heat can be extracted by another heat exchanger and used to supply room heaters. It reduces the microbial activity in the surface layers of the slurry thereby reducing emissions.

The second is Air-Washing (scrubbing). This system takes all the stale air exhausted from a building through a chemical washer. This can be simple just using water or composite using a multi stage chemical system. This system is highly efficient and can potentially take as much as 0.90 of the ammonia from the air as well as removing dust and odour. This may provide the answer in circumstances where the level of ammonia released is critical – for example near sensitive habitats or domestic housing. These systems have been widely adopted in the Netherlands and Germany on larger units. Both these techniques may sound complicated but they may merit consideration if below-slat storage remained an option. Neither of these techniques has been widely used in the UK for agricultural situations, but widely used in other industrial sectors and would generally be considered only where circumstances demand their use.

The list of acceptable designs is not exclusive and other techniques can be used as long as they provide at least equivalent reductions in ammonia emissions. The onus is on the producer, however, to persuade the Environment Agency that non-listed techniques will provide the required reductions.

Costs

It is difficult to compare the cost of different housing systems. Most of the systems documented in the Bref have not even been tested extensively in the UK. Typically the cheaper systems e.g. Part slatted with reduced slurry channels might cost 5% - 10% more than the typical fully slatted system. The more sophisticated systems, e.g. surface cooling or air-washing, may cost in the region of 15-20% more and have on-going running costs.

Costs depend very much on individual circumstances and have been estimated for different systems. Details are included in the Bref but they are it seems difficult to compare and are not very helpful. Table 1 provides a guide to the efficiencies of ammonia reduction and costs for a number of systems.

Table 1. Efficiency and cost of ammonia reduction systems

Housing type	Reduction (%)	Ammonia emission (kg/pig/place/ year)	Estimated % cost increase over reference system
Fully slatted floor	Reference	3.0	Reference
Partly slatted (c 0.50) floor	20	2.5	0 – 5%
Vacuum system	25	2.2	5
Partly slatted floor - metal slats	40	1.8	5
Flushing system with clarified aerated slurry	55	1.4	10 - 15
Partly slatted floor - metal slats plus reduced manure pit surface to max. 0.18m^2	65	1.0	10
Manure cooling system (to 12°C max.)	60	1.2	15 - 20
Air Washing	70 – 90	0.3 -0.9	15 - 20
Solid floor, with straw bedding scraper passages	0	3.0	0 - 5

It is worth noting that straw-based systems produce a similar level of ammonia to the fully slatted base system.

In performance terms, the building designs in the Bref document are unlikely to compromise pig performance, although most of the systems have been developed and tested in northern Europe (largely in the Netherlands). Many of the designs have not before been used commercially in the UK. Some have been tested on an experimental scale. In some cases development has been undertaken at different stocking rates and in different types of housing than we are used to in the UK.

Slurry storage – IPPC & NVZ

On 1 January 2009, additional NVZs were designated taking the area of agricultural land in England within zones to around 0.70 from 0.55. The Action Programme rules were revised and made more prescriptive. Closed periods for spreading slurries now apply to all soil types and pig farmers have to install a minimum of 6 months slurry storage (excluding any they export). Minimum nitrogen efficiency factors have to be used when calculating contribution of nitrogen to crops, and manure nitrogen content has to be assessed following prescribed methods.

These new rules are leading to more spreading in the spring, especially on growing crops. Farmers are having to reassess their whole storage and spreading systems, the positive outcome being recognised is the fertiliser value of manures that is driving investment in spreading technology to deliver better results. This will

have a net positive benefit on the environment, reduce reliance on manufactured fertilisers and cut green house gas emissions. Typically storage in an above-ground tank may cost of the order of £35-40 per m^3. Covered storage may add perhaps 20% depending on the method used.

Producers' responsibilities whilst holding a permit

TERMS OF THE PERMIT

As previously mentioned, IPPC permits have now been transposed into EPR Permits. At the present time, when a unit's permit is issued, a range of legally-binding conditions are included. Many of these are fixed conditions affecting all units whilst others are specific to the unit or installation. Perhaps of most concern from the producers' viewpoint is that most units will also be required to prepare Improvement Plans (IPs) reviewing housing and drainage systems with the emphasis on reducing emissions either foreseen or unforeseen.. The IPs have to be agreed with the Environment Agency and be implemented in stages agreed between both parties. As anticipated, some IPs have been the subject of some discussion with perhaps some disparity between the expectations of the two parties. The producer must carefully prepare a case and negotiate strongly.

There is an expectation that the severe financial circumstances endured in the last year will at least postpone any costly requirements in the short term but enforced investment in for example slurry tank covers will eventually be required.

There will be at least two visits to the unit annually to review progress and agree priorities.

RECORDING

Producers are being asked to record a wide range of information under a number of headings. Under "General Management" records relating to areas like maintenance schedules are required along with details of any incident under the heading "accident management". Accident management plans need to be reviewed at least every 4 years. Energy records are required and again need to be reviewed along with the use of raw materials, medicines, biocides, pesticides and water use. Any complaints from sensitive receptors – odour, noise etc - need to be recorded along with whatever action was taken.

Details of the number of animal places and animal movements are required

together with records of all slurry and FYM spreading under the unit's Manure Management plan. Where slurry and FYM are exported from the unit details of dates, amounts, destination and land area available for spreading are required. All waste exported from the site must be detailed.

Progress with items listed in the improvement plan needs to be documented and the EA informed within 14 days of completion

Unless otherwise agreed with the EA, all records are to be retained for at least 6 years from the date of recording and will be supplied within 14 days of a written request from the Agency. There have been circumstances where producers have refused to accede to demands and, in these early stages, there have been some stand-offs yet to be resolved. There does appear to be some scope for producers to make their case heard.

MANURE SPREADING

The impact of the revised NVZ Regulations on manure spreading have previously been highlighted.

There is now a whole-farm limit for organic N from livestock of 170 kg/ha across the farmed area with a standardised calculation to assess the overall amount of slurry that can be applied (Table 2).

There is also to be an overall "Crop N limitation" investigating overall crop requirements and taking into account all sources of Nitrogen, e.g. soil N levels, slurry/manure (at an assumed efficiency / availability factor) and manufactured N fertiliser. This will seriously challenge the cropping knowledge of many pig producers and training will be required; the British Pig Executive (BPEX) is addressing this need in several ways.

The original NVZ rules included a closed period in the autumn when slurry could not be applied on shallow and sandy soils. Extended closed periods (see below) and all soil types are now included. The closed periods generally extend from the autumn through to January with some variation in timing depending on local rainfall, soil type and cropping. Restrictions on spreading are published in Defra's NVZ leaflet (Table 3).

These restrictions do not apply to FYM. Where straw muck is scraped from pig buildings and stored on concrete, run-off collection is required and, depending upon the situation, it may be classified as slurry, so having to come within the 6 months storage calculation, or dirty water, in which case there is no spreading restriction as far as closed periods are concerned. Field heaps may then be used taking into account drains, watercourses and boreholes, but these sites must not be used for more than 12 months with a 2 year non-return policy.

Table 2. Limits to animal occupancy in terms of N loading

Pigs	Occupancy (%)	Total N produced (kg/year)*	Volume of excreta (m³/ month)	Animal places per ha to comply with maximum N loading (170 kg/ha N per year)
1 weaner place, 7 to 13 kg	71	1.0	0.03	170
1 weaner place, 13 to 31 kg	82	4.2	0.05	40.5
1 grower place, 31 to 66 kg (dry fed)	88	7.7	0.10	22.1
1 grower place, 31 to 66 kg (liquid fed)	88	7.7	0.18	22.1
1 finisher place, 66 kg and over (dry fed)	86	10.6	0.13	16.0
1 finisher place, 66 kg and over (liquid fed)	86	10.6	0.26	16.0
1 maiden gilt place, 66 kg and over	80	11.1	0.13	15.3
1 sow place, 66kg and over, with litter up to 7 kg, fed on lower protein diet but supplemented with synthetic amino acids	100	16.0	0.33	10.6
1 sow place, 66kg and over, with litter up to 7 kg, fed on a diet without synthetic amino acids	100	18.0	0.33	9.4
1 breeding boar place from 66 kg to 150 kg	100	12.0	0.15	14.2
1 breeding boar place, 150 kg and over	100	17.5	0.26	9.7

* N produced in excreta is per pig place and includes an allowance for N losses from livestock housing and manure storage.

Table 3. Restrictions on spreading manure imposed by the UK Department for Environment, Food and Rural Affairs (Defra)

You must not apply organic manures with a high readily available nitrogen content (e.g. Slurry, poultry manure and liquid digested sludge) to land during the following periods (inclusive dates):

Grassland		Tillage Land	
Sandy or shallow soils	All other soils	Sandy or shallow soils	All other soils
1 Sep – 31 Dec	15 Oct – 15 Jan	1 Aug – 31 Dec*	1 Oct – 15 Jan

*On tillage land with sandy or shallow soils, application is permitted between 1 August and 15 September inclusive, provided a crop is sown on or before 15 September.

This means there is now a much-reduced window for slurry to be applied. Application in the late summer/early autumn is restricted to the period after early crops are harvested. There may be a requirement for a cover crop on land which would otherwise be bare over winter. The closed period ends between mid December and the end of January depending on cropping soil type and average rainfall. Slurry application is limited to spring crops or direct application to the growing crop. In a wet Spring there is significant risk of damage to land. There is a requirement to undertake field inspections before spreading. Where spreading is on third-party sites there may be some conflict of interest between recipients wary of soil damage and producers with full slurry tanks. Although the statutory storage requirement would be 6 months on some soil types and in some seasons, this may not be enough. There are also implications on the capacity and type of spreading equipment which are likely to further increase costs.

IPPC AND SLURRY SPREADING

Under IPPC requirements the application form asked if "on site" spreading of slurry and FYM is undertaken according to a Manure Management Plan. It also asked about analysis of the material and of the land it was being spread on – specifically mentioning Phosphorus.

The form asked producers if manure or slurry is spread "off site". The "How to comply" manual states that written evidence of the arrangements in place when exporting slurry should be maintained – quantities and dates, the names and addresses of recipients and the land area available. Where a third party accepts responsibility for removing manure or slurry from the unit "acceptable confirmation" that it will be spread according to the relevant Code of Good Agricultural Practice should be provided and that it will be spread according to a Manure Management Plan for the receiving land.

Since the applications have been processed, EA officers generally appear to have had few concerns about spreading arrangements on land farmed by the producer under a Manure Management Plan. More detailed questions have been asked, however, relating to off-site spreading, for example asking to see the lists of receiving landowners and the acreage of land available.

Slurry is an increasingly valuable commodity at current manufactured fertiliser prices. Even so great care needs to be taken that requirements put onto the receiving landowner are not so onerous that they choose to decline the manure and opt to use purchased fertiliser instead. If the landowner is in an NVZ it is already their responsibility to apply the material correctly according to the rules. It seems excessive to expect the producer supplying the slurry to become involved in details relating to spreading.

There are some practical concerns about "contingency" areas. This is land in excess of calculated requirements expected to be allocated by the recipient landlord. The idea is that it is available for emergency spreading if other allocated land is inaccessible due to water-logging, for example. Few landowners will be willing to hold land back from some form of fertiliser on the off chance that it might be required later.

Diffuse sources of pollution consultation

In addition to the consultation on the Nitrates Directive, there is a further consultation looking at Diffuse Sources in England. The probable outcome of this is the introduction of designated Water Protection Zones (WPZs) with further controls on activities such as manure spreading and also on outdoor pig keeping. These WPZs could target any particular pollutant but perhaps the most significant in this context is Phosphorus.

In areas where there have historically been high livestock numbers, the soil P index is likely to be higher. In these circumstances, application of slurry or FYM may be restricted to the amount of P removed by the following crop. This probably will become the limiting factor in the amount of organic manure that can be applied rather than the N application limits.

The following points are perhaps the main issues concerning slurry storage and spreading under the proposed rules:-

1. Many producers will not have sufficient storage for slurry and, in the current financial climate, will be unable to afford to invest in extra capacity.
2. There will be increased pressure on planning authorities for consents for slurry storage.
3. The IPPC requirement for more "out of building" storage will further exacerbate the situation
4. More land will be required for spreading
5. Slurry will need to be spread in a shorter time-frame and in many cases to a growing crop.
6. There will be increased pressure on spreading especially in a difficult Spring.
7. There is a significant risk that third party landlords will be unwilling to take a chance with spreading on their land
8. More expensive and greater capacity equipment will be required to spread the slurry.
9. Farms unable to comply will be viewed as a greater risk by financial institutions

10. Farms may risk losing their "permit".
11. There will be significantly more planning and bureaucracy involved with spreading manures and recording.
12. Many producers may need training to meet requirements.
13. There may be a possible requirement for a cover crop over winter

Welfare

The IPPC requirements for lower ammonia emissions from part-slatted floors tend to support the welfare perspective of those floors for the pigs. However there is a distinct problem with straw-based systems that have the same ammonia emissions as a fully slatted system.

There is some scope here for further work to allow pigs to have more comfortable lying areas in slatted buildings – part-slatted but with sufficient undisturbed slurry area under the slats to reduce the ammonia emissions to a satisfactory level. This may well be combined with better flushing and more regular emptying as discussed earlier.

Some years ago in the UK, most sows farrowed in 'Solari' farrowing pens, a long thin pen with a sloping roof, rails round the edge of the lying area and a totally inaccessible creep area at the back. The industry has progressed subsequently over the previous four or so decades through all types of maternity systems including crates for a week followed by multiple suckling (Brent, 'Pig World', 2009). Gerry Brent was the Manager of the UK National Agricultural Centre Pig Demonstration Unit at Stoneleigh and tried out most of the systems now under discussion as alternatives to the farrowing crate.

They tended to give three main problems: increased piglet mortality, poor piglet observation and access, and decreased safety for the stockperson.

Many sows produced good litters with little mortality but the problem came with the odd sow which gave total litter loss attributable to savaging, from chilling due to the litter not finding the warm creep, simple overlying or even sows trampling their litter in a bizarre expression of maternal protection when the stockperson enters the pen.

Getting past a sow with a bucket of expensive creep feed to service the 'creep' area at the back of a 'Solari' pen typifies the problem of piglet observation and access as well as the problem of staff safety.

As Gerry Brent states, "....This free access arrangement, along with other variants which caused massive rises in piglet losses, [are] now being proposed by those who fail to realise that it has been tried and abandoned by people who were desperate to make them work".

The farrowing crate has become the farrowing system of choice for the majority of producers but, as weaning age has increased from 21 days to 28 days, can the impact on sow welfare be justified in terms of keeping the sow confined for all this time when research has shown that the greatest problems occur in the first 5 to 8 days after birth.

There is need for some new thinking in this area whereby the sow can be confined for the time when her litter most demands it for their welfare but is given more freedom to move in the latter part of the lactation. A return to a two-stage system with a 'maternity' phase followed by a move to more spacious accommodation where the sow is allowed to move freely and the piglets still have a 'refuge' or creep area is a possibility. Unfortunately this introduces considerable labour input into the system associated with moving the sow and litter as well as extra cleaning out between animals.

A German-designed system appears to allow the sow and litter to stay in the same pen, with the sow confined for as long as necessary but able to move freely once the stockperson decrees it is safe for the piglets (and stockperson). Piglet observation and access even when the sow is 'free' is still good and the stockperson can always return the sow to confinement for a short time when there is litter work to do which might upset the sow. The manufacturer is reporting that, despite the increased cost of the system, repeat orders for it are being taken.

Acknowledgements

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MYCOTOXIN ISSUES IN FARM ANIMALS AND STRATEGIES TO REDUCE MYCOTOXINS IN ANIMAL FEEDS

GIUSEPPINA AVANTAGGIATO AND ANGELO VISCONTI
Institute of Sciences of Food Production ISPA-CNR, Via Amendola 122/O, 70126 Bari, Italy

Introduction

MYCOTOXINS

Mycotoxins are natural products of filamentous fungi that cause acute toxic or chronic carcinogenic, teratogenic or oestrogenic responses in higher vertebrates and other animals. Exposure is usually by consumption of contaminated feeds but may also be by contact or inhalation. Mycotoxins are not required for the growth of the producing fungus and, therefore, are considered secondary metabolites. Presumably these compounds play some role in the ecology of the fungus, but their function has not been clearly defined. Not all fungal growth results in mycotoxin formation and detection of fungi does not imply necessarily the presence of mycotoxins. Only some moulds produce mycotoxins, and they are referred to as toxigenic. Most mycotoxins of concern are produced by three genera of fungi, namely, *Aspergillus*, *Penicillium* and *Fusarium*. The fungal toxins are chemically diverse – representing a variety of chemical families – and range in molecular weight from about 200 to 500. They are non-proteinaceous compounds derived primarily from amino acids, shikimic acid or acetyl and malonyl coa. Due to modern analytical methods and to a growing interest in this field of research, more than 300 different mycotoxins have been differentiated to date. In 1981, around 120 metabolites of common hyphomycetous fungi that were toxic to higher animals were listed (Cole and Cox, 1981). The mycotoxins that pose the greatest potential risk to human and animal health as food and feed contaminants are aflatoxins, ochratoxin A, zearalenone, trichothecenes (e.g., deoxynivalenol), and fumonisins. Both acute and chronic mycotoxicoses reduce animal production and increase costs. When farm animals are exposed either to

high levels of mycotoxins or to lower levels over a longer period of time, there is also a risk that significant amounts of the mycotoxins will be carried over into animal products such as milk, eggs and meat. Control of mycotoxins in animal feed is thus of great importance.

TOXIC EFFECTS OF MYCOTOXINS

Mycotoxins can have a very wide range of effects on animals depending on quantitative and qualitative aspects of their presence in animal feedstuffs. The diseases in animals caused by mycotoxins are referred to as mycotoxicoses. Mycotoxins act within the animal to: modify nutrient quality, absorption and metabolism; alter endocrine and neuroendocrine functions; depress the immune response. While obvious clinical mycotoxicoses can result in lesions, abortion and death, even moderate amounts frequently result in lower feed consumption, decreased feed conversion, reduced disease resistance, and increased reproductive problems. All species of livestock can be affected by mycotoxins. Non-ruminants (pigs, horses) are most sensitive, followed by ruminants and poultry. In general, young stock and animals under environmental, nutritional and production stresses are most sensitive. Safe levels of mycotoxins in feed, below which there are no effects on animal health or production, are not well established. The adverse biological effects exerted by mycotoxins have been subjected to various risk assessment strategies. The European Food Safety Authority (EFSA) has generated a series of evaluations of mycotoxins, including aflatoxins, ochratoxins, zearalenone, trichothecenes and fumonisins that specifically address adverse animal health effects (EFSA, 2004a; b, c; d; 2005).

Aflatoxins: Aflatoxins are mycotoxins produced by species of the genus *Aspergillus* and represent one of the most potent carcinogenic substances known so far (IARC, 1993). Aflatoxin B_1 has been classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans and animals (group 1), based on sufficient evidence for carcinogenicity in humans and experimental animals (IARC, 1993). Its metabolite aflatoxin M_1 appears in milk and milk products as a direct intake of aflatoxin B_1-contaminated feed. The aflatoxins are potent liver toxins and most animal species exposed to these mycotoxins show signs of liver disease raging from acute to chronic. Clinical signs in animals, associated with aflatoxin exposure, consist of anorexia, icterus, depression, weight loss, nasal discharge, gastrointestinal affections, haemorrhages, ascites and pulmonary oedema. Aflatoxins are known to impair the cellular and humoral immune system, rendering animals more susceptible to bacterial, viral, fungal and parasitic infections. This immunosuppressive effect also impairs acquired resistance following vaccination,

and may occur at a sub-clinical level of intoxication. Whereas acute clinical intoxications are rarely seen under the conditions of modern agricultural practise, sub-optimal weight gain, lower milk and egg production, as well as an increased susceptibility towards infectious diseases may lead to considerable economic losses in animal production due to aflatoxin exposure. The scientific Panel CONTAM expressed on 3 February 2004 an opinion relating to aflatoxin B_1 as an undesirable substance in animal feed (EFSA, 2004a). The Panel underlined that under circumstantial maximum exposure from feed materials (albeit in compliance with the levels set for feed materials), milk obtained from high-yielding milk-producing animals might contain aflatoxin M_1 levels exceeding the present statutory limits. The Panel concluded that the current maximum levels of aflatoxin B_1 in animal feed (EC, 32/2002) not only provide adequate protection from adverse health effects in target animal species but, more, importantly seems successfully to prevent undesirable concentrations of aflatoxin M_1 in milk.

Ochratoxins: Ochratoxins are metabolites of both *Aspergillus* and *Penicillium* species showing nephrotoxic effects in all animal species (EFSA, 2004b). Ochratoxin A inhibits protein synthesis, impairs blood coagulation, glucose metabolism and is carcinogenic. At present, the mechanism of the renal carcinogenicity remains unresolved, albeit a genotoxic mechanism cannot be excluded unequivocally (FAO/WHO, 2001). Ochratoxin A is also immunotoxic and teratogenic but usually at higher than nephrotoxic doses. It has been classified by the IARC (1993) as possibly carcinogenic to humans (group 2B), based on inadequate evidence in humans but sufficient evidence for carcinogenicity in animal studies. Pigs, dogs and poultry are particularly sensitive to the nephrotoxicity but a No-Observed-Adverse-Effect Level (NOAEL) has not been established in pigs or dogs; ruminants are less sensitive due to degradation of ochratoxin A to the less toxic ochratoxin-alfa by the rumen microflora. Upon absorption from the gastro-intestinal tract, ochratoxin A binds to serum proteins resulting in considerable variation in elimination half-lives across species depending on the affinity and degree of protein binding. It is only metabolised to a small extent systemically. Accumulation occurs in blood, liver and kidney, and significant lower residue concentrations are found in muscle tissue, fat and milk. Carry-over into eggs has been demonstrated under experimental conditions using high toxin concentrations.

Zearalenone: Zearalenone is a mycotoxin produced by several field fungi, including *F. graminearum* and *F. culmorum*. The structure of zearalenone allows its binding to mammalian oestrogen receptors (EFSA, 2004c). Subsequently, zearalenone induces oestrogenic effects in mammals and interferes with conception, ovulation, implantation, foetal development and viability of newborn animals. Zearalenone causes alterations in the reproductive tract of both laboratory and farm animals,

but no teratogenic effects are found. Female pigs of all age groups are considered to be the most sensitive animal species, but the hormonal effects vary in intensity according to age and reproductive cycle. Limited experimental studies indicate that, next to pigs, sheep are rather sensitive to the adverse effects of zearalenone, whilst cattle are less sensitive. Poultry (chicken and turkey) are the least sensitive to the hormonal effects of zearalenone. No reliable data exist from other species such as rabbits, horses, cats and dogs. There is only limited tissue deposition of zearalenone in meat and other edible tissue, and a low transmission rate into milk and eggs.

Trichothecenes (Deoxynivalenol): The trichothecenes are a large class of mycotoxins produced by several fungal genera, with the *Fusarium* spp. being the most notable. Deoxynivalenol (or vomitoxin) is the most common occurring trichothecene and exhibits toxic effects in all animal species (FAO/ WHO, 2001; EFSA, 2004d). Susceptibility varies considerably amongst species, but pigs are generally recognised as the most sensitive animal species. The adverse effects observed after deoxynivalenol exposure are decreased feed conversion, vomiting and feed refusal that lead to a reduced body weight gain, particularly in growing animals. Deoxynivalenol affects the immune response, and the release of pro-inflammatory cytokines is one of the earliest manifestations of exposure. At present, the available data do not allow the establishment of a NOAEL for pigs; the lowest reported levels with a negative effect on feed intake vary between 0.35 and 0.9 mg/kg feed. With respect to other animal species, it seems that healthy ruminants tolerate several mg deoxynivalenol/kg dry matter in the diet, due to the degrading capacity of the rumen flora. Poultry are also less sensitive than pigs to the effects of deoxynivalenol on feed intake and weight gain. Other species, including rabbits, horses, cats and dogs seem to have a higher tolerance towards deoxynivalenol than pigs. Deoxynivalenol is rapidly metabolised in the animal and the carry-over into edible tissues, milk and eggs is very low.

Fumonisins: The recently discovered fumonisin B mycotoxins are produced by *Fusarium* spp. that contaminate maize worldwide (EFSA, 2005). The fumonisins are non-mutagenic in different bacterial assays and lack genotoxicity in short-term DNA repair assays *in vivo* and *in vitro*, but exhibit clastogenic effects in primary hepatocytes (FAO/WHO, 2001). They are cytotoxic and inhibit both protein and DNA syntheses. Fumonisin B_1 is the most toxic and has been shown to promote tumours in rats and to affect the immune system. Intoxications associated with the occurrence of fumonisins in animal feeds comprise distinct syndromes such as equine leukoencephalomalacia (ELEM) and porcine pulmonary oedema (PPE), as well as hepatocellular injury and renal tubular necrosis. Fumonisins exhibit toxic effects in all animal species so far investigated. Susceptibility varies considerably

amongst species with pigs, rabbits and horses (and other *Equidae*) being the most sensitive. With respect to other animal species, adult ruminants are significantly less sensitive than calves. For broiler chickens the lowest observed effect level is approximately 2 mg/kg bw/day and data from duck, duckling and turkeys do not provide evidence that these species are more sensitive than chickens. Available data on carry-over of fumonisins from animal feeds into edible tissues, including milk and eggs, indicate that transfer is limited.

SYNERGISTIC INTERACTIONS AMONG MYCOTOXINS

Many fungal species are capable of simultaneously producing several mycotoxins, thus an individual grain may be naturally contaminated with several mycotoxins. Increased international trading of feedstuffs has contributed to the severity of mycotoxicoses as this increases the chance that a given compound feed will contain components of widely varying geographical origins showing mixtures of different mycotoxins. This can result in interactive toxicological effects which can be classified as additive, less than additive, synergistic, potentiative or antagonistic. A summary of mycotoxin interaction studies has been published by the CAST-Report (2003). Results of the review indicated that additive or less than additive effects were the predominant interactions observed (0.78), followed by synergistic interactions (0.19) and antagonistic interactions (0.03). These studies were conducted under laboratory conditions where animals generally are not exposed to many of the environmental stressors (heat, ammonia, disease, etc.) that occur under commercial conditions. Moreover, in these studies, only pairs of mycotoxins were evaluated, whereas under commercial conditions contaminated feeds may contain more than two mycotoxins. Multiple mycotoxin-induced immune suppressions may make livestock species more susceptible to disease agents present in commercial production systems.

MYCOTOXIN CONTAMINATION OF FEEDSTUFFS AND RISK ASSESSMENT

A variety of crops is susceptible to fungal invasion and in turn might be contaminated with mycotoxins. Different mycotoxins are more commonly found in or associated with certain feedstuffs. Some develop in the growing crop due to its susceptibility to certain toxigenic fungi, while infection and toxin production by others can be facilitated by insufficient preservation and storage systems. Some mycotoxins are associated only with crops from a certain region due to its climatic or ecological conditions. Most feeds are produced from crops at the farm

and consumed by the animals some time later. However, some straight feeds and particularly mixed feeds are also produced and sold by feed mills. Feed raw materials are divided into: (1) cereals and by products, (2) oilseed by products, (3) leguminous seeds, (4) roots and tubers, (5) animal by-products, (6) green crops/ pasture, (7) silages, (8) hays, (9) straws. They can be used straight, single or in combination in the feeding regime. Component types 1-5 are often combined and used as mixed feed and concentrates. The other raw material groups (6-9) are combined as roughage and mainly used for ruminants and horses. Mixed feed and concentrates are mainly produced at feed mills and, to a lesser extent, on farms.

   Mycotoxins of major concern associated with these feedstuffs are listed below.

- **Aflatoxins** – Aflatoxin B_1, B_2 (AFB), G_1, G_2 – are expected in tropical and subtropical products, such as groundnut, copra, palm kernel, cottonseed, babassu, maize and products derived from processing. With respect to feed materials originating from Europe, only few data are available, as aflatoxin formation was previously considered to occur mainly in geographic regions with a tropical or subtropical climate. However, in 2003, the formation of AFB_1 was observed in samples of maize originating from the Po valley in Italy (EFSA, 2004a). High temperatures, drought and strong insect damage were conducive for the growth of *A. flavus* and production of aflatoxins. Subsequently, milk samples taken at the farm level in that region in early autumn 2003 exceeded the 0.05 µg/kg EU limit for aflatoxin M_1 in milk, as a result of incorporating this locally-grown contaminated maize into the diets of dairy cows.
- **Ochratoxins**, particularly ochratoxin A (OTA), in Northern Europe, have to be expected in cereals (maize, wheat, barley, oats, triticale, rye) as animal feed components, nuts, dried peas and beans, soybean, sunflower seeds and sometimes in grass pellets. OTA occurrence in animal feed and feed components is predominantly a problem of poor or inadequate drying of cereals prior to storage, or poor storage conditions leading to 'hot-spots' of contamination.
- **Zearalenone** (ZEA) is common in maize and maize products, but can be found in soybeans and various cereals and grains, and additionally their co-products. ZEA can also occur on grass, hay and straw resulting in additional exposure of animals from roughage and bedding. ZEA occurs as a field (pre-harvest rather than a storage) contaminant and often co-occurs with other *Fusarium* toxins such as deoxynivalenol.
- **Deoxynivalenol** (DON) is particularly found in wheat, but may also contaminate barley and maize, maize silage and maize cob mix, and even

hay. Next to DON, other trichothecenes such as nivalenol (NIV) can be present in the same commodities. DON is also found in milling co-products at various levels.

- **Fumonisins**, of which the major representative is fumonisin B_1 (FB_1), are unique amongst the mycotoxins in being almost exclusively contaminants of maize.. Only incidentally fumonisins have been found in wheat, asparagus, tea and cowpea. Extensive world-wide survey data on fumonisins in maize indicate that the vast majority of maize is contaminated with fumonisins. It is in fact very difficult to obtain uncontaminated maize as, even when contamination is not significant, fumonisins can still be found at low background levels. Information about the possible occurrence of fumonisins in silage is slender, but incidental studies suggest that maize silage may be contaminated with fumonisins at low levels.

The global frequency of mycotoxin contamination of feedstuffs and the severity of mycotoxicoses in livestock and poultry appear to be increasing in recent years (Binder *et al.*, 2007). This may be due, in part, to increased monitoring of suspect materials and an increased awareness of the symptoms of mycotoxicoses by veterinarians and producers. Global climate change has also contributed to an increased frequency of mycotoxin contamination of feed grains. Drought, excessive rainfall and flooding can all promote mould growth (Smith, 2006). Current knowledge of exposure and of dose-effect relationships in farm animals is limited (Fink-Gremmels, 2008). Data on the occurrence of mycotoxins in feed materials reported officially to the EU are scarce and, in many cases, it is not clear whether the commodities evaluated are intended for human consumption or for animal feed(s). Data from the feed industry (voluntary quality control programs) generally remain unpublished. A recent 2-year survey program aimed to evaluate the incidence of mycotoxins (DON, T-2 toxin, ZEA, fumonisins, OTA, and AFB_1) in feed and feed raw materials in some of the major animal production regions demonstrated the worldwide occurrence of mycotoxins in animal feeds (Binder *et al.*, 2007). In the survey, a total of 2753 analyses were performed on samples sourced from European and Mediterranean markets, and 6391 analyses were undertaken on samples originating from the Asian-Pacific region. More than half of materials sampled in Europe were found to be contaminated at levels above the limit of quantification of methods applied, while one third of tests on Asian-Pacific sourced samples were positive. European samples had DON, ZEA and T-2 toxin as major contaminants, materials from Asia and the Pacific tended to be contaminated with DON, ZEA, fumonisins, and aflatoxins. Despite the large number of samples analysed, these data are too small for detailed exposure assessment for individual animal species. So, further data of mycotoxin occurrence in animal feeds are needed for assessment of animal exposure to mycotoxins.

EU REGULATION OF MYCOTOXINS IN ANIMAL FEEDS

The large uncertainties in exposure assessment also make it difficult to establish realistic maximum tolerance levels in individual feed components for all animal species. The first feed legislation worldwide addressed the levels of AFB_1 and total aflatoxins in the feed for dairy cows. These levels were established because aflatoxin M_1 can be carried over into milk and not because of adverse clinical signs in cattle. Directive 2002/32/EC (EC, 32/2002) of the European Parliament establishes maximum levels for AFB_1 and rye ergot. Regarding *Fusarium* mycotoxins (DON, ZEA, fumonisins B_1 and B_2, T-2 and HT-2 toxins) and OTA in feed, the 2006/576/EC document provides recommendations to monitor the presence of these mycotoxins in feed (EC, 576/2006). These recommendations provide orientation to the Member States on the acceptability of cereals and cereal products and compound feed for animal feeding and avoid disparities in the values accepted by the different Member States. However, in applying these guidance values, Member States should take into account the fact that the guidance values were determined for the most tolerant animal species and are therefore to be considered as upper guidance values (Verstraete, 2006). For feed for more sensitive animals, Member States should ensure that lower guidance values are applied by feed manufactures taking into account the sensitivity of the animal species. Feed business operators should use in their Hazard Analysis and Critical Control Points (HACCP) system (EC, 183/2005) the guidance values to determine the critical limits at critical control points which separate acceptability from unacceptability, for the prevention, elimination or reduction of identified hazards.

Concerning the Regulatory framework for contaminants in feed, the Directive 2002/32/EC (EC, 32/2002) provides that:

- products intended for animal feed may enter for use into the community, be marketed and used in the community only if they are sound, genuine and of merchantable quality and therefore do not represent any danger to human health, animal health or to the environment or do adversely affect livestock production;
- maximum levels and action levels can be set for contaminants in all products intended for animal feed;
- products intended for animal feeding containing levels of an undesirable substance that exceed the maximum level cannot be marketed and/or used for animal feeding; they may not be mixed for dilution purposes with the same, or other, products intended for animal feed;
- detoxification is allowed by chemical treatment and Member States shall ensure that measures are taken to guarantee the correct application of these

detoxification processes and to guarantee the conformity of the detoxified products intended for animal feed with the provisions of the Directive.

ECONOMIC IMPACTS OF MYCOTOXINS

The presence of mycotoxins in maize and small-grained cereals may have serious economic implications for crop, livestock and poultry producers, grain handlers, and food and feed processors. Economic losses caused by mycotoxin-producing fungi and mycotoxin contamination originate from reduced crop and animal production, and from the cost of programs designed to monitor and regulate mycotoxin concentrations in crops to minimize health risks to humans and animals. Losses could amount to millions of dollars each year in a grain-and livestock-producing country. The economic costs of mycotoxin contamination of crops are not limited to direct economic losses due to crop and livestock production losses, but also include effects on other sectors of society. Indirect economic losses, although more difficult to quantify, still represent major economic losses. The costs of chemical analyses and quality control programs, research and development, extension services, law suits and out-of court settlements, regulatory expenses and the cost of human illness and medical treatments must all be borne by the national economy. Of course the actual value of the losses encountered each year depends on several factors, such as grain, animal, and animal product prices; interest rates; the degree and extent of contamination, which would depend on the prevailing weather conditions; and many other economic variables (Charmley *et al*., 1994). The potential annual cost (US figures) of three mycotoxins – aflatoxin, deoxynivalenol, and fumonisin - contamination of crops is estimated to range from US$418 million to US$1.66 billion, with the mean estimated cost about US$932 million. In addition, mitigation costs and livestock losses could add another US$466 million and US$6 million, respectively, to the mean simulated costs (CAST, 2003). Far more severe can be the economic and health impacts of mycotoxins in the developing world (Wu, 2004).

Economic impacts of mycotoxins in animal feed are more difficult to measure, because information regarding animal illnesses and productivity losses due to chronic low-level exposures have been largely unreliable. Costs to animal producers (livestock and poultry) should include: higher feed costs, reduced animal growth and reproductive performance; increased disease incidences; unsafe mycotoxin residues in animal products; loss of markets; increased monitoring, testing, and detoxification costs, possible health risks to farm staff. So far the livestock losses, potential or actual, remain unknown (CAST, 2003). Recently, Wu (2007) presented an original economic model that took the first steps in measuring the various impacts of *Fusarium* toxins in animal feed. Two different

categories of losses were considered: losses related to animal health, and trade losses related to grain rejection in the marketplace. Of animal health losses, both mortalities and morbidities were considered. The relevant trade losses concerned impacts in both the domestic and international feed grain markets. A case study regarding fumonisin in US maize intended for animal feed was presented. It was estimated that, in a normal year (without a significant *Fusarium* ear rot outbreak), the losses due to fumonisin in animal feed would total US$ 1–20 million. In a year with a significant *Fusarium* ear rot outbreak, losses would total US$ 31–46 million. Sublethal morbidities of various animal species could drive this number slightly higher.

Prevention and management of mycotoxins in pre-harvest and during storage

Mycotoxins are produced in cereal grains as well as forages before, during and after harvest, when portals of entry are provided and environmental conditions are appropriate (Magan *et al*., 2003). Thus, methods for the prevention of mycotoxin contamination can conveniently be divided into pre-harvest, harvesting and post-harvest strategies. In general, mould growth and mycotoxin production are related to plant stress caused by weather extremes, to insect damage, to inadequate storage practices and to faulty feeding conditions (Bilgrami and Choudhary, 1998).

Several codes of practice have been developed by Codex Alimentarius for the prevention and reduction of mycotoxins in cereals, peanuts, apple products and raw materials, including the annexes on OTA, ZEA, fumonisins and trichothecenes (Codex, 2002). The elaboration and acceptance of a General Code of Practice by Codex can provide uniform guidance for all countries to consider in attempting to control and manage contamination by various mycotoxins. The recommendations for the reduction of various mycotoxins in cereals are divided into two parts: recommended practices based on Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP). A complementary management system to consider is the use of Hazard Analysis Critical Control Point (HACCP) (Codex, 2002). Control parameters would include time of harvesting, temperature and moisture during storage and transportation, selection of agricultural products prior to processing, processing/decontamination conditions, addition of chemicals and final product storage and transportation. In this regard, Commission Recommendation 2006/583/EC of 17 august 2006, on the prevention and reduction of *Fusarium* toxins in cereals and cereal products, contains general principles for the prevention and reduction of *Fusarium* toxin contamination (zearalenone, fumonisins and trichothecenes) in cereals (EC, 583/2006).

Pre-harvest control of mycotoxins

Prevention through pre-harvest control is the first step in ensuring a safe final product (Lopez-Garcìa and Park, 1998). Some seeds are contaminated with mycotoxin-producing fungi in the field and, during the growing period, can be exposed to environmental factors, such as weather, that are impossible to control. Once the crop becomes infected under field conditions, fungal growth will continue during post-harvest stages and storage. Thus, pre-harvest management is focused on controlling critical factors that have been shown to enhance mycotoxin production. This includes management of insect infestation and crop residues, planting adapted varieties, proper fertilization, weed control, necessary irrigation and proper crop rotation (Brown *et al.*, 1998; EC, 583/2006). Maintaining good cultural and management practices that promote the general health of crops can reduce but not eliminate pre-harvest mycotoxin contamination (Cleveland *et al.*, 2003). For example insect-resistant germplasm, such as maize transformed with the gene encoding *Bacillus thuringiensis* Berliner crystal protein (Bt maize), reduces levels of fumonisins. Irrigation of peanut essentially prevents aflatoxin contamination of this crop, probably by preventing drought stress known to induce aflatoxin contamination. However, optimization of management practices to control mycotoxins is not always possible for reasons of production costs, geographical location or the nature of the production system for the particular crop vulnerable to mycotoxins. In addition, even the best management practices are sometimes negated by biotic and abiotic factors that are hard to control, and by extremes in environmental conditions. Significant inroads have been made in establishing various control strategies such as development of atoxigenic biocontrol fungi that can out-compete their closely related, toxigenic cousins in field environments, thus reducing levels of mycotoxins in the crops (Cleveland *et al.*, 2003). Potential biochemical and genetic resistance markers have been identified in crops, particularly in maize, that are being utilized as selectable markers in breeding for resistance to aflatoxin contamination. Prototypes of genetically-engineered crops have been developed which: (1) contain genes for resistance to the phytotoxic effects of certain trichothecenes, thereby helping reduce fungal virulence, or (2) contain genes encoding fungal growth inhibitors for reducing fungal infection. Gene clusters housing the genes governing formation of trichothecenes, fumonisins and aflatoxins have been elucidated and are being targeted in strategies to interrupt the biosynthesis of these mycotoxins. In conclusion, a combination of strategies using bio-competitive fungi and enhancement of host-plant resistance may be needed to adequately prevent mycotoxin contamination in the field. To achieve this, plants may be developed that resist fungal infection and/or reduce the toxic effects of the mycotoxins themselves, or interrupt mycotoxin biosynthesis. This

research effort could potentially save affected agricultural industries hundreds of millions of dollars during years of serious mycotoxin outbreaks.

Control of mycotoxins at harvesting

During harvesting, it is important to control factors such as timeliness, clean-up and drying of the agricultural product (CAST, 2003; EC, 583/2006). Such control is essential for preventing mycotoxin formation during storage. Harvesting may inflict mechanical damage on commodities. When damage is kept to a minimum during this phase, subsequent contamination is significantly reduced. Field crops should be harvested in a timely manner to reduce the moisture or water activity levels to a point where mycotoxin formation will not occur. Harvesting should take place as soon as the crop is fully grown and the crop cycle is completed. Crops left on the field for longer periods may present higher levels of toxin contamination.

Control of mycotoxins during storage

Grains at harvest and entering initial storage contain a wide range of potential spoilage and toxigenic organisms, such as fungi, insects and mites, which directly or indirectly affect grain quality. Interactions of several factors, such as water activity, moisture content, rapidity of drying, temperature, time, composition of the substrate, mechanical damages to the seed, oxygen and carbon dioxide availability, fungal abundance, prevalence of toxigenic strains, spore load, microbial interactions and invertebrates are responsible for fungal growth in stored crops and mycotoxin production (Abramson, 1998). The recent review of Magan and Aldred (2007) examines some of the important post-harvest control strategies that have been developed for effective management to minimize the entry of mycotoxins in the food/feed chain.

MOISTURE CONTROL

Moisture is the single most important factor in determining if and how rapidly moulds will grow in feeds. Moisture in feeds comes from three sources: (1) feed ingredients, (2) feed manufacturing processes and (3) the environment in which the feed is held or stored. To control the moisture content of feeds successfully, moisture from all these three sources should be controlled. Thus, control of adequate aeration and periodical monitoring of the moisture content of silos

plays an important role in the restriction of mycotoxin contamination during the storage period.

The objective of raw material drying is to maintain the commodity at a "safe" level where fungal growth and mycotoxin production are not possible. An important parameter when considering moisture and fungal growth is water activity (a_w), i.e. the measure of the fraction of the water content of the substrate which is "free" and immediately available for fungal growth. The value of a_w of a sample is directly related to the equilibrium relative humidity (ERH) the sample can generate by the expression (a_w = ERH/100). Water activity is measured on a scale from 1.0 (pure water) to 0.0 (completely desiccated material). The growth limit for the most xerophilic fungal types is around 0.7 (Lacey and Magan, 1991). In general, mycotoxin formation may occur if the water content of stored products increases above 130-160g/kg, and maximum production may occur at 200-250g/kg (Bullerman *et al.*, 1984). In order to obtain a good preservation of stored crops from fungal growing, the water content of grains must be reduced by drying the crops until the necessary safe water contents which vary from one commodity to another (Lacey and Magan, 1991). At 20 °C this is around 140g/kg for maize and about 150g/kg for wheat, while for groundnuts it is much lower, at about 70g/kg. This is a reflection of the chemical makeup of the commodity and the relative proportion of "free" water. Too rapid heating may cause stress cracks in maize kernels increasing susceptibility to fungal invasion, while overheating may alter the relationship between water availability and water content (Lacey and Magan, 1991).

To control mould growth, obvious sources of moisture in the feed handling and storage equipment must be eliminated. These sources may include leaks in feed storage tanks, augers, roofs (either at the barn or at the feed mill), and compartments in feed trucks. With respect to storage facilities Codex recommend they be dry, well-constructed structures that provide protection from rain, drainage of ground water and protection from the entry of rodents and birds, ideally with minimum temperature fluctuations (Codex, 2002).

TEMPERATURE

Fungal contamination is also influenced by the temperature and each fungal species has characteristic minimum, optimum and maximum temperature requirements for growth. Some fungal species may have a minimum close to or below 0 °C, whereas others have a maximum up to 55-60 °C (Lacey, 1989). It is important to note that, for a given water content, water activity increases by increasing the temperature, with the practical implication that a temperature rise during storage

may lead to an increase in water activity to unsafe levels, and allow the initiation of fungal growth, hence the importance of temperature control in the storage situation (Lacey and Magan, 1991). The control of temperature of the stored grain at several fixed time intervals during storage may be important in determining mould growth. A temperature rise of 2–3 °C may indicate mould growth or insect infestation.

FRESHNESS OF FEEDS AND EQUIPMENT CLEANLINESS

Time is required for both mould growth and mycotoxin production to occur. It is therefore important to have feeds delivered often in order to be fresh when used (NC, 2007). Feeds should generally be consumed within 10 days of delivery, and feeder systems should be turned off weekly, and cleaned to remove caked and old feeds. Old feed is often very mouldy and may "seed" the fresher feed it contacts, increasing the chances of mould growth and mycotoxin formation. If the feeder system is allowed to keep the feed pans full at all times, the feed in the pans will be significantly older than that in the storage tank. The animals will tend to eat primarily the feed in the top layer and the feed at the bottom of the pans will age, providing greater opportunities for moulds to grow. The animals' performance may suffer as a result.

SUBSTRATE

Mycotoxigenic fungi can grow on different substrates, and the nature and amount of mycotoxin production depend on the substrate physical and chemical characteristics, such as water availability, mechanical resistance to packing and thermal conductivity, fat and protein content, trace mineral, amino acid and fatty acid composition (Ominski *et al.*, 1994; Lacey and Magan, 1991). Certain essential trace elements (zinc) and amino acids (proline) can stimulate aflatoxin production. On the other hand, some substrates may contain compounds that are inhibitory to fungal growth and mycotoxin production. Cloves, allspice and cinnamon bark were found to inhibit mould growth of *A. flavus*, *A. ochraceus*, and *A. versicolor*, while other spices including thyme, sage, dill seeds, turmeric and oregano inhibited only toxin production (Ominski *et al.*, 1994).

PRESERVATIVES

Moist grain specifically destined for animal feed is often treated with aliphatic acid-based preservatives (Magan and Aldred, 2007). Weak organic acids including formic, benzoic, acetic, sorbic and propionic acids are known to inhibit fungal

growth by acidifying the cytoplasmic content of fungal cells. Generally, the acid form of a mould inhibitor is more active than its corresponding salt. However, these are fungistats and thus the coverage of the grain must be efficient to prevent under-treated pockets. Poor coverage can lead to growth of spoilage fungi, especially mycotoxigenic moulds which can sometime metabolise these aliphatic acids. When formic acid was used as an antifungal compound on stored grain, levels of $> 400\mu g/kg$ of AFB_1 were subsequently detected but not when propionic acid was used. In consideration of these findings, the use of formic acid for this purpose has been discouraged in the EU since 1999 (EFSA, 2004a). This measure may not be applied in the new Member States and outside the EU.

Thus, there is interest in finding alternative compounds either to enhance or to replace such acids. Research has been carried out on both essential oils and anti-oxidants (Galvano *et al*, 2001). These studies have suggested that only a few essential oils such as cinnamon and clove leaf oil have the capacity for control of mycotoxigenic *Fusarium* species, *P. verrucosum*, *A. ochraceus* and DON and OTA production depending on environmental conditions. However, there are many economic and technological hurdles associated with this type of approach. In tests on wheat grain, butylhydroxyanisole, propyl paraben, cinnamon oil and resveratrol gave greater than 90% reduction in DON and NIV accumulation. Resveratrol has been demonstrated to have a particularly wide spectrum of mycotoxin control, although at present this is a relatively expensive product and may be uneconomical (Fanelli *et al*., 2003). However, should costs decrease then it may become a viable alternative to existing preservation systems for animal feed grain.

COPPER SULPHATE

The practice of recommending copper sulphate as a treatment for fungal diseases in animals goes back many decades (NC, 2007). The effectiveness of copper as a mould inhibitor is difficult to document. Although copper sulphate in the diet has been shown to improve body weight and feed conversion efficiency in broilers, excessive levels of copper may be toxic to young animals and will accumulate in the environment. In addition, recent research has indicated that feeding copper sulphate to poultry causes the formation of mouth lesions similar to those formed by some mycotoxins. Similar mouth lesions might be formed in other animal species.

SULPHUR DIOXIDE GAS

Sulphur dioxide (SO_2) is one of the oldest food additives. It has a long history as a disinfectant by the burning of elemental sulphur and the use of the resultant

fumes (Magan and Aldred, 2007). It is commonly used as a fungal inhibitory treatment of grapes and sometimes raisins. It is also used in the process of wine making and it is a necessity for the storage and preservation of white wines. During processing, golden raisins are often treated with SO_2 in order to prevent the enzymatic browning and additionally to prevent growth of moulds, yeasts and bacteria. SO_2 fumigation has also been examined for medium term storage of cereals. However, there is very little detailed information on the actual tolerance and sensitivity of mycotoxigenic fungi to SO_2. Some studies examined the effect of different concentration of SO_2 in relation to different temperatures (15, 25 °C) and a_w levels *in vitro* and *in situ* on grain for control of *Penicillium* and *Aspergillus* spp (Magan, 1993). The growth of *Aspergillus* species (*A. flavus*, *A. ochraceus*, *A. terreus*) was inhibited by 50 mg/l dissolved SO_2 on a malt extract-based medium. Some *Penicillium* species and *A. niger* were tolerant of up to 250 mg/l, in contrast growth of *Penicillium* spp. was stimulated by 100 mg/l.

MODIFIED ATMOSPHERES

For many years modified atmospheres or alternative gases have been examined for the medium and long term storage of cereal grain destined for food/feed (Magan and Aldred, 2007; Ominski *et al*., 1994). While fungi involved in biodeterioration of grain are considered to be obligate aerobes, many are actually micro-aerophilic, being able to survive and grow in niches where other species cannot grow and thus dominate specialised grain ecosystems. In many cases decreasing O_2 to < 0.14% is required before growth can be substantially reduced. Increasing CO_2 to > 50% is required for inhibition of mycelial growth. Some species, e.g. *P. roqueforti*, are able to grow and infect grain at > 80% CO_2 provided at least 4% O_2 is present. The use of integrated post-harvest systems for prevention of deterioration entails modifying O_2 and CO_2 simultaneously. The tolerance to low O_2 and high CO_2 is also influenced by interactions with grain type and water availability. The drier the grain, the more effective the treatment. Modified atmosphere storage can be used for control of both moulds and insects in moist stored grain. Regimes sufficient for moulds may not however be effective against some storage insects, which can survive and grow over a wider equilibrium relative humidity range.

MECHANICAL DAMAGE AND INSECT INFESTATION

Mechanical damage resulting from harvesting or from the action of insects disrupts the seed coat and facilitates the penetration of inoculum into the interior of the grain (Dowd, 1998). Certain kinds of stored-grain insects such as the grain weevil, in

which larvae and pupae develop within the infested kernel, carry numerous spores of storage fungi. Prevention of insect damage is relevant to control mycotoxins because insects, by feeding on stored material, can also cause local heating and moisture generation due to their metabolic activity. These warmer and damper areas containing nitrogenous waste products from insects provide ideal conditions, and favourable carbon-nitrogen ratio, for the initiation and development of local fungal growth and mycotoxin production. These localised effects lead to the development of heterogeneous conditions in large bulk stores (CAST, 2003). In general, it appears that the relationship between storage fungi and insects is unpredictable. Although some species of storage insect spread storage fungi, others are capable of decreasing fungal growth. In addition, some storage fungi attract storage insects, leading to increases in their population, whereas others are detrimental to them (Ominski *et al.*, 1994).

IRRADIATION

Radiation is typically categorized as either ionizing (IR) or non-ionizing (NIR), with IR involving X-rays and gamma-rays and NIR involving UV rays, microwaves, infrared rays and radio waves. Despite much public debate on the safety of irradiated foods, it is becoming more frequently used in the sterilization of a wide variety of food/feed commodities (Raso and Barbosa-Canovas, 2003). In 1980, the FAO/IAEA/WHO Expert Committee on food irradiation (JECFI) indicated that irradiation of any food commodities up to an average dose of 10 kGy causes no toxicological hazards and no special microbiological or nutritional problems (Molins *et al.*, 2001). Thus in relation to mycotoxin prevention, irradiation has been used to inhibit mycotoxin biosynthesis during storage period, and many studies have been conducted to access the use of gamma-irradiation in particular to prevent mould growth and mycotoxin formation (Sharma, 1998). Results accumulated from studies of the effects of radiation on mycotoxins have revealed several points of concern: 1) dosages required to degrade a pure mycotoxin vary, depending on the state of the toxin being tested and the concentration; 2) dosages required to destroy a pure mycotoxin are not the same as those needed for its detoxification in feeds; 3) dosages needed to degrade any particular toxin might depend on the moisture content of products; 4) irradiated fungal inocula may produce large amounts of toxins; 5) mycotoxin production on irradiated grains is sometimes significantly higher that on non-irradiated material; 6) dosages required to degrade most mycotoxins are well above those permitted for use in food preservation (up to 10 kGy) (Sharma, 1998). Therefore, at the present time, irradiation cannot be regarded as a practical means for mycotoxin elimination. However, studies which will address the safety of the process (at doses relevant to

the destruction of mycotoxins) along with the application of the Hazard Analysis Critical Control Point (HACCP) system in a food irradiation plant, might pave the way towards implementing this means for eliminating mycotoxins.

Feed processing and decontamination/detoxification of mycotoxins

Decontamination/detoxification of contaminated raw materials and feedstuffs plays a major role in the prevention of economic loss and animal disease. Mycotoxins are, in general, chemically and thermally stable compounds. Once a mycotoxin contaminated ingredient is screened and enters the milling process, mycotoxins will probably be retained in the finished product, and further removal of mycotoxins is difficult. In practice, decontamination or detoxification of mycotoxins can be achieved by removal or elimination of the contaminated commodities or by the inactivation of toxins present in the commodities through various physical, chemical and biological means depending on the commodities (Lopez-Garcia and Park, 1998; Visconti and De Girolamo, 2002). Food/feed processing that may involve physical and/or chemical decontamination can be considered as a strategy to destroy or redistribute mycotoxins (Scott, 1984; Scott, 1998). Processes used in the manufacture of animal feeds generally cause little loss of mycotoxins (Scott, 1998). Processed feeds are very complex systems because processing not only alters the product but also adds new ingredients and conditions, so many new interactions can occur. Treatments known to reduce mycotoxins in contaminated commodities include density segregation and flotation, cleaning and washing, sieving, de-hulling, hand picking and electronic sorting, wet and dry milling, thermal degradation such as baking, cooking, extrusion and roasting. In general, factors that may influence the fate of a mycotoxin during feed processing include the presence of other constituents and enzymes, moisture content, temperature, pH, pressure, buffer conditions, the mycotoxin concentration and whether it is introduced into the matrix for experimental study by natural contamination or by spiking (Scott, 1984). Ideally each treatment of food/feed processing and/ or decontamination, in addition to assuring an adequate wholesome food/feed supply, should: inactivate, destroy, or remove the toxins; destroy fungal spores and mycelia, so that new toxins are not formed; not produce or leave toxic residues in the food/feed; retain nutritive value and food/feed acceptability of the product; not alter significantly the technological properties of the product; be economically feasible and thus the cost should be considerably less than the value of the decontaminated crop (Lopez-Garcia and Park, 1998). Although a variety of decontamination methods have been tested and several show potential for commercial application, large-scale, practical and cost-effective methods for complete mycotoxin decontamination are currently not available. Moreover, no

single decontamination method that is equally effective against the variety of mycotoxins that can occur has been developed.

With regard to mycotoxin decontamination, the European Union is in favour of the use of physical decontamination processes and sorting procedures for both food and feed products (Verstraete, 2006). It does not allow the use of chemical decontamination processes for products intended for human consumption. Chemical detoxification is allowed for animal feeds, but Member States shall guarantee the correct application of the detoxification processes and the conformity of the detoxified products intended for animal feed with the provisions of the Directive 2002/32/EC (EC, 32/2002). Blending of contaminated crops with batches of good-quality material can reduce the mycotoxin concentration in the feedstuff, but this is not allowed as a decontamination procedure. The practice has been forbidden within the EU countries, at least for food and feed containing regulated mycotoxins. It is, however, probably used at farm level for low-quality crops, which may contain mycotoxins at low or unknown levels. In the U.S., blending contaminated feed with uncontaminated feed to reduce mycotoxin concentrations is regulated by federal agencies. Blending aflatoxin-contaminated commodities is not permitted. However, under certain circumstances, U.S. FDA may not object to blending feeds containing mycotoxins at concentrations higher than a limitation (Carlson, 2003).

Physical methods of mycotoxin removal

CLEANING AND SEGREGATION

Once a contaminated product has reached a processing facility, clean-up and segregation are the first control options. These procedures are usually non-invasive and, except for milling, do not alter the product significantly. In some cases, they are the best methods of reducing mycotoxin presence in final products and represent a good alternative for industry (Scott, 1984; 1991; 1998; Lopez-Garcia and Park, 1998; Visconti and De Girolamo, 2002).

Cleaning methods for cereals can be very effective in reducing mycotoxin contamination and extensive cleaning is in practical use for lowering mycotoxin contamination of feed cereals. The efficacy partly depends on the degree of fungal growth within the kernels. The highest fungal infestation and mycotoxin concentrations of cereals are found in the husks, debris, hull and the outer layer of the kernel. Mould-damaged kernels are often also of smaller size than sound kernels. The cleaning process often consists of scouring, aspiration, sieving and specific gravity separation. Dust, husks, hair and loose superficial particles are blown away by scouring and aspiration. Extensive aspiration can considerably

reduce the quantity of mould spores and mycelia on the kernel surface. Sieving and specific gravity separations (by density segregation in certain liquids or fractionation by gravity tables) remove small kernels, kernel debris, weed seeds and other impurities which differ in size and gravity in comparison to normal kernels. The results from the various studies indicate that sorting for quality (discarding scalpers, stained floaters and hand pick-outs) removes a large part of the aflatoxin present at harvest. Manual, mechanical and electronic methods can all be used for the segregation of crops. While manual selection is the simplest way for the physical removal of contaminated grains, it is a time-consuming procedure and this in many cases limit its applicability. A partial removal of aflatoxin can be achieved by fluorescence sorting of the maize, cotton seed and dried figs, where contamination can be observed by fluorescence following illumination with UV light with a positive correlation being reported between the observation—bright greenish–yellow fluorescence under long wave (365 nm) UV light and the presence of aflatoxin in these commodities. Separation of mould-damaged maize and/or screening can also significantly reduce fumonisin concentrations. In addition, the removal of rot from apples significantly reduces the patulin content in the final product.

Density segregation and removal of kernels buoyant in water and saturated sodium chloride solution can reduce DON, ZEA and aflatoxins in cereals. The use of specific gravity tables which allow removal of the least dense fractions containing the tombstone kernels can reduce DON contamination by 68-85% in wheat.

The bran fraction obtained from de-hulling is often associated with concentrated mycotoxin residues in whole maize grain. Removing the outer part of the kernel in dehuller studies have shown that a 40-100% reduction of DON and ZEA levels can be achieved with a concomitant loss of 13-19% of the grain material in barley, wheat and rice. The efficacy of this procedure depends on the degree of fungal penetration into the kernel.

Some years ago, it was a common practice to wash wheat prior to the milling process. Because of the resulting disposal problems and also because it added another stage to the overall process, the washing stage was abandoned. The effect of washing on the level of *Fusarium* toxins in grain has been examined. Simple washing procedures, using distilled water, results in 2-61% reductions of ZEA levels in contaminated barley and maize. Washing using sodium carbonate solution (0.1-1 mol/l) increases the removal of ZEA up to 87%. Similarly, washing barley and maize with distilled water reduces the DON level by 65-69%. Washing might be a useful treatment to use prior to wet milling or ethanol fermentation, otherwise the cost of drying grains remains prohibitive.

In conclusion, cleaning and segregation represent a primary source of the removal of hazard in mycotoxin contaminated feed. In some cases, these

procedures will eliminate the problem. However, in most cases, further processing is needed to reduce the risks associated with mycotoxin contamination.

WET AND DRY MILLING

Wet and dry milling are procedures that distribute mycotoxins in the different fractions depending on the commodity and the type and level of contamination (Scott, 1998; Lopez-Garcia and Park, 1998; Visconti and De Girolamo, 2002; Scudamore, 2004). Therefore, it is important to identify the fractions that remain toxic so that they can be diverted to lower-risk uses or subjected to decontamination procedures.

Wet milling is a process widely used for maize. Maize wet milling products include germ, gluten, fibre and starch. In wet-milling of ZEA contaminated maize, it was showed that mycotoxin was mainly concentrated in the gluten (0.49-0.56), followed by milling solubles (0.17-0.26), while the starch fractions were relatively free of ZEA (Bennett *et al*., 1978). In a study of commercial scale wet milling, ZEA was found in germ, fibre and gluten fractions at a much higher level (2.2-4.8 mg/kg) than in the concentrated steep liquor (0.6 mg/kg) (Lauren and Ringrose, 1997). In wet-milling of DON-contaminated maize, much of the toxin went into steep liquor, although measurable amounts remained in the starch. In addition, fibre, a fraction composed mainly of pressed fibre and concentrated steep liquor, contained high levels of the toxin. The use of such products for animal feed represents a potential risk of toxicosis in some animals (Trigostockli, 2000). Analysis of the fractions obtained from laboratory-scale wet milling of maize contaminated with fumonisins revealed that the starch fractions did not contain measurable amounts of FB_1; 0.22 of the toxin was found in the steeping and process water, while the fumonisin content of the other fractions diminished in the order gluten > fibre > germ and represented 0.10-0.40 of the concentration found in the original maize (Bennett and Richard, 1996). The presence of fumonisins in gluten, fibres and germ can be again a hazard to animal, since these products are used as feed ingredients. Laboratory studies have reported that during wet milling of aflatoxin contaminated maize, AFB_1 went primarily into the steep water (0.39-0.42) and fibre (0.30-0.38) with the remainder found in gluten (0.13-0.17), germ (0.06-0.10) and starch (only 0.01) (Lopez-Garcia and Park, 1998). In conclusion, all these studies show that, if a mycotoxin-contaminated lot of maize is processed by wet milling, toxin-free starch is produced. However, other products generally used in animal feed may have much higher levels of toxin than the starting maize.

Dry milling can also fractionate the toxin(s). Thus, identification of the toxic fractions is needed to adequately use milling as a decontamination procedure (Lopez-Garcia and Park, 1998). In dry milling of naturally contaminated maize,

the highest levels of AFB$_1$ occurred in the germ and hull fractions; but distribution varied with the contamination levels. In commercial dry milling, fumonisins are found in flaking grits, flour, germ and bran. The pattern of distribution after experimental dry milling varies slightly for different types of maize but, in general, the levels are lower in grits and higher in germ, bran and fines. The distribution of DON in the various dry milling fractions of wheat depends on the degree of fungal penetration of the endosperm, therefore milling grain having surface contamination results in flour with low mycotoxins levels.

In conclusion, from the industrial perspective, milling can be used as an effective method of separation. If the distribution of toxin is determined and alternative management of the toxic fraction is developed, milling can be considered a good cost/benefit method. A combination of cleaning, sorting and milling can significantly reduce the risks associated with mycotoxins.

THERMAL INACTIVATION

Thermal inactivation is a good alternative for products that are usually heat-processed. However, most mycotoxins are relatively heat-stable within the range of conventional feed processing temperatures (80–121°C), so little or no destruction can occur under normal cooking conditions such as boiling and frying, or even following pasteurization (Scott, 1998; Lopez-Garcia and Park, 1998; Visconti and De Girolamo, 2002; Scudamore, 2004). Degradation by heat treatment depends on the type of mycotoxin and its concentration, the extent of binding between the mycotoxin and the food constituents, the degree of heat penetration, as well as the heating temperature and the processing time. Thus, thermal inactivation should be evaluated for the conditions of a particular process. In some cases, a change in conditions such as moisture content, pH, and ionic strength of feed can contribute to the chemical modification of a particular toxin.

Aflatoxins have decomposition temperatures ranging from 237-306°C. Solid AFB$_1$ is quite stable to dry heat up to its thermal decomposition temperature of 267°C (Rustom, 1997). It has been observed that increasing the moisture content of the food product results in an enhanced degradation of aflatoxins. For example, 75% of aflatoxins (B$_1$+B$_2$) were degraded by heating at 100°C for 1 h at a moisture content of 300g/kg in contaminated cottonseed meal, while 33% of the degradation occurred in a similar meal containing 66g moisture/kg, under similar conditions. It has been suggested that the presence of moisture in food products helps in opening the lactone ring in AFB$_1$ to form a terminal carboxylic acid; which then undergoes a heat-induced decarboxylation.

Studies on the effect of heat processing of OTA-contaminated flour have reported that considerable reduction of OTA (76%) occurred in samples of

white flour heated to 250°C for 40 min (Scott, 1984). OTA appears to be more readily destroyed in dry cereal than in the presence of water. Processing of other commodities naturally contaminated with OTA has shown that after blanching, salting, and heat-processing beans, about 53% of OTA remained (Lopez-Garcia and Park, 1998). In this case, it is important to determine if the heat processing will yield products with unchanged technological characteristics.

Fumonisins are considered to be fairly heat stable compounds (Visconti and De Girolamo, 2002). No loss of FB_1 was observed when *F. verticillioides* culture material was boiled in water for 30 min and dried at 60° for 24h or during cooking of polenta for 20-30 min in boiling water. Moreover, several investigations, focused on the effect of thermal processing on the stability of fumonisins, showed that sometimes thermally processed maize products contained lower concentrations of fumonisins than unprocessed products depending on the time and the temperature of the processes. In particular, frying polenta or autoclaving maize meal produced reductions in fumonisins of 70-80% with no conversion to the hydrolyzed forms. The rate and extent of fumonisins decomposition in aqueous solutions increase with processing temperature; in particular from < 27% at ≤ 125°C to > 80% at ≥ 175°C, for 60 min, depending on buffer pH. Losses of FB_1 and FB_2 exceeding 70% were obtained in dry maize meal heated at 190°C for 60 min and complete loss at 220°C for 25 min. In another study, it was observed that fumonisins in spiked and naturally contaminated maize meal were unstable under roasting conditions (218°C for 15 min) but were stable under canning (121°C up to 87 min) and baking conditions (204-232°C for 20 min), probably because the canned and baked products reached lower internal temperatures than the roasted products.

Extrusion processing is one of the most versatile technologies available to the feed industry and it is used in the production of feeds. During extrusion cooking, high temperature and pressure are reached causing gelatinization of maize starch. The stability of FB_1 and B_2 during processing of cornflakes was investigated by analyzing the naturally contaminated raw material (maize flour), intermediate product (extruded, but not roasted cornflakes) and final product (roasted cornflakes). It was observed that about 60-70% of the initial amount of fumonisins was lost during the entire cycle of cornflakes processing, with less than 30% losses occurring during the intermediate extrusion step (70-170°C for 2-5 min) (De Girolamo *et al*., 2001). However, cornflakes processing may vary considerably from plant to plant depending on the time and temperature of cooking, the kind and amount of additives (salts, iron, vitamins, sugars, etc.) and on the quality of the raw material (maize variety, with or without germ and bran layers, etc.). These different parameters, while being a determinant for the quality of the final product, may also affect the degree of fumonisin reduction during processing (De Girolamo *et al*., 2001).

Heating ZEA-contaminated maize at 150°C resulted in only 0-28% reduction of ZEA, depending on the duration of the process, while a greater reduction (69%) was observed by heating wheat flour cake at 200°C for 60 min (Visconti and De Girolamo, 2002). Similar or greater ZEA reductions were achieved at temperature as low as 120°C with extrusion cooking.

Trichothecene mycotoxins are stable at 120°C, moderately stable at 180°C, and decompose within 30-40 min at 210°C (grilling) (Scudamore, 2004). A study on the effects of pH, salt and temperature on stability of standard solutions of DON and NIV showed both toxins to be relatively stable in buffer solutions over the pH range 1-10. Quite harsh conditions (pH 12, high salt concentration, 80°C, and prolonged exposure) were needed to give substantial breakdown. When present in ground maize substrate, DON and NIV were further stabilized relative to the solution tests. A study regarding the effects of heating procedures on DON and NIV levels in naturally contaminated barley and wheat resulted in a time-temperature-dependent decomposition of the toxins. Probably, during feed processing, trichothecenes are protected from decomposition by low penetration of heat, or stabilization resulting from interactions with other constituents within the sample. In a study of the effect of high temperature and high pressure processing of feed spiked with DON, no significant toxin reduction was found after processing in extruded maize grits, extruded dry dog food, or autoclaved moist dog food. Autoclaved cream-style maize showed a reduction of only 12% (Wolf-Hall *et al.*, 1999). DON was stable at 98°C during a feed pelleting process. DON levels (0.2-1.0 mg/kg) in a naturally contaminated wheat ingredient in the feed mash remained at the same level when they were processed using a laboratory pellet mill into feed pellets (Trigo-Stockli *et al.*, 2000).

In conclusion, the use of elevated temperatures to detoxify contaminated commodities is largely hindered by the impairment of both the nutritional and organoleptic properties of the foods, coupled with doubts regarding the potential generation of toxic pyrolysate. However, treatments providing high temperatures and pressures such as extrusion cooking may prove useful in future decontamination regimes.

MICROWAVES

When a rapidly oscillating radio frequency of microwave field (500 MHz-10GHz) is applied, the water molecules reorient with each change in field direction, creating intermolecular friction and generating heat. Aflatoxins (pure or in a food model) are destroyed when exposed to microwaves, but the rate of destruction depends on the microwave power and exposure time (Shapira, 2004). A reduction of at least 95% in aflatoxin content in peanuts occurred following a 16 min treatment at a

microwave power level of 1.6 kW or a 5 min treatment at a power level of 3.2 kW. Microwave treatment was only partially successful in lowering DON levels but it was most effective at the highest temperatures. There is no additional evidence of the destruction of other mycotoxins by microwaves.

Chemical methods of mycotoxin inactivation

A wide variety of chemicals, including calcium hydroxide monomethylamine, sodium bisulphite, moist and dry ozone, chlorine gas, hydrogen peroxide, ascorbic acid, hydrochloric acid, sulphur dioxide, formaldehyde, ammonia and ammonium hydroxide, have been found to be effective (to different extents) against several mycotoxins, including DON, ZEA, T-2 toxin, aflatoxins and fumonisins (Lopez-Garcia and Park, 1998; Visconti, 1998; Scott, 1998; Sinha, 1998; Visconti and De Girolamo, 2002; Pettersson, 2004; Shapira, 2004). However, their detoxification ability depends on parameters related to the contaminated products (e.g. moisture content), parameters associated with the process (such as temperature and pressure), the incubation time and the level of the mycotoxin in the product.

Ammoniation is an approved procedure for the detoxification of aflatoxin-contaminated agricultural commodities and feeds (Park *et al.*, 1988). It is the method of choice in some North American States (Arizona, California, Texas, Georgia, Alabama), Mexico, Senegal, Brazil and South Africa for detoxifying aflatoxins in contaminated peanut, cotton and maize meals. The US Food and Drug Administration (FDA) does not permit interstate shipment of ammoniated cottonseed or maize. In some EU countries, e.g. France and the UK, use of ammoniated contaminated feed is also approved. It is, however, not allowed to be used for dairy animals, due to the risk that residual aflatoxin may become higher than the maximum permitted level. The ammoniation process uses ammonium hydroxide or gaseous ammonia, both of which are equally effective in detoxifying aflatoxins. The success of aflatoxin detoxification by ammonia treatment is governed by the quantity used, the reaction time, as well as the temperature and pressure levels employed. Two major ammoniation processes are used for aflatoxin decontamination. The high pressure/high temperature (HP/HT) process is used for feed-mill operations, while the atmospheric pressure/ambient temperature (AP/AT) process is primarily for on-farm use. High pressure treatments are more effective in aflatoxin detoxification than treatments under atmospheric pressure, with the additional benefit that ammoniation under high pressure requires lower levels of ammonia with less processing time. AFB_1 degradation by ammonia occurs as a result of hydrolysis of the lactone ring followed by decarboxylation. However, the mutagenicity of aflatoxin-contaminated groundnut meal extracts

was not completely eliminated by ammoniation in a European safety study of the ammoniation procedure (Heidenreich *et al.*, 2001).

Ammoniation at ambient temperature and pressure has also been studied for OTA, ZEA and fumonisins in cereals (Visconti, 1998; Sinha, 1998; Pettersson, 2004). OTA, ZEA and DON were nearly completely degraded. In studies in which the ammoniated OTA contaminated barley was fed to pigs, some toxicity and lower nutritional value was observed. Ammonium hydroxide (3%) was able to reduce by 64% ZEA concentration in naturally-contaminated maize with 33.5 mg/kg of ZEA after 16 h of exposure. DON content was reduced by 9% and 85% in contaminated maize (1 g/kg) exposed to 100% ammonia for 1h and 18h, respectively. Ammoniation treatment combined with heat and pressure was able to reduce fumonisin level by 79% in maize contaminated with 86 mg/kg of FB_1. Ammoniation of *F. verticillioides* culture material as well as naturally contaminated maize, for 4 days at 50°C and atmospheric pressure, resulted in the reduction of FB_1 by 30-45% even if the toxicity of the culture material in rats was not altered by that treatment.

The effect of alkaline agents such as sodium, potassium, or calcium hydroxides on the destruction of mycotoxins is slightly less than ammonia treatment. The destruction order of different alkali on AFB_1 in solution at 110°C is potassium hydroxide > sodium hydroxide > potassium carbonate > sodium carbonate > potassium bicarbonate > ammonium hydroxide > sodium bicarbonate > ammonium carbonate. The treatment of peanut meal (containing 300g moisture/kg) with a 2% sodium hydroxide at 100°C for 120 min reduced AFB_1 to trace quantities.

Several oxidizing agents have successfully been used to detoxify mycotoxins from contaminated agricultural products (Shapira, 2004; Visconti and De Girolamo, 2002). Ozone or triatomic oxygen (O_3) is a powerful oxidant and reacts across numerous chemical groups, although it is particularly effective with olefinic double bonds. Treatment of cottonseed and peanut meals with aqueous O_3 has been shown to destroy aflatoxins, such AFB_1, AFG_1, and AFM_1. However, aflatoxins which lack a double bond in the terminal furan ring such as AFB_2, AFG_2, and AFM_2 are resistant to oxidation by ozone. Several studies have indicated that the O_3 treatment reduces AFB_1 levels by more than 90% in cotton-seed meal without any effect on animals fed with the treated material. Degradation of DON by O_3 has also been reported with the presence of moisture being critical for degradation of the toxin. O_3 was also effective in degrading patulin, OTA, cyclopiazonic acid, fumonisin and ZEA. However, the rate of destruction for several of these mycotoxins was dependent upon the level of O_3 and the duration of application. In the case of fumonisin, degradation did not correlate with detoxification.

Hydrogen peroxide (H_2O_2) has been used on a commercial scale to detoxify aflatoxins, ZEA, and DON (Scott, 1998). H_2O_2 has been used on a commercial scale in India to detoxify peanut protein contaminated with aflatoxin. Trials to

decontaminate aflatoxin-containing oil-seeds have also been carried out on an industrial scale using aqueous calcium hydroxide and monomethylamine, with the conclusion that the procedure was effective. Calcium hydroxide monomethylamine has been used to decontaminate feeds containing T-2 toxin and diacetoxyscirpenol at 10-20 mg/kg; the success of the procedure was dependent on the moisture content of the feed and the processing temperature. In particular, about 50% mycotoxin reduction was observed when the treatment was performed at about 25°C and 100g moisture/kg in 4 hours; when the moisture content was increased to 250g/kg, T-2 toxin level was reduced by 95-99%.

The use of sodium bisulphite as a detoxification reagent is worth special mention as it is a common food additive. Sodium bisulphite is commonly used as a food and drink additive due to its enzymatic degradation inhibitor, antioxidant, and bacteriostatic properties. The main product of reaction with AFB_1 is a sulphonate, AFB_1-S, formed by addition of bisulphite to the furan ring present in AFB_1 and G_1 but not in AFB_2 and G_2. It has been shown that AFB_1 can be destroyed in maize and dried figs with sodium bisulphite and that peroxide and heat increase detoxification in the figs. Sodium bisulphite solution greatly reduces DON levels in contaminated maize and wheat. These treatments resulted in the formation of DON-sulphonate conjugate which is unstable due to its alkaline hydrolysis into DON under certain baking and processing conditions. For this reason and because DON-sulphonate conjugate affects the rheological properties of flour, this treatment might not be suitable for direct application to human foods; nevertheless DON-sulphonate conjugate appeared to be nontoxic to pigs and reduced the short-term toxic effects of DON-contaminated maize on feed intake and body weight gain in pigs, then this treatment has been proposed for decontaminating DON-contaminated maize destined for use in pig feeds. To date there are no reports of attempts to scale up this procedure for possible use on feedstuffs for pigs.

A variety of other chemicals such as chlorinating agents, formaldehyde, potassium permanganate, sodium borate have been shown to be effective in the detoxification of several mycotoxins. Aqueous chlorine is commonly used in the food/feed industry to sanitize processing equipment and to wash a variety of raw materials such as fruits, nut meats, fish, frog and meat prior to processing. Chlorinating agents such as chlorine and sodium hypochlorite have been reported to destroy mycotoxins (Scott, 1998; Sinha, 1998; Visconti and De Girolamo, 2002). Chlorine at concentrations of 11 mg gas per gram of copra meal spiked with AFB_1 resulted in more than 75% reduction of mycotoxin. In addition, the application of gaseous chlorine at 10% degraded more than 90% of aflatoxins in peanut meals. Chlorine at a gas concentration of 300 mg gas per gram of maize was able completely to reduce DON level in contaminated maize within 30 min. Formaldehyde in vapour form or in aqueous solutions reduced ZEA concentration in both naturally contaminated maize meal and spiked maize grits. A complete

reduction of ZEA in maize grits containing 3 or 5 mg ZEA/kg was observed after exposure for 16h at 50°C to 3.7% of formaldehyde solution. The heating of FB_1 in an aqueous solution with reducing sugars such as D-fructose or D-glucose resulted in the formation of N-(carboxymethyl)-FB_1 that seems to be less toxic than FB_1 when tested on cell tissue culture. Further studies are needed to compare the toxicity of N-(carboxymethyl)-FB_1 and FB_1.

In conclusion, many chemical treatments may be effective in destroying mycotoxins present in foods/feeds. However, often they significantly decrease the nutritional value of the food products or produce toxic metabolites or other products with undesirable effects; thus limiting their widespread use.

Biological inactivation of mycotoxins

The presence of other microorganisms, such as bacteria or other filamentous fungi, may alter fungal growth and mycotoxin production (Ominski *et al*., 1994). A promising approach in the post-harvest prevention of mycotoxin contamination is the potential use of antagonistic bacteria, fungi, and yeast (Karlovsky, 1999; Varga and Toth, 2005; Shetty and Jespersen, 2006). These biological methods have been explored as options for mycotoxin decontamination/detoxification (Karlovsky, 1999). Biological detoxification can be defined as the enzymatic degradation or biotransformation of mycotoxins that can be obtained by either the whole cell or an enzyme system. For example, a black yeast (*Exophiala spinifera*) and a gram-positive bacterium strain (*Caulobacter* spp.) isolated from maize stalks completely metabolizes FB_1 with release of CO_2. These microorganisms have been used as gene source for the production of transgenic maize plants. The 12,13-epoxide ring is essential for the toxicity of trichothecene mycotoxins, and removal of this ring results in a significant loss of toxicity. Ruminal or intestinal microflora are capable of detoxifying DON by enzymatic reduction of the epoxide ring resulting in the metabolite DOM-1 that is known to be non-toxic. A pure anaerobic bacterial strain (*Eubacterium*), capable of the biotransformation of DON to DOM-1 has been isolated from an enriched mixed culture obtained from bovine rumen contents. This bacterium transformed DON and other trichothecenes within 24-48 h in *in vitro* experiments using segments of pig intestine. A soil bacterium strain belonging to the *Agrobacterium-Rhizobium* group was able to convert DON to the less toxic 3-keto-4-deoxynivalenol. The bacterium was also able to transform 3-acetyldeoxynivalenol but not NIV and fusarenone X. Fermentation by *Saccharomyces cerevisiae* of wort-containing ZEA results in conversion of 69% toxin to beta-zearalenol, a metabolite with lower activity than the parent compound. Of the microorganisms screened for their ability to degrade OTA, *Acinetobacter calcoaceticus*, *Phenylobacterium immobile* and a non-toxigenic strain of *Aspergillus niger* were reported to

convert OTA to the less toxic alpha-OTA. The microbial flora of the mammalian gastrointestinal tract, including rumen microorganisms of the cow and sheep, were also reported to degrade OTA to alpha-OTA although the microorganisms responsible for OTA degradation were not identified. *Flavobacterium aurantiacum* was the only microorganism able irreversibly to remove AFB_1 from both solid and liquid media, among one thousand strain tested including yeast, moulds and bacteria. The ability of this microorganism to remove AFB_1 from foods was demonstrated in vegetable oil, peanut, maize, peanut butter and peanut milk. The possibility of using culture extracts containing the semi-purified active protein in the food/feed industry was suggested as a means of removing aflatoxins from contaminated foodstuffs/feedstuffs. Other microorganisms including *Rhizopus* spp., *Corynebacterium rubrum*, *Candida lipolytica*, *Aspergillus niger*, *Trichoderma viride*, *Mucor ambiguous*, *Neurospora* spp., *Armillariella tabescens* can inhibit AFB_1 biosynthesis. When degradation compounds were investigated, formation of aflatoxicol, a compound having the same carcinogenicity of AFB_1, was observed.

Alternatively, lactic acid bacteria or their antifungal metabolites have been studied as natural preservatives to inhibit mycotoxigenic mould growth and mycotoxin production (Shetty and Jespersen, 2006). Lactic acid bacteria are of special interest as preservation organisms since they have a long history of use in food and are GRAS "generally regarded as safe" organisms, and as such any compounds isolated from lactococcal species, may be particularly useful in the preservation of foods/feeds from mould spoilage and mycotoxin contamination. Even though in many cases the antifungal and antimycotoxigenic potential of lactic acid bacteria are still unknown, it is widely believed that inhibition of mycotoxin synthesis is due to microbial competition, the depletion of nutrients, low pH, and also due to the production of heat-stable low-molecular weight of metabolites which are produced by lactic acid bacteria (Bata and Lasztity, 1999).

Biological methods of mycotoxin decontamination/detoxification are a good option for the fermentation industry. In this regard, it has been found that aflatoxins are not degraded during fermentation; they concentrate in the spent grains and are not found in the alcohol fraction after distillation. When contaminated products are used for fermentation, it is therefore important to determine the end use of the contaminated by-products. Accumulation of aflatoxin in spent grains is a potential problem when using this material as animal feed, and further decontamination procedures must be used. Similarly, Bennet and Richard (1996) reported that, although pure fumonisins exhibit considerable water solubility, residues of toxin remain in the distillers' dried grains. This fermentation product accounts for 31-35% of the total fumonisins in the initial maize. The fermentation process did not destroy fumonisins. Products from ethanol fermentation of fumonisin-contaminated maize generally used as animal feeds could be detrimental if consumed by pigs or horses, animals sensitive to relatively low levels of these toxins.

Feed additives for prevention of mycotoxin effects

An alternative approach to detoxification, which seeks to minimise the impact of mycotoxins on the animal itself by treating or modifying the contaminated diet prior to its consumption, has also been attempted with varying degrees of success. If unacceptable mycotoxin levels occur, removal of the contaminated feed is preferable. While it is often not possible completely to replace the feed, particularly the forage ingredients, obviously mouldy feeds should be removed. Acidic diets may intensify the effects of mycotoxins and should be avoided in these situations. Dietary manipulations include improvement of the nutritional content of the diet to compensate for mycotoxin-induced reductions in intake, and addition of potential mycotoxin-binding agents to the diet to inhibit the absorption of mycotoxins by the animal (Trenholm *et al*., 1991). Increasing the energy, crude protein, mineral and vitamin content of a contaminated diet by the 20% can improve weight gain in pigs, but only if feed intake has been reduced by 20% or less. The possible use of inorganic or organic binders as mycotoxin-detoxifying agents in feeds has received a lot of research attention recently.

NUTRIENT SUPPLEMENTATION

Galvano *et al*. (2001) reviewed various dietary strategies to contain the toxic effects of mycotoxins using antioxidant compounds (selenium, vitamins, provitamins), food components (phenolic compounds, coumarin, chlorophyll and its derivatives, fructose, aspartame), medicinal herbs and plant extracts. Selenium, some vitamins (A, C, and E) and their precursors have marked antioxidant properties and are believed to work as free radical scavengers and to protect against membrane damage by mycotoxins. Some antioxidants, selenium and vitamins can also induce or stimulate detoxifying enzyme systems in the liver and other tissues and thereby increase the metabolic detoxification of mycotoxins. Interesting results have been obtained by food components contained in coffee, strawberries, tea, pepper, grapes, turmeric, *Fava tonka*, garlic, cabbage and onions. Additionally, some medicinal herbs and plant extracts could potentially provide protection against AFB_1 and FB_1. As summarized in the review (Galvano *et al*., 2001), available data are primarily from *in vitro* studies and mainly focus on AFB_1, whereas much less information is available about other mycotoxins. More feeding studies with antioxidants and vitamins carried out with farm animals are needed for an evaluation of their practical advantages.

MYCOTOXIN-BINDING AGENTS

The use of mycotoxin-binding agents is commonly recommended to farmers in order to protect animals against the harmful effects of mycotoxins occurring in contaminated feeds. Reviews of mycotoxin binders have been published (Ramos *et al*., 1996; Phillips, 1999; Huwig *et al*., 2001; Galvano *et al*., 2001; Diaz and Smith, 2005; Avantaggiato *et al*., 2005). These adsorbent materials should act like 'chemical sponge' and adsorb mycotoxins in the gastrointestinal tract, thus preventing the uptake in the blood and subsequent distribution to target organs. Therefore, this approach is seen as prevention rather than therapy. The efficacy of the adsorption depends on the chemical structure of both the adsorbent and the mycotoxin. The most important feature for adsorption is the physical structure of the adsorbent, i.e. the total charge and charge distribution, the size of the pores and the accessible surface area. On the other hand, the properties of the adsorbate molecules (the mycotoxins) like polarity, solubility, shape and charge distribution also play a significant role. Several studies have shown that a variety of adsorbent materials have high affinity for mycotoxins by the formation of stable linkages. These linkages can also occur in several liquid systems such as water, beer, wine, whole and skimmed milk and peanut oil. Potential absorbent materials include aluminosilicates (clay, bentonite, montmorillonite, zeolite, etc.), activated carbon, complex indigestible carbohydrates (cellulose, polysaccharides in the cell walls of yeast and bacteria such as glucomannans, peptidoglycans, and others) and synthetic polymers such as cholestryamine and polyvinylpyrrolidone and derivatives.

Results obtained with some extensively studied adsorbents, such as aluminosilicates, are quite satisfactory with respect to aflatoxins. It is suggested that this specific silicate minerals can bind with aflatoxin by chelating the beta-dicarbonyl moiety in aflatoxin with uncoordinated metal ions in the clay materials. Other silicates that have been studied include bentonites, zeolites, clinoptilolites and various others that are often not completely characterized. Hydrated sodium calcium aluminosilicates included at 5-20g/kg diet can adsorb aflatoxin and prevent aflatoxicosis across species, including chickens, turkeys, pigs, lambs, dairy cows, dairy goats and mink. Responses to aluminosilicates appear to be dose-dependent. Aluminosilicates are allowed by the U.S. Food and Drug Administration when used at a level up to 20g/kg complete diets as "anti-caking" agents. Aluminosilicates, however, show a number of disadvantages, not least being the impairment of mineral utilization at levels above 20g/kg and a narrow range of binding efficacy. In animals, aluminosilicates are selective in their "chemisorption" of aflatoxins and show little or no beneficial effect against ZEA, FB_1, OTA, and trichothecenes, including DON, T-2 toxin, or diacetoxyscirpenol. A number of studies have

examined chemically modified silicates and shown that chemical modifications can increase the binding of aluminosilicate with aflatoxin, ZEA and OTA.

Studies on the elimination of mycotoxins other than aflatoxins (e.g. trichothecenes, ZEA, OTA or fumonisins) from contaminated feedstuffs by the use of adsorbents show controversial results. While some authors suggest a positive influence caused by the addition of glucomannans or clays, others observed no or only slight reduction of toxic effects. Beneficial effects of activated carbon and sodium bentonite have been shown in rats intoxicated with T-2 toxin. These effects were associated with their ability to bind the mycotoxin, thus preventing toxin absorption. Bentonite was ineffective against ZEA and NIV in pigs. Synthetic anion exchange zeolite was found to alleviate the adverse effects of ZEA in rats. Cholestyramine (anion exchange resin) has been used to adsorb ZEA *in vitro* from simulated gastric and intestinal fluids. Divinylbenzene-styrene polymers (anion-exchange resins) exhibited beneficial effects when added to the diets of T-2 toxin or ZEA intoxicated rats. Polyvinylpyrrolidone added to the diets of pigs contaminated with DON did not appear to alleviate the toxic effect of the toxin. Extensive screening tests have been performed to evaluate the efficacy of various adsorbent materials in binding *Fusarium* mycotoxins (Avantaggiato *et al*., 2003; 2004; 2005; 2007). Most of the commercially available mycotoxin-binders failed in sequestering *Fusarium* mycotoxin *in vitro*. Only a small number of adsorbent materials possessed the ability to bind more than one mycotoxin. Cholestyramine was proven to be an effective binder for fumonisins and ZEA *in vitro*, which was confirmed for ZEA in experiments using a dynamic gastrointestinal model simulating the gastrointestinal tract of pigs and for fumonisins in *in vivo* experiments with rats by using the sphinganine/sphingosine ratio as biomarker of fumonisin exposure. In these studies, no adsorbent materials, with the exception of activated carbon, showed relevant ability in binding DON and NIV. The *in vitro* efficacy of activated carbon toward fumonisins was not confirmed *in vivo* with rats by the biomarker assay.

LEGAL STATUS, CHARACTERISTICS OF MYCOTOXIN BINDERS AND CONCERNS

Research with mycotoxin binders has been conducted for over 20 years and well over a hundred products are on the market. Experimentally, mycotoxin binders have been effective at partially reducing the effects of some mycotoxins but currently and despite their large-scale use, no product has been approved. Ideally, a mycotoxin binder should be effective at sequestering the mycotoxin(s) of interest and show a large spectrum of action as feedstuffs are commonly contaminated

with more than one mycotoxin. A binder should significantly prevent animal toxicity. There should not be serious detrimental effects on the animal, or at least detrimental effects should not outweigh the benefits. Costs should render its use practical and profitable. Animal/product residues of mycotoxins should not increase. There should be no detrimental effects on the animal food product. Mycotoxins in feeds should not be masked such that feed contamination cannot be verified. The binder should be physically usable in commercial feed manufacturing situations and should not occupy a large portion of the complete diet. It should be free of impurities, off-flavours and odours. No product currently meets all these characteristics.

Although responses in farm or laboratory animals to some of these products have been very encouraging, the primary concern in using these products as feed additives is that the *in vivo* efficacy in binding mycotoxins and the safety towards livestock of most of the commercial products have not been thoroughly tested. Moreover, there is no official method to check their efficacy, and each laboratory developed its own *in vitro* and/or *in vivo* technique.

In vitro evaluations are a key step in the development and quality control of binders, and are useful as a screening method for potential binder products. However, *in vitro* methods are not standardized and therefore are not comparable across all laboratories. The *in vitro* techniques have not produced results that correlate well with *in vivo* results. Therefore, *in vitro* data should not be used to make decisions about products to use in practice.

In vivo experiments are the best way to evaluate the efficacy of mycotoxin binders. Of course, *in vivo* models are ideal in theory and difficult to perform. Gathering the definitive information is complex, expensive, time consuming. In *in vivo* studies, individual bioassays should be conducted using the same strain, age, body weight and diet type in order to obtain consistent results. Variations in housing conditions, health status, growth rate and maturity can also influence results. Binders should be evaluated with different inclusion rates, with different mycotoxins, across animal species, ages and gender, and under different environmental conditions. Moreover, according to the EU Guideline 2001/79/EC on additives for use in animal nutrition, the *in vivo* efficacy of binders should be proven by using an experimental design justified according to the claim for the use of the additive, and by using specific biological markers such as tissue residues or changes in biochemical parameters. The main difficulty with mycotoxins is to get specific and reliable biomarkers. Until now, most of investigations on the efficacy of mycotoxin binders were limited to feed intake and performance measurements, which are rather non-specific and not suited to prove the efficacy. Few *in vivo* studies investigated possible side effects of these agents on performance or health of animals.

In conclusion, the contribution of mycotoxin binders to the safety of the feed/ food chain has not been fully exploited and this is one of the reasons they had no legal status and were not approved for the marketplace anywhere. However, very recently, on 12 May 2009, the European Union with the Regulation (EC) No 386/2009 has regulated the position of additives intended for the control of mycotoxins in feed. According to this regulatory status, a new functional group in the category of technological feed additives was established and substances that suppress or reduce mycotoxin absorption, promote the excretion of mycotoxins or modify their mode of action, were recognized. The purpose of these new feed additives shall not be to clean up or mask adulterated feed, but to help further reducing contamination once the limits fixed or recommended have been attained through other means. It might also allow better management of the risk posed from non-regulated mycotoxins and for synergistic effects between mycotoxins at low concentrations. Due to EC Regulation No 386/2009, in the European Union companies will be able officially to apply for authorisation of substances for the control of mycotoxins. Of course, the authorisation of these feed additives should comply with the general safety and efficacy rules provided by the feed additive Regulation (EC) 1831/2003. It should also be without prejudice to the existing risk management measures of mycotoxins, in particular those set under council Directive 2002/32 on undesirable substances in feeds.

Recommendations and future research

Use of an integrated management program based on observance of HACCP principles represents a useful tool to prevent the risk of mycotoxin contamination in pre-harvest, harvesting and post-harvest stages.

Although certain treatments have been found to reduce concentrations of specific mycotoxins in foods and feeds, no single method has been developed that is equally effective against the wide variety of mycotoxins that may co-occurs in different commodities. In addition, detoxification processes that may appear effective *in vitro* do not necessarily retain their efficacy when tested *in vivo*. More work is needed to study the fate of mycotoxins during food/feed decontamination and processing; in particular the reduction of toxicological risk associated with processed mycotoxins-contaminated commodities as well as prevention of recontamination during storage, should be evaluated.

Data gathered by feed processors about the fate of mycotoxins during processing are rarely or only partially made available to the public.

Contaminated feeds are frequently more toxic than the pure toxin in animals, indicating possible interactions. It is recommended to perform further studies on

mycotoxins occurring concomitantly in feeds, their possible interactions and how toxicological significance of such interactions could be assessed.

The use of feed additives to prevent absorption and toxic effects of mycotoxins in farm animals is of growing commercial interest and new products will appear. The effectiveness of the additives for the various mycotoxins and in the different farm animals has to be proven, e.g. by peer-verified studies. The use of cell lines or artificial models to simulate animals is recommended as an important alternative to the use of living experimental animals.

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9

THE EFFECT OF NUTRITION ON STRESS AND IMMUNITY

T. A. NIEWOLD

Professor Nutrition and Health, Faculty of Bioscience Engineering, Katholieke Universiteit Leuven, Kasteelpark Arenberg 30, 3001 Heverlee, Belgium

Introduction

It is generally recognized that there is a relationship between nutrition and immunity or health. In the last 20 years, it has become much clearer that psychological and neurological factors also influence immunity (Irwin, 2008). A healthy diet, not deficient in energy, nutrients and micronutrients is essential to prevent and fight off diseases. Stress influences immunity through the hypothalamic pituitary adrenal (HPA) axis and autonomic mechanisms, and it has become clear that immune mediators influence behaviour. Furthermore, immune mediators have a profound influence on feed intake and on the energy and nitrogen balance. During inflammation, the release of pro-inflammatory cytokines not only leads to activation of immune cells but also to a decrease in appetite and increased catabolism of muscle tissue. It has also become clear that inflammation (and other parts of the immune system) can be influenced by dietary components, either directly or indirectly. In conclusion, there is an intricate relationship between stress, nutrition and immunity. This is not a simple relationship as is evident from the comments above. In order to maintain the integrity of the body, the whole system is tightly regulated. It is especially important to realize that the regulation in time and dose is very important. For instance, transiently elevated levels of cortisol after stress or inflammation are beneficial and essential for the body's function; however, when chronically elevated, cortisol may lead to immune dysfunction and an inflammatory state. This also implies that the same (e.g. feed) compound when interfering with the above-mentioned regulation can have both beneficial and detrimental effects, depending on dose and also dependent on the timing of other processes such as inflammation. To further complicate matters, the immune system

consists of two functionally distinct parts: the systemic part, which is oriented towards reaction, and the larger mucosal immune system, which is oriented towards tolerance. Furthermore, the genetic background has influence. It is also important to realise that, like the brain, the immune system is a learning system. This means that immunological reactions are influenced by past experience. The latter also includes perinatal imprinting, meaning that paternal and maternal feeding can influence gene expression in the offspring in an epigenetic way. In production animals feed itself is in particular one of the most important environmental factors influencing gene expression and hence health (Figure 1).
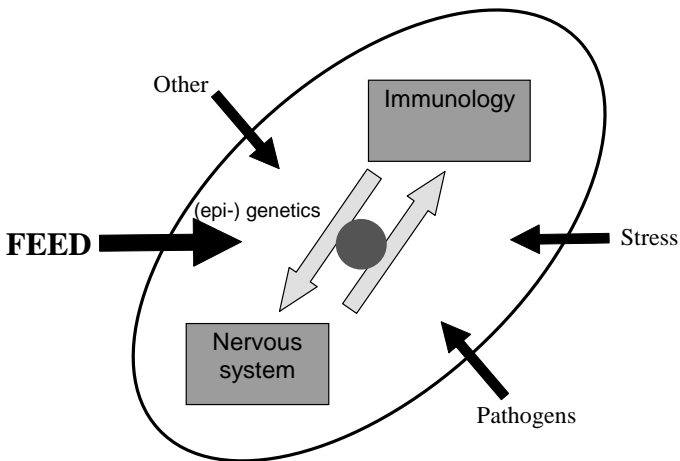
Figure 1. Feed itself is, particularly in production animals, one of the most important environmental factors influencing gene expression and health.

Each meal provokes a host of physiological responses, the most well-known response is the initiation of the process of digestion and absorption of nutrients. Much less known is the postprandial (low-grade) inflammatory response in the intestines, which is also referred to as a metabolic inflammation. This is a normal physiological response of the body to a meal, and the degree of inflammation is related to the dietary energy value, the glyceamic index and specific constituents including fatty acids (Margioris, 2009). If not properly regulated, postprandial inflammation could ultimately lead to unfavourable (in production animals) phenomena such as muscle catabolism and inappettance. Since the intestines are constantly exposed to foreign substances, it is not surprising that the body has evolved an intestinal system to control inflammation and immunity. This is the so-called nervous anti-inflammatory reflex which plays a pivotal role in the control and containment of the intestinal defence system, and is therefore essential for health and survival of the individual (Tracey, 2002). This also means that, in

contrast to the often-suggested need to enhance intestinal defences, the organism benefits somewhat from down regulation of the intestinal inflammatory responses (as discussed subsequently). This regulatory mechanism is important because it can be overwhelmed by risk factors such as large amounts of (high) energy feed. Some production animals face some of the risk factors that may compromise the process. Some feed components are potentially pro-inflammatory that may aggravate the problem, whereas others could be anti-inflammatory. The latter could be very helpful if included in the diet to mitigate the effect of the postprandial inflammation.

There follows a brief review of a selection of feed-related factors known to influence the immune system and their significance. A distinction can be made between nutrients as exogenous factors, and hormones as examples of endogenous factors. Finally, a few recommendations for further progress are made.

Macro and micronutrients (Table 1)

Table 1. Feed components modulating immune function (modified after Field *et al*., 2002)

Macronutrients		*Miscellaneous*	
	Carbohydrates		Polyphenols/Tannins
	Protein		Phyto-estrogens
	Amino acids (Table 2)		Essential oils
	Fat		Herbal compounds
	MUFA		Organic acids
	PUFA (n-6)		Probiotics
	PUFA (n-3)		Prebiotics
Vitamins			
	A (Carotenoids)		
	B6	Other	Antibiotics
	B12		Mycotoxins
	C		Dioxins
	D		
	E		
	Folic acid		
Minerals			
	Cu		
	Fe		
	Se		
	Zn		
	Many other		
Other			
	Nucleotides		

MUFA: Mono unsaturated fatty acid, PUFA: Poly unsaturated fatty acid

It has long been established that the nutritional status influences immunity, and a host of different feed components have been implicated in this process. An important distinction needs to be made between malfunction of the immune system because of (simple) deficiencies, and the purported enhancement or modulation of the immune system above the mere nutritional requirements by certain compounds, the so-called functional feeds. Furthermore, other compounds such as toxins, chemicals and pharmaceuticals may be inadvertently present in feed, also exerting their effects on the immune system.

Protein / energy malnutrition is known to be major cause of immune deficiency in humans, and has the same effect in animals. Deficiencies in amino acids, (essential) fatty acids, vitamins and minerals have a similar negative effect on the immune system. Whereas there is in general at least some idea about the daily minimum requirement, there is very little knowledge (if any), about the optimum dosages for each component, and especially when combining them in feed. This is particularly true for the micronutrients because, although it is known that selected vitamins and minerals influence each other's bioavailability on a one to one basis, it is much less clear what happens in a complex mixture such as feed. Furthermore, caution should by taken when administrating (micro)nutrients since unpredicted adverse effects may occur (Prentice, Ghattas and Cox, 2007). Finally, requirements can differ considerably depending on the species and on the physiological state, i.e.; during disease, lactation, pregnancy etc.

FUNCTIONAL ASPECTS

It is hard to find a feed component without any physiological effect beyond the mere nutritional value. Therefore, the current section will only consider a selection of the more important components having a possible impact on immunity. Concerning carbohydrates, the most relevant for feed are probably the non-digestible poly- and oligosaccharides (prebiotics). Proteins are of interest since several have possible bioactivity when ingested. Apart from several candidates in plants, plasma and milk contain peptide hormones such as leptin, insulin, ghrelin and insulin-like growth factor-1 (IGF-1) with immunological function. Whether these really retain their activity *in vivo* is much less certain. Furthermore, the digestion of proteins could yield peptides with possible bioactivity, and amino acids and their derivatives. Whereas there is still limited *in vivo* evidence for actual bioactivity of peptides when feeding whole protein, there is much more evidence about a role for amino acids and derivatives.

Amino acids and derivatives (Table 2)

Table 2. Major functions of amino acids (modified after Li *et al*., 2007)

AA	Products	Major functions
Alanine	Alanine	Stimulation of lymphocyte proliferation, enhancement of antibody production
Arginine	NO	Signaling molecule; killing of pathogens; regulation of cell metabolism and cytokine production; immunity
Cysteine	Taurine	Antioxidant
Glutamate	GABA	Neurotransmitter; inhibition of T-cells and inflammation
Glutamine	Glutamine	Upregulation of immune cell metabolism and function
	Glu, Asp	Neurotransmitters; cell metabolism
	Glucosamine	Glycoprotein and ganglioside formation; inhibitor of NO synthesis
Glycine	Serine	Ceramide and phosphatidylserine formation
Histidine	Histamine	Allergic reaction; vasodilator; gastric acid & central acetylcholine secretion
Leucine	HMB	Inhibition inflammation, enhancement specific immunity (1)
Lysine	Lysine	Regulation of NO synthesis; antiviral activity; ketogenesis; collagen crosslinks (lysine or hydroxylysine)
Methionine	Homocysteine	Oxidant; inhibitor of NO synthesis
	Betaine	Methylation of homocysteine to methionine
	Choline	Synthesis of betaine, acetylcholine and phosphatidylcholine
	Cysteine	Glutathione synthesis, production of H2S
Phenyl-alanine	Tyrosine	Synthesis of bioactive substances regulating neuronal function and cell metabolism
Proline	H_2O_2	Killing pathogens; intestinal integrity; a signalling molecule; immunity
	P5C	Cell proliferation; ornithine formation; gene expression;
Serine	Glycine	Antioxidant; neurotransmitter; immunomodulator
Threonine	Threonine	Synthesis of mucin protein intestinal integrity; immunity
Tryptophan	Serotonin	Neurotransmitter; inhibition of inflammation
	Melatonin	Bio-rhythms; free radical scavenger; antioxidant
	ANS	Inhibiting production of proinflammatory cytokines; enhancing immunity
Tyrosine	Dopamine	Neurotransmitter; control of behaviour, immune response
	EPN, NEPN	Neurotransmitters; glycogen and energy metabolism
	Melanin	Free radical scavenger; inhibition of inflammation
Arg, Met	Polyamines	Gene expression; DNA and protein synthesis; antioxidants; cell function, proliferation and differentiation
Arg, Met, Gly	Creatine	Energy metabolism (muscle, nerve); antioxidant; antiviral
Arg, Pro, Gln	Ornithine	Glutamate, glutamine and polyamine synthesis
Cys, Glu, Gly	Glutathione	Free radical scavenger; antioxidant; formation of leukotrienes; immunity

Table 2. Contd.

AA	Products	Major functions
Gln, Asp, Gly	Nucleic acids	Genetic information; gene expression; cell cycle and function
Gln, Glu, Pro	Citrulline	Free radical scavenger; arginine synthesis
Gln, Trp	NAD(P)	Coenzymes for oxidoreductases
Lys, Met, Ser	Carnitine	Oxidation of LCFA, storage of energy as acetylcarnitine; glucocorticoid-like function in immunity (2)
Ile, Leu, Val	Glutamine	Upregulation of immune cell metabolism and function

1. Buyse *et al*., 2009, 2. Buyse *et al*., 2007.
ANS: anthranilic acid, EPN: epinephrine, GABA: gamma-amino-butyrate, HMB: beta-hydroxy-beta-methylbutyrate, LCFA: log-chain fatty acids, NEPN: norepinephrine, P5C: pyrroline-5-carboxylate

For a long time, amino acids have been considered simply as building blocks. More recently, it has become clear that amino acids and their derivatives have an important role in immune responses by regulating immune cell function. Furthermore, several amino acids are also precursors for neurotransmitters, linking the nervous system with the immune system. Amino acids have been predicted to be good candidates for neutraceuticals, although not much is known about the molecular mechanisms that regulate the action of amino acids on immune cell function (Li, Yin, Li, Kim and Wu, 2007). A lot of data were derived from what can be best described as deficiency studies, and hence it is not always known whether feeding an increased amount of a particular amino acid will lead to an increase in the levels of the desired functional derivative, or enhancement of the associated function. Finally, as with other active compounds, beneficial as well as adverse effects can be anticipated depending on the dosage. In most cases the association with the immune system or actual health is not always clear. Nevertheless, there is clear evidence for at least three compounds that do influence immunity. Initially it was demonstrated that dietary inclusion of tryptophan can indeed reduce stress (Koopmans, Guzik, van der Meulen, Dekker, Kogut, Kerr and Southern, 2006), and inflammation associated growth retardation (Trevisi, Melchior, Mazzoni, Casini, De Filippi, Minieri, Lalatta-Costerbosa and Bosi, 2009) in pigs. Furthermore, addition of HMB (beta-hydroxy-beta-methylbutyrate) (Buyse, Swennen, Vandemaele, Klasing, Niewold, Baumgartner and Goddeeris, 2009) and carnitine (Buyse, Swennen, Goddeeris, Niewold, Klasing, Baumgartner and Janssens, 2007) to broiler diets affected immunological parameters.

Fat and fatty acids

Fat is involved in the postprandial inflammation in three different ways. First, it can be responsible for the high energy value of a meal. Second, the lipid profile of the meal determines the severity of the postprandial inflammation. The most important lipids in this respect are probably the n-3 and n-6 polyunsaturated fatty acids (PUFA), and in particular the ratio between the two (Margioris, 2009). The n-3 PUFA are anti-inflammatory, whereas the n-6 PUFA are pro-inflammatory. Diets have been reported to have low n-3 to n-6 ratio mainly due to low n-3 levels. Supplementation of the diets with higher ratios leads to better performance in chickens, and to attenuated growth retardation after the inflammatory challenge if not exceeding the optimum dosage (e.g. Korver, Roura and Klasing, 1998). The anti-inflammatory effect of n-3 PUFA was confirmed by microarray studies in pigs showing down-regulation of inflammatory marker genes was associated with the oil fraction of linseed (Jansman, Niewold and Hulst, 2007). Anti-inflammatory activity has also been attributed to short chain fatty acids such as butyrate, a hindgut fermentation product of poly- and oligosaccharidesI that may be instrumental in certain effects of prebiotics. Third, fatty acids can induce cholecystokinin (CCK), which plays a role in the anti-inflammatory reflex.

Miscellaneous compounds (See Table 1)

These comprise a group of compounds which can be categorized as non-essential and functional feed components. They modulate the immune system, and anti-inflammatory effects have been demonstrated for polyphenols (Chen, Li and Wang, 2006) and essential oils (Marsik, Kokoska, Landa, Nepovim, Soudek and Vanek, 2005). These effects are not always reproducible *in vivo*, which may have been caused by the other feed components present in the different studies, and the different dosages. A similar variability in results is seen with pro- and prebiotics, and even adverse reactions have been described. Dose-dependent deleterious effects of mycotoxins (e.g. Tiemann and Dänicke, 2007), and dioxins (Rhind, 2002) on the immune system have been documented. Furthermore, the beneficial effect of antibiotics on growth has been demonstrated frequently, most probably due to the known direct (non-antibiotic) anti-inflammatory effect of antibiotics on immune cells (Niewold, 2007).

Probiotics and prebiotics

Probiotics are defined as living organisms that are beneficial to the health of the host. There is a wide variety of organisms and strains available that are not

necessarily similar. Results in terms of growth and health vary widely even within strains, making it very hard to draw firm conclusions. It may very well be that there are effective probiotics to combat infections in certain species. However a fundamental point is that some probiotics have been advertised as enhancers of host resistance. The growth retardation seen in piglets when fed with certain probiotics (SCAN, 2000) is consistent with this. Furthermore, microarray studies in pigs demonstrated up-regulation of intestinal inflammatory marker genes associated with a *Lactobacillus* strain (Gross, van der Meulen, Snel, van der Meer, Kleerebezem, Niewold, Hulst and Smits, 2008). It is concluded that probiotics are more likely to have a role in preventing and combating infections than as growth promoters in their own right.

Prebiotics are non-digestible poly- and oligosaccharides that are used to stimulate the growth of beneficial bacteria in the hindgut. There is surprisingly little evidence about whether these bacteria are indeed beneficial or not, and at which levels they should be present. As with probiotics, results in terms of growth and health vary widely, probably also because of the wide variety of sources of prebiotics, the chemical type and concentrations used, and the experimental protocol. In contrast to probiotics, however, there is at least one possible mechanism for a positive effect of prebiotics on immunology and growth. Fermentation products such as butyrate have been described to have *in vitro* anti-inflammatory properties (Park, Lee, Lee, Kim and Kim, 2007). Whether this is really the case in vivo is still under investigation. What is already clear is that the dosage of prebiotics is very important, adverse effects on health and growth can occur at relatively low inclusion rates (e.g. 2%), and in a rat model, the fructo oligosaccharide inulin appeared to increase translocation of Salmonella (Rodenburg, Keijer, Kramer, Roosing, Vink, Katan, van der Meer and Bovee-Oudenhoven 2007).

Hormones (Table 3)

Many publications have focused on the endocrine and immune system as two separate systems. Recent research has shown that both systems are intimately connected, and that many hormones have pleiotropic actions in both systems (Ahima and Lazar 2008, Ganjavi and Shapiro, 2007). Table 3 summarizes the current knowledge of immune functions of selected hormones (partly based on Renaville and Renaville, 2007). Of considerable interest in the context of this paper are the hormones which levels respond to feed, the adipokines, ghrelin and cholecystokinin (CCK). This means that the immune response can be modified through feed composition. CCK is a very interesting example. In recent years, it became clear that in contrast to the often suggested need to enhance intestinal defences, the organism rather benefits from down regulation of the intestinal

Table 3. Major functions of different types of hormones (After Renaville and Renaville, 2007)

Hormones	
Growth hormone	Stimulates growth, gluconeogenesis etc. Growth hormone (GH) acts either direct or indirect via insulin-like growth factor (IGF-I). GH administration improves weight gain.
	Immunology: GH is important for the development and function of the immune system. It increases NK-cell, neutrophil and macrophage activity, lymphopoiesis, and granulopoiesis.
Insulin-like growth factor I	Stimulates growth in concert with GH.
	Immunology: The IGF-I stimulates the proliferation and/or numerous functions of lymphocytes B and T which have an important role in immunity. Proinflammatory cytokines also suppress IGF-I resulting in growth impairment.
Prolactin	Prolactin(PRL) is mainly secreted by the anterior pituitary gland, contributing to the control of over 300 functions in vertebrates. The majority is related to reproduction.
	Immunology: Has a key role on immune system by acting as an immunostimulatory cytokine. Lymphoid cells produce PRL especially after activation. PRL plasma levels are directly related with NK cells cytolytic axtivity.
Adipokine system	Adipose tissue produces a host of hormones (adipokines such as leptin, resistin, adiponectin) involved in the regulation in wide range of complex processes, such as fat metabolism, feeding behaviour, energy balance and immunology.
Leptin	Adipokine, anorectic, after fat storage. Has a key role in the regulation of the energy balance. The biological action is not restricted to its effects on appetite and food intake, but has a pleotropic action including reproduction, hematopoiesis, hypothalamo-pituitary-adrenalin axis endocrinology, specific and innate immunity.
	Immunology: Leptin is considerd as a pro-inflammatory cytokine having structural similarity with IL-6, IL-12 and IL-15. Leptin receptors are expressed in all cell types of innate and adaptive immune cells. Leptin is believed to bias the immune system toward a proinflammatory rather than anti-inflammatory phenotype.
Adiponectin	Adipokine, anorectic.
	Immunology: Adiponectin has an anti-inflammatory effect in various diseases, suppressing the production of pro-inflammatory cytokines TNF and IL-6, and inducing various anti-inflammatory cytokines, such as IL-10.

Table 3. Contd.

Hormones	
Resistin	Adipokine, resistin is produced by adipocytes in rodents but blood monocytes are the major source of human resistin. Named for its relationship with insulin resistance.
	Immunology: resistin has a proinflammatory function up-regulating IL-6 and TNF.
Ghrelin	Orectic, the stomach is the major source of peripheral ghrelin. Ghrelin stimulates GH secretion and food intake. The wide distribution of ghrelin receptors throughout several major organ systems including the immune system suggest additional functions such as an immunoregulatory one.
	Immunology: During chronic inflammation, ghrelin inhibits and leptin increases inflammatory cytokines as a direct consequence of reduced ghrelin and increased leptin levels in the circulation. Ghrelin inhibits expression of the proinflammatory cytokines IL-1 IL-6 and TNF. The loss of appetite or anorexia associated with inflammation and illness is believed to be mediated through proinflammatory cytokines (IL-1 , 1L6 and TNF) under the control of ghrelin and leptin balance.
Cholecystokinin	Produced in the small intestine, anorectic after fat consumption.
	Immunology: CCK can down regulate macrophage function either directly, or through the nervus vagus, thus exerting an anti-inflammatory effect (Luyer *et al*, 2005).

inflammatory responses. The nervous anti-inflammatory reflex plays a pivotal role in the control and containment of the intestinal defence system, and is therefore essential for health and survival of the individual (Tracey, 2002). In vitro research shows the tissue macrophage to be central in this process. Excretion of pro-inflammatory cytokines by stimulated macrophages can be down regulated by acetylcholine from cholinergic neurons of the nerves vagus, and nutritionally induced CCK modulates the nervus vagus response (Luyer, Greve, Hadfoune, Jacobs, Dejong and Buurman 2005). Further nervous modulation occurs by (nor) adrenalin (sympathic neurons), and by substance P (pain fibres; up regulation). Apart from this reflex, a slower modulation occurs via the circulation by anti-inflammatory hormones and cytokines (e.g. glucocorticoids, IL10). Although it is clear that the interaction between neurons and macrophages is pivotal, also other cell types are crucially involved (e.g. epithelium, endothelium, and leucocytes (Metz and Tracey, 2005)). CCK is most effectively induced by fatty acids (Luyer *et al*., 2005), which may explain the beneficial effects of the latter.

Mechanisms

Feed components can work either directly or indirectly. Exogenous factors such fatty acids can influence the immune system either directly, or indirectly through endogenous factors such as hormones (e.g. via CCK). Prebiotics are most likely to work indirectly by the fermentation product butyrate. Concerning antibiotics, most evidence points towards a direct inhibitory effect on inflammatory cells (Fig. 2).
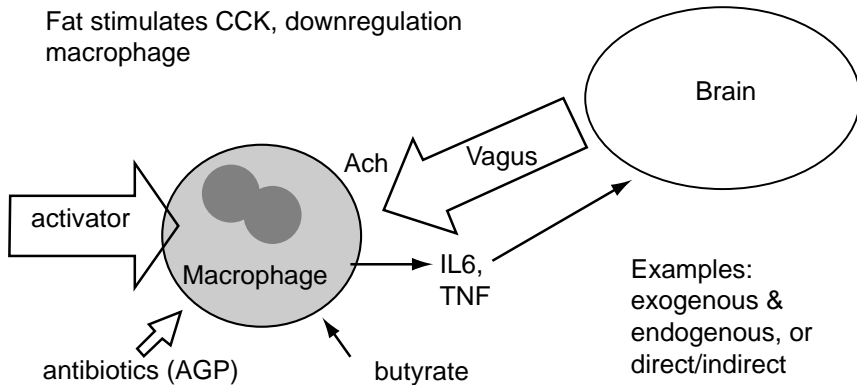
Figure 2. The anti-inflammatory reflex, and how it can be influenced. Inflammatory cells can be activated, and produce pro-inflammatory cytokines (e.g. IL6, and TNF) which reach the brain. The inflammatory response is subsequently down regulated by acetylcholine (Ach) from cholinergic neurons of the nervus vagus. Exogenous factors can help down regulate either directly (e.g. antimicrobial growth promoters (AGP) or indirectly through endogenous factors such as hormones (e.g. CCK), or through fermentation products such as butyrate.

Conclusions

Stress is described to modulate immunity, and in general is thought to stimulate inflammation, and to weaken the specific immune response (Irwin, 2008). However, stress has also been found to either weaken or enhance various parts of the immune reaction (Boersma, van der Meulen and Niewold, 2009). The variation in experimental designs in the literature makes it very hard to establish whether these results are indeed conflicting or not. Furthermore, it is very hard to translate in vitro functional cell studies to in vivo health, and it is often equally hard to translate immune parameters measured in vivo to health. Anyway, as is demonstrated in this paper, since feed and immunity and the nervous system are interconnected, stress should be open to what Field, Johnson and Schley, 2002 call "nutrient-directed management of immune-related syndromes". However, as

long as the physiological and immune effects of stress on health are not always entirely clear, it is at present still difficult to unravel the exact mechanisms involved. The enormous complexity of the interactions requires research techniques which can cope with such a complexity, and real detailed insight is possibly only to be expected by application of genomic (microarray) analysis (Niewold, 2006). Finally, but most important, a lot of studies in the area of feed (and food) are empirical trials often build on an inadequate theoretical basis. A high priority for fundamental research remains (Klasing 2007, Prentice *et al.*, 2007).

What is also clearly needed are reliable physiological and immunological markers. What is available is markers which indicate a biological response, although it is not always known what the response means in terms of health and growth. For example, certain products claimed to increase intestinal health and resistance indeed change immunological parameters such as interleukins in the intestine. Whereas it is clear that these parameters are involved in immunology, and hence defence, it is entirely unclear whether they really translate into improvement or deterioration in animal health and growth, or whether they don't make a difference at all (Niewold, 2008). What is evident however, is that inflammation is central in stress, disease and growth retardation. There are useful markers of inflammation, not only on the intestinal level such as PAP (Niewold, Kerstens, van der Meulen, Smits and Hulst, 2005, Gross *et al.*, 2008), but also systemically, the acute phase proteins (Gruys, Toussaint, Niewold, Koopmans, van Dijk and Meloen, 2006). By measuring plasma levels of acute phase proteins postprandial inflammation (Margioris, 2009) and inflammation in general can be quantified. Inflammation whether or not it results from stress or disease is inversely related to growth (Korver *et al.*, 1998, Niewold, 2007). Therefore, research in feed should focus on anti-inflammatory compounds and anti-inflammatory feed composition.

Acknowledgement

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10

POTENTIAL APPLICATIONS OF GM TECHNOLOGY

GREG TUCKER

Division of Nutritional Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK

Introduction

Products from genetically modified (GM) organisms, and indeed the organisms themselves, have been used commercially for several decades as processing aids within the food industry. The first genetically modified organisms, given regulatory approval for inclusion in the food chain, were introduced into commercial trade in the 1990s. These were a tomato modified for better shelf life and processing qualities (Schuch, Kanczler, Robertson, Hobson, Tucker, Grierson, Bright and Bird, 1991) and soyabean that had been modified for resistance to the herbicide glyphosate (Padgette, Kolacz, Delannay, Re, Lavallee, Tinius, Rhodes, Otero, Barry, Eichholtz, Peschke, Nida, Taylor, Kishore, 1995). This was followed by the introduction of maize which had been modified for insect resistance (Koziel, Beland, Bowman, Carozzi, Crenshaw, Crossland, Dawson, Desai, Hill, Kadwell, Launis, Lewis, Maddox, McPherson, Meghji, Merlin, Rhodes, Warren, Wright and Evola, 1993). Since then, this technology has consistently developed and about 30 different commercialised GM crops are currently being grown, quite extensively worldwide (Stein and Rodriguez-Cerezo, 2009). The technology has been the subject of intense controversy, however, with a significant consumer backlash, especially within the EU countries. Although the acreage of GM crops continues to grow year on year, the number of applications for which this technology has been targeted has remained relatively small, with the vast majority of crops having been modified for either herbicide or insect resistance; this despite the fact that fundamental scientific research has in the meantime established a multitude of additional potential applications. It has been predicted that the use of GM crops is likely to expand in the future so now is a good time to consider the drivers for

the potential expansion of this technology-food security, sustainability, healthy diet- and also the potential timeline for introduction of these novel GM organisms, and the types of traits that may be targeted. It has been estimated that by 2015 the number of commercialised GM crops may have reached 120 (Stein and Rodriguez-Cerezo, 2009). The vast majority of these new applications will be new GM crops, although micro organisms and perhaps even applications in animals may also be included. It is also relevant to note that most if not all of the currently approved GM plants within the EU are approved for use as animal feeds. This is likely to be the case for new introductions as well, at least in the near future.

Current status of GM crops in the EU

Genetically modified crops began to be grown commercially around 1996. Worldwide cultivation in 1996 was only around 2 million hectares, but this has increased consistently over the intervening years until in 2008 it stood at about 125 million hectares (GMO Compass, 2009, Stein and Rodriguez-Cerezo, 2009). Uptake of the technology has not been universal across all regions, but 25 countries across the world have some cultivation of GM crops; there are probably around 30 commercialised GM "events" which are grown extensively and several others with more localised production (Stein and Rodriguez-Cerezo, 2009). The bulk of production resides in North and South America, with the USA thought to contribute about 62.5 million hectares and Argentina and Brazil between them about 37 million hectares (GMO Compass, 2009). Other major sources of GM crops are India, Canada, Paraguay and South Africa. In comparison to these other countries, cultivation of GM crops within the EU has been extremely low, with around only 0.1% of global GM production being associated with the EU (Stein and Rodriguez-Cerezo, 2009). This does not mean that utilisation of GMOs in food and feed has been similarly restricted in the EU. In fact, although few GMOs have been approved for cultivation within the EU, use of imported GMO in both food and feed is widespread. The reticence of EU countries towards GM crops may be largely due to resistance from several NGOs and the public.

There has always been a requirement for GMOs entering commercial trade to be subject to scrutiny and approval by the relevant national regulatory bodies. In the EU this process is co-ordinated by the European Food Safety Authority (EFSA). All GMOs with applications in the food or feed area need to be submitted for approval under regulation (EC) No 1829/2003. This regulation has been designed to have a single risk assessment for all GMOs within the EU and is centralised within the EFSA. Final approval is actually given by the EC and this is carried out after consideration of the final opinion of the EFSA and requires a validated event-specific detection method, along with the availability of certified reference

material. Once approved, details of the GMO are added to the Community Register of Genetically Modified Food and Feed. This has public access and can be viewed through the internet at:

http://ec.europa.eu/food/dyna/gm_register/index_en.cfm.

This site thus lists details of all currently approved GMOs within the EU. As of July 2009, this list contained 27 approved GMOs for use in animal feeds. All but two are GM crops, the other two being micro organisms that have been approved for use as protein sources. In relation to the approved GM plants, most approved applications are for maize (12), followed by cotton (6), oil seed rape (3), soyabean (3) and sugar beet (1). In all cases the applications are either for herbicide or insect resistance, or in some cases organisms in which transgenes conferring these two independent traits have been "stacked" together.

The list of approved GMOs, and their applications within the EU, matches quite closely with the general commercial application of this technology worldwide. A recent report by the EU Joint Research Centre (JRC) recorded the major commercial GM crops as of early 2009 (Stein and Rodriguez-Cerezo, 2009); a summary of their findings for soyabean, maize and rapeseed is shown in Table 1. Similarly, a survey by the New Zealand Ministry of Agriculture and Food (MAF) of "authorised" GM plants in 2007 showed that there were about 64 independent "events", the majority in maize (26) with cotton (13), oil seed rape (6) and soyabean (4) constituting the other major GM crops. Other GM crops included tomato, sugar beet, lucerne and rice. (MAF Biosecurity, New Zealand, 2007)

The approved applications within the EU, also reflect those prevalent in the worldwide application of this technology with three - herbicide resistance (24), Insect resistance (10) and stacked (herbicide and insect resistance combined 19) - accounting for the vast majority of applications. Viral resistance (4) and modification of composition (3) were two of the other applications.

There is a legal requirement, within the EU, to declare the presence of any GMO in food or animal feed. For any GMO on the approved list this declaration must be made if the content of the GMO exceeds 0.9%. It is permissible to use non-approved GMOs, providing that they have a favourable risk assessment associated with them. In this case, however, presence of the GMO at levels above 0.5% must be declared on the label. This requirement is event specific, i.e. it relates to a very specific GMO. For example whilst there may be several commercial maize varieties which have been modified with exactly the same gene, to confer say herbicide resistance, these will have been produced independently and the "transgene" is most likely to have inserted into the host DNA in different places. These are referred to as separate "events" and are treated as completely separate crops for regulatory purposes. Thus, whilst many GMOs may contain the same, or

similar transgenes, any detection method must be able to clearly differentiate them. This will be of importance later when considering detection of GMO material. It is important to note that whilst these legal requirements apply to material that is to be used for animal feed there is no legal requirement to label livestock, or products from livestock, that have been fed GM material. This has given rise to concern in some areas of society.

Table 1. Current (2009) commercialised GM soyabean, maize and rapeseed "events" worldwide.

Commodity	Developer	Product name	Trait	Unique Identifier
Soyabean	Monsanto	Roundup Ready	Glyphosate tolerance	MON-04032-6
Maize	Monsanto	Yield Gard Corn Borer	Insect resistance	MON-00810-6
Maize	Monsanto	Roundup Ready Corn 2	Glyphosate resistance	MON-00603-6
Maize	Monsanto	Yield Gard Root worm	Insect resistance	MON-00863-5
Maize*	Monsanto	Yield Gard VT	Insect resistance	MON-88017-3
Maize	Dow / Pioneer	Herculex 1	Insect resistance	DAS-01507-1
Maize	Dow/Pioneer	Herculex RW	Insect resistance	DAS-59122-7
Maize	Syngenta	Agrisure CB	Insect resistance	SYN-BT011-1
Maize	Syngenta	Agrisure GT	Glyphosate tolerance	MON-00021-9
Maize*	Syngenta	Agrisure RW	Insect resistance	SYN-IR604-5
Rapeseed	Monsanto	Roundup Ready	Glyphosate tolerance	MON-00073-3
Rapeseed	Bayer	Liberty Link	Glufosinate tolerance	ACS-BN008-2
Rapeseed	Bayer	InVigor	Glufosinate tolerance and male fertility	ACS-BN005-8 x ACS-BN003-6

*Until early 2009, event not yet authorised in the EU.
Data from (Stein and Rodriguez-Cerezo, 2009).

Basic science behind herbicide and insect resistant crops

Given that the two most prevalent current applications are herbicide and insect resistance, it would be useful to go through the basic science involved in their generation.

Firstly, genetic modification of plants has become relatively straightforward and is a lot easier today than in 1996. The major hurdles to successful modification of a plant are firstly to identify the gene(s) controlling or affecting the trait to be modified. This is often a complex process and outside the scope of this review. Once the "transgene" has been identified, and isolated, the next hurdle is to

transfer this to the host plant in such a way that the gene functions "normally", expressing mRNA and eventually the functional protein. This requires addition of a suitable promoter to control gene expression and in this area great advances have been made since the early days of genetic modification. Many early GMOs contained genes that were controlled by a "constitutive" gene promoter such as the CaMV35S promoter from the Cauliflower mosaic virus which was thought to function in all plant tissues. Nowadays a wider range of more sophisticated promoters are available, and whilst CaMV35S may still be used, the industry now has at its disposal genes that can confer tissue specific expression of the transgene. For example, promoters could be chosen to ensure that expression occurs in leaf tissue but not in fruit, or that expression could be induced by stress conditions such as in response to drought.

The transgene is often introduced into the host cell by a vector, a commonly used vector being the Ti plasmid from Agrobacterium. This process is often inefficient and as such requires a marker to enable the researcher to identify and isolate those cells which have been successfully transformed. Early GMO technologies often used genes conferring resistance to antibiotics, such as kanamycin, or herbicides for this marker. This has led to concern over the safety of this technology. Current legislation prevents the inclusion of such markers in new GMOs. The efficiency of the transformation process has, in many cases, been improved over the years to such an extent as to make the use of a marker redundant. Alternatively, genetic technologies can be employed to remove the marker gene from the successful transformant once identified.

The aim of herbicide resistant crops was, in the first instance, to provide resistance to the herbicide glyphosate (N-phosphonomethyl-glycine) the active ingredient in the non-selective herbicide Roundup. This herbicide inhibits 5-enolpyruvylshikimate-3-phosphate synthase, the first enzyme in the biosynthesis of the aromatic amino acids – tryptophan, tyrosine and phenylalanine. Several strategies have been examined to achieve this aim, including over expression of the target enzyme. However, one strategy that has proved very effective was the identification of a bacterial gene that encoded a herbicide resistant form of the target enzyme. This bacterial gene was transferred to the plant under the control of the CaMV35S promoter and expression of this new protein induced resistance to the herbicide (Padgette *et al.*, 1995). This strategy, of expressing herbicide resistant enzymes, has been further refined over the years.

A second approach to generating herbicide tolerant plants is exemplified by the LibertyLink plants developed to exhibit tolerance to glufosinate. This herbicide inhibits the enzyme glutamine synthetase and as such results in the build up of toxic levels of ammonia within the plant. In this case a bacterial enzyme encoding another enzyme -phosphinothricin acetyl transferase- is used to genetically modify the crop. This enzyme is able to metabolise the glufosinate herbicide into

a non-lethal product. The expression of this enzyme within the plant thus confers herbicide resistance (Hinchee. Padgette, Kishore, Delannay and Fraley, 1993).

In the case of insect resistance, proteins from bacteria such as the Cry1Ab from *Bascillus thuringiensis* were employed (Koziel *et al.*, 1993). This is a member of a large group of proteins, many of which have been used in GM plants, which on ingestion by the insect degrade in the gut to form a toxin. There is no evidence that this class of protein has any toxicity in relation to humans. The strategy in this case was to transfer the gene encoding the Cry1Ab protein to the host plant, again under the control of either the CaMV35S or more tissue specific promoters, and thus confer resistance to insect attack, in the case of maize specifically the European corn borer (Koziel *et al.*, 1993). The Cry1Ab protein confers resistance to lepidopteran insects such as the corn borer, another commonly employed protein is the Cry3Bb1 which confers resistance to coleopteran insects.

Perceived problems with GM technology limiting exploitation

Although genetic modification has been used experimentally to generate literally thousands of GM plants with a host of altered traits, commercialisation of these has been relatively limited. This is due in part to the perceived safety and health risks that might be associated with these crops. This review will not attempt to cover the arguments for or against such claims. It will simply examine the impact such perceptions (be they true or false) may have on the future uptake of this technology within the agricultural sector, especially in terms of animal feed.

Concerns raised relate to environmental risk, animal welfare or human health (Weaver and Morris, 2005). It is beyond the scope of this review to consider these concerns in relation to GM in general. In relation to the use of GM animal feed two major concerns have been voiced - the potential impact on animal welfare of long term feeding of GM feed and whether subsequent consumption of these animals, or products derived from them, have any adverse effects on human health.

The use of GM feed for livestock has been both common and widespread over the last 10 to 20 years and there is no evidence, as far as the author is aware, to suggest that this has had any adverse impact on animal health or welfare. The primary safety assessment for GM foods is normally a compositional analysis, to assess substantial equivalence. However, feeding studies in laboratory animals are also often carried out to complement compositional analysis (Delaney, 2007). Many feeding trials have thus been carried out to examine the impact of feeding GM material on animal health and metabolism. Although some of these laboratory studies claim to have seen effects from long term feeding of GM material, many others have concluded that there was no observable difference between animals

fed GM as opposed to conventional feed. At the moment, therefore, there is no convincing body of scientific evidence to suggest that feeding GM material to animals has any significant long term impact on either their health or welfare.

The potential impact of GM fed livestock on human health is much clearer. For a GMO to have a direct impact on human health would require the GM-fed animal to either, acquire and express a functional transgene, or to accumulate the protein product of the transgene to pass onto the human in the food chain. In both cases there is very strong evidence to show that this is extremely unlikely to occur. Firstly, processing of the feed is very likely to degrade or denature proteins and to cause DNA to fragment. Secondly, during the process of digestion, within the gastrointestinal tract of the animal, the majority of protein and the DNA will be digested into small fragments. The majority of protein can only be absorbed across the intestine as amino acids, or as small fragments, and these would be subject to further metabolism once absorbed. Similarly nucleic acids are only absorbed as small fragments and are again subject to further degradation in the body. It is thus very unlikely that any functional protein or DNA would survive this digestive process.

There is no reason to assume that the transgene, or the protein product (which is often nature identical), should behave any differently to the rest of the dietary DNA and protein. There is no evidence for any recombinant DNA sequences appearing in the tissues or fluids of animals fed GM material. There is some evidence for trace amounts of fragments from high copy number DNA from plants (chloroplast DNA) in some animal fluids, but these are small nucleotide fragments and by no means a fully functional gene. There is no evidence at all for fragments of plant DNA being incorporated into the animal genome and since the transgene should be no different to the bulk of the plant DNA this would apply to recombinant DNA as well as conventional DNA. The evidence for transmission of recombinant DNA has been fully evaluated by the EFSA and they have made the following unequivocal statements on the fate of GM material in animal feed:

(1) Biologically active genes and proteins are common constituents of foods and feed in varying amounts. After ingestion, a rapid degradation into short DNA or peptide fragments is observed in the gastrointestinal tract of animals and humans.

(2) To date, a large number of experimental studies with livestock have shown that recombinant DNA fragments or proteins derived from GM plants have not been detected in tissues, fluids or edible portions of farm animals like broilers, cattle, pigs or quails.

(European Food Safety Authority, 19th July 2007, http://www.efsa.europa.eu)

Detection of genetically modified organisms

Practical concern within the feed industry lies with the detection of genetically modified plant material. There have been several reviews on the methodologies for detection of GMO in food and feed (Bardsley and Tucker, 2004; Marmiroli, Maestri, Gulli, Malcevschi, Peano, Bordoni and De Bellis, 2008). Most, if not all, of the current detection methods rely on monitoring the DNA content within the feed and, although DNA can be degraded during processing, it is not fully destroyed and short fragments persist which can be employed in subsequent detection systems.

Before considering the methodologies used to detect and quantify GMOs, it is useful to describe the general nature of rDNA. This consists of several elements; firstly a promoter (required to direct expression of the transgene within the plant tissue); secondly, a coding region which contains the genetic information used for the synthesis of RNA and eventually the protein; and lastly a terminator region. The structural gene obviously varies between GMOs because it confers the trait of interest. However, there may be several different GMOs available which employ the same structural gene, for example bt11 and bt176 maize, which are two separate GMOs but both have the Cry1Ab structural gene that encodes for the bacterial toxin that confers insect resistance onto these crops. These are considered as distinct varieties and the genetic modifications referred to as separate "events".

There are two requirements of a detection methodology. Firstly to identify the actual variety(ies) or "events" of GMO present to check that they comply with those allowed under EU regulations and secondly to quantify the level of GMO to comply with labelling regulations. The usual approach to provide "event" specific identification of the GMO is to utilise PCR primers targeted to the insertion junction; one primer is specific to a region within the rDNA, the other to a region of the host genome immediately adjacent to the site of insertion. Because it is extremely unlikely that the rDNA will insert in the same place in separate transformation events, this approach is highly specific. One disadvantage is that the site of insertion of the rDNA must be mapped. With current advances in molecular biology this is becoming much easier.

A straight PCR followed by gel electrophoresis of the product can be used for detection of GMOs. The final level of sophistication is quantification of the GMO content. The most reliable current technique is Real-Time PCR and this has become the current method of choice. In order to quantify accurately there is a need not only for rDNA specific primers but also for species-specific targets with which to compare. These include endogenous genes like invertase and lectin. Methods for accurate quantification of several event-specific GMOs have been described and many of these are commercially available. It is often necessary to quantify levels of several different GMOs within the same raw material and to this

effect multiplex PCR is most useful. Again there are several published methods as exemplified by that of Huang and Pan (2004) who have described a multiplex Real-Time PCR for the simultaneous detection of insect-resistant maize MON810 and herbicide-tolerant maize NK603.

Future potential applications

The use of GM material within agriculture will continue to expand in the future. However, the range of crops involved, or indeed whether this technology is deployed in say the modification of animal species, relies on the drivers –economical and social - influencing agriculture in general. The same applies to traits that may be targeted by GM in the future; the drivers are diverse and often interactive. A major driver will be the need to improve agricultural productivity. The demand for food is expected to double by 2050, due to the combined effects of population growth and an increasing expectation of better lifestyles from much of the population in the developing world. A significant part of this is likely to arise from greater consumption of meat and other animal products in these countries. This in turn will require development of more efficient livestock production methods, including more efficient production of the basic animal feed stocks. This is likely to be complicated by an increase in land area devoted to the production of biomass for bioenergy. This has already resulted in some competition between food and energy use for grain stocks. It is likely that the use of grain for biofuel production will decline considerably in the future as the so called "second generation" bioenergy crops come on stream. These will use either dedicated bioenergy crops, such as *Miscanthus*, or agricultural by-products such as straw. Adoption of these second generation biomass sources may reduce direct competition for grain, but there is still a potential for competition for land use. These drivers, along with the response of crops to climate change, are all influencing the current important debate on food security. Introduction of new agricultural practices and technologies will be needed to meet these challenges, for example use of new practices and development of drought resistant crops to reduce water use. Application of GM has great promise in many of the key areas that need to be addressed, and although GM alone will not solve the problems, it is likely to play a significant role.

Acceptance of GM by the public, especially within the EU, is likely to remain as a hurdle to its further exploitation. As stated earlier, GM crops have been grown extensively over the last 10 years and to date there has been no evidence to suggest that this has affected significantly either the environment or human health. However, consumer perception of this technology has been tainted by the controversies during its introduction 10 to 15 years ago and it is likely to take a generation (25 years) for this perception to erode. One obvious way to improve

public acceptance of this technology would be to use it for the clear benefit of the consumer. Most applications in the past, and many potential future applications, have been directed at improving agricultural productivity with minimal perceived benefit for the consumer. One obvious driver in respect of consumer benefit would be to target crops for enhanced nutritional value. There are two major areas in which this might be particularly beneficial. The first would be elimination of nutrient deficiencies. The World Health Organisations web site lists three major worldwide nutritional deficiencies - iodine, anaemia (iron) and vitamin A. The second would be a more general concept of enhanced nutritional value in terms of, for example antioxidants and vitamins such as folate. Although such a driver, in terms of GM, may be targeted primarily at applications within the human diet, this may also have impact and use within the animal feed industry. This aspect of GM application will obviously require support from the food industry and the final consideration is obviously whether or not the industry is willing to contemplate introduction of GM products in the current consumer climate.

One way to attempt to predict the more short term (5 year) trend in the application of GM within agriculture is to examine the list of GM products that are currently under consideration by the EC for approval under regulation (EC) No 1829/2003. These are listed under the register of questions and can be found on the EFSA WEB site at:

http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?panel= GMO&questiontype=2

The list, when examined in August 2009, contained 78 relevant applications for the approval of new GM products. Not surprisingly the majority of these were for new "events" within maize (43), cotton (13), soyabean (9), oil seed rape (2) and sugar beet (2). Thus the first obvious thing to note is that the range of crops subject to GM technology is unlikely to expand significantly in the near future. The remaining applications involved six for GM bacteria for inclusion in animal feed, and modifications to potato (2) and Arabidopsis (1). The second thing to note is that the vast majority of applications were for herbicide and insect resistance and as such the range of applications is also unlikely to change considerably. In many cases new introductions relate to "stacked" genes in which herbicide and insect resistance have been combined and where insect resistance is being maintained by "stacking" genes for several of the cry proteins discussed earlier. It is interesting, however, to note that applications also include modifications to composition of crops. This is particularly the case in less common species, for instance starch modification in potato. Compositional modification is likely to be a significant application in the longer term as discussed later.

Another way to assess the likely short term future applications of GM technology is to examine the literature related to commercial plans of the major industrial companies involved in its exploitation. Thus Monsanto have recently committed to the introduction of at least 7 High Impact Technology (HIT) products over the coming years with a timescale of perhaps one product per year.

The most recent introduction from Monsanto is their "Genuity SmartStax corn". This received registration from the US Environmental Protection Agency (EPA) and the Canadian Food Inspection Agency in July 2009, and is on track for commercial release in 2010. This product exemplifies the current general trend in GM crop development, i.e. a combination of traits to enhance productivity. The SmartStax corn combines several well proven technologies. The combination of Dow AgroSciences' proven HERCULEX® Insect Protection with Monsanto's Genuity™ VT Triple PRO™ is designed to provide above-ground insect control, whilst below-ground insect control is provided by a combination of Monsanto's YieldGard VT Rootworm/RR2 with Dow AgroSciences' HERCULEX® RW Insect Protection technology. These pest control traits are further combined with broad-spectrum weed and grass control by adding Monsanto's Roundup Ready® 2 and Bayer CropSciences' LibertyLink® herbicide tolerance.

The major crops to be targeted by this HIT are maize, soyabean and cotton, but sugar beet and canola are also included. The major traits to be combined in these crops are herbicide and insect resistance but also include "weather protection", this being resistance to drought and cold, and also traits for increased productivity. Thus in this instance a clearer expansion of the targeted traits can be observed, but these are all in response to the increase in agricultural productivity driver and not immediately aimed at improving the quality or composition of the crop.

Lists of GM crops, worldwide, which are either in the commercial pipeline (events authorised in at least one country but not yet commercialised), regulatory pipeline (events in the regulatory pipeline for commercialisation in at least one country) or the advanced R&D pipeline have been given in a recent report for the EU (Stein and Rodriguez-Cerozo, 2009). This report covers a wide range of crops that may be targeted for GM applications in the next 5 or so years. A summary of some of these applications in the case of the major feed crops-soyabean, maize and rapeseed is listed in Tables 2, 3 and 4, along with a general prediction of the timeline for their introduction. These tables primarily list those crops in which new GM "events" have taken place. In addition there are a large number of new potential GM crops that represent varieties in which current "events" have been stacked.

A more long term view of potential introductions and applications, within soyabean, has been prepared by the American Soybean Association. In November 2007 they published an estimated pipeline of expected biotechnology events in relation to soyabean, which can be accessed at:

http://associationdatabase.com/aws/SOYOIL/asset_manager/get_file/6324

From these lists it is again evident that the key driver for many of these potential products will be agricultural productivity and these traits again include herbicide, insect, fungal and nematode resistance. However, it is also evident that the list contains a significant number of applications related to changes in quality, especially in terms of compositional modifications, to either assist processing or to enhance nutrition. These latter targets include high omega-3, low phytate and high protein products.

Table 2. Potential new commercialised GM soyabean "events" worldwide.

Pipeline	Developer	Trait	Possible Commercialisation
Commercial	Monsanto	Glyphosate tolerance	Imminent
Commercial	Bayer	Glufosinate tolerance	Imminent
Commercial*	Bayer	Glufosinate tolerance	Imminent
Commercial*	Pioneer	Glyphosate tolerance and ALS inhibitors	Imminent
Regulatory *	Pioneer	High oleic acid	Near future
Regulatory*	BASF	Imidazolinone tolerance	Near future
Regulatory*	China	Insect resistance	Near future
Advanced R&D*	Syngenta	Nematode resistance	2011
Advanced R&D*	Monsanto	Omega-3	2012
Advanced R&D*	Monsanto	Dicamba tolerance	2012
Advanced R&D*	Monsanto	Glyphosate tolerance and insect resistance	2013
Advanced R&D*	Dow	Herbicide tolerance	2013
Advanced R&D*	Monsanto	High oleic acid	2014
Advanced R&D*	Syngenta	HPPD inhibitor- herbicide resistance	2014
Advanced R&D*	Bayer	Herbicide tolerance to HPPD and glufosinate	2015

*Until early 2009, event not yet authorised in the EU. Data from (Stein and Rodriguez-Cerezo, 2009).

Table 3. Potential new commercialised GM maize "events" worldwide.

Pipeline	Developer	Trait	Possible Commercialisation
Commercial*	Monsanto	Insect resistance	Imminent
Commercial*	Monsanto	High Lysine content	Imminent
Commercial*	Syngenta	Amylase content	Imminent
Regulatory*	Syngenta	Insect resistance	Near future
Regulatory *	Pioneer	Herbicide tolerance ALS and glyphosate	Near future
Regulatory*	China	High lysine content	Near future
Regulatory*	China	Insect resistance	Near future
Regulatory*	China	Phytase enzyme	Near future
Advanced R&D*	Monsanto	High oleic acid	2010
Advanced R&D*	Pioneer	Insect resistance	2010
Advanced R&D*	Monsanto/ BASF	Drought tolerance	2012
Advanced R&D*	Dow	Herbicide tolerance	2012
Advanced R&D*	BASF	Protein, amino acid and phytase content	2015
Advanced R&D*	Syngenta	Drought tolerance	2015

*Until early 2009, event not yet authorised in the EU. Data from (Stein and Rodriguez-Cerezo, 2009).

It is more difficult to predict the longer term applications of GM technology. Perhaps a good way to assess this would be to examine applications related to field trials carried out within EU countries. Data from GMO Compass (2009) on the deliberate release of GMOs into the environment for field trials show that the number of trials, within the EU as a whole, peaked at just over 250 in 1997. The number then declined until 2002, since when there has been an increase with just over 100 trials in 2007 and just under 100 in 2008. A total of 66 species have undergone field trials, but the majority of these trials have been carried out in maize (772), rapeseed (379), beet (304) and potato (277). Other plants trialled include tomato (75), rice (35) and wheat (34). Again the major proportion of these trials were targeted towards traits likely to influence agricultural productivity, thus herbicide (47%), insect (16%), fungal (4%) and viral (5%) resistance traits dominate this group. It is interesting that 17% of the trials involved compositional modifications (GMO Compass, 2009).

Table 4. Potential new commercialised GM rapeseed "events" worldwide.

Pipeline	Developer	Trait	Possible Commercialisation
Advanced R&D*	Bayer	Herbicide tolerance	20101-2013
Advanced R&D*	Bayer	Disease resistance	2011-2013
Advanced R&D*	Bayer	Oil content	2014
Advanced R&D*	BASF	Fatty acid content	2013
Advanced R&D*	BASF	Oil content	2015

*Until early 2009, event not yet authorised in the EU. Data from (Stein and Rodriguez-Cerezo, 2009).

The use of GM to enhance nutritional value of a food is not a new concept. There have been several reviews of this area (Tucker, 2002; Davies, 2007; Zhu, Naqvi, Gomez-Galera, Pelacho, Capell and Christou, 2007; Key, Ma and Drake, 2008; Mayer, Pfeiffer and Beyer, 2008). Indeed, at the laboratory level, GM plants with modified nutritional characteristics have been available for several years. The obvious best known example of this is "Golden rice" (Ye, Al-Babili, Kloti, Zhang, Lucca, Beyer and Potrykus, 2000). This has been modified for enhanced levels of beta-carotene, a precursor for the formation of vitamin A in the diet. Other examples are enhanced vitamin E in maize (Cahoun, Hall, Ripp, Ganzke, Hitz and Coughlan, 2003), folate in tomato (Diaz de la Garza, Gregory and Hanson, 2007), flavonols in tomato (Bovy, de Vos, Kemper, Schijlen, Pertejo, Muir, Collins, Robinson, Verhoeyen, Hughes, Santos-Buelga and van Tunen2002) and iron in rice (Lucca, Hurrell and Potrykus, 2002). More recently, these nutritional traits have also been "stacked" within maize (Naqvi, Zhu, Farre, Ramessar, Bassie, Breitenbach, Conesa, Ros, Sandmann, Capell and Christou, 2009) to provide enhanced levels of beta-carotene, vitamin C and folate within a single plant.

The technological approaches used for these nutritional enhancements are quite diverse, but most commonly involve introduction of transgenes encoding for enzyme(s) within the biosynthetic pathway for the nutrient that are either rate limiting or not expressed at all in the target tissues of the crop. Thus, in the case of folate enhanced tomato fruit, the combined expression of two rate limiting enzymes - GTP cyclohydrolase I and aminodeoxychorismate synthase - which control the pteridine and p-ABA branches of the folate pathway, respectively, have resulted in a 19-fold increase in folate (Daiz *et al.*, 2007). A similar approach has been taken to enhance vitamin E levels in maize. Overexpression of the enzyme – homogentisic acid geranylgeranyl transferase - resulted in a 6 fold increase in total vitamin E antioxidants - tochotrienols and tocopherols (Cahoon *et al.*, 2003). Another approach is to introduce a transgene encoding for a transcription factor(s) that can enhance expression of an entire biosynthetic pathway. An example of this

is in enhancement of flavonoids within the flesh of tomato fruit. In this case the genes for this pathway are all present in the tomato, but are not expressed within the pericarp (flesh) of the fruit. The introduction of transcription factors LC and C1 from maize resulted in accumulation of kaempferol in the fruit (Bovy *et al.*, 2002). These nutritionally enhanced crops, whilst providing potential health benefits for the human consumer, may also benefit animals if they were to be utilised as animal feed.

Other potentially relevant compositional changes that could be addressed by GM, and which could enhance livestock production, include modifications to the amino acid composition of seeds, in particular enhancement of lysine and methionine, and reduction in phytase levels within animal feed.

Most cereal grains are deficient in the amino acid lysine. Thus this nutrient often needs to be added, representing additional cost and complexity to feed manufacture. One approach to overcome this deficiency would be to introduce a lysine-rich protein into the seed. This approach is exemplified by the high lysine maize line Y642 (Yu, Peng, Zhang, Zhao, Zhy, Sun, Liu, and Ao, 2004). In this case a pollen-specific gene from potato that encodes a lysine-rich protein was introduced into maize and expressed in the seed. A hybrid line (Y642) was then generated by crossing with a conventional non-GM maize. Compositional analysis of the hybrid line demonstrated that both total protein and lysine content were higher in the transgenic seed, and feeding rats for 90 days showed no adverse diet-related effects (He, Tang, Luo, Li, Cao, Yu, Delaney and Huang, 2009).

In maize, the amino acid deficiency is thought to be due to the fact that the major storage proteins – zeins - which make up about 60% of the total maize seed protein are almost completely devoid of lysine and tryptophan (Coleman and Larkins, 1999). In an alternate approach to enhance lysine levels, gene silencing technology has been used to reduce accumulation of the 19 and 22KD zeins (which comprise 40% and 20% respectively, of total zein); this resulted in an increase in the level of lysine from 2.83% to between 5.23 and 5.62% and also an increase in tryptophan from 0.69% to between 1.12 and 1.22 % (Huang, Frizzi, Florida, Kruger and Luethy, 2006). Total protein in the transgenic maize seed was not significantly different to the control and no other major compositional change, for example in starch or oil, was detectable.

Increasing biosynthesis of lysine may also be important for enhancing levels of this amino acid in tissues. This could be achieved by the expression of a novel dihydrodipicolinic acid synthase in transgenic plants. This is an enzyme involved in biosynthesis of lysine and is normally subject to feedback inhibition by this amino acid. This can restrict the normal accumulation of lysine in tissues. The expression of a form of the enzyme resistant to such feedback inhibition may result in enhanced lysine levels. A high lysine maize (Event LY038), in which this has been achieved, has been patented (Patent US07157281, 2007).

Phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate) is the major form in which phosphorus is stored in several plant seeds including soyabean. Phytate (the mixed salt form of phytic acid) typically comprises around 75% of the total phosphorous in the seed (Raboy, 2001). However, this compound poses several problems for the feed industry. Firstly, there is a need to break down phytate to release phosphorous for uptake by the animal; in plants phytate is naturally broken down by the enzyme phytase. Phytate can also have an environmental impact; if it is not digested then it can contribute to phosphorous contamination of the environment. Finally, phytate acts as a chelator of important minerals such as calcium and as such can be classed as an anti-nutritional factor (Lott, Greenwood and Barton, 1995). It is thus common practice to degrade phytate by addition of phytase enzyme to animal feed. There is commercial interest, therefore, in reducing either levels of phytate in seeds or expression of phytase within the crop itself.

Although rare, low phytate mutants have been isolated in a number of crops, including maize (Reboy, Gerbasi, Young, Stoneburg, Pickett, Bauman, Murthy, Sheridan and Ertl, 2000) and soyabean (Wilcox, Premachandra, Young and Raboy, 2000). Low phytate seeds can also be generated through the application of GM. Phytate levels in maize have been shown to be controlled by a number of different genes. These include genes encoding inositol polyphosphate kinases (Shi, Wang, Wu, Hazebroek, Meeley and Ertl, 2003), myo-inositol kinases (Shi, Wang, Hazebroek, Ertl and Harp, 2005) and multidrug resistance-related protein (MRP) (Shi, Wang, Schellin, Bailin, Faller, Stoop, Meeley, Ertl, Ranch and Glassman, 2007). These are thus potential targets for manipulation using GM. Silencing of MRP in maize resulted in a 32-75% reduction in phytate levels (Shi *et al.*, 2007). This group also demonstrated that silencing of a homologous gene in soyabean also reduced phytate levels, suggesting that this may be a generic approach for several target species.

The other approach involving use of GM, is to generate transgenic plants in which the phytase enzyme is expressed within the seed tissue. An example of such an approach is the expression of a fungal phytase gene in transgenic maize (Chen, Xue, Chen, Yao, Yang, Ma, Fan, Zhao, Tarczynski and Shi, 2006). In this case the *Aspergillus niger* phyA2 gene, driven by the maize embryo specific globulin-1 promoter, was transformed into maize. Phytase activity reached about 2,200 units per kg of seed, which is about 50-fold higher than non-transgenic maize seed.

Conclusions

The use of GM worldwide is going to increase in the future. This technology has the potential, along with many other technologies, to help us meet the growing challenges of food security, climate change and healthy diets.

One requirement is to enhance agricultural productivity and the major GM crops currently under cultivation address this by being targeted primarily at herbicide and insect resistance. The number of GM crops exploited commercially is very small compared to the number that could be modified. Similarly, the number of traits that have been modified to date is also very small compared to the number of traits that have been manipulated in the laboratory or even in small field trials. The short term extension of GM technology is likely to be targeted at increased exploitation of current traits, especially by "stacking" traits within a single commodity, in a wider but still limited range of crop plants. The targeted traits, and range of crops, are both likely to be extended in the longer term. These new traits are likely to include those that will further enhance productivity, such as drought, cold or salt tolerance, along with the ability to better utilise resources such as nitrogen. Another potentially significant application will be in modification of the composition of crops. These modifications may be designed to make subsequent processing of the crop into for example oils more efficient, or may be targeted at improving the nutritional value of the crop for human and potentially animal diets. Ultimately, expansion of GM technology will depend on consumer perception and on the subsequent perceived potential profitability of these products.

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11

INFLUENCE OF FEED PROCESSING TECHNOLOGY ON PIG PERFORMANCE

L.A. DEN HARTOG[1,2] AND S.R. SIJTSMA[1]

[1]*Nutreco R&D and Quality Affairs, PO Box 220, 5830 AE Boxmeer, The Netherlands*
[2]*Wageningen University, Department of Animal Nutrition, Wageningen, The Netherlands*

Abstract

Feed processing technology is essential in the concept of *sustainable precision livestock farming*. This concept aims to optimize productivity and efficiency in pig production by an integrated approach taking into account as many relevant factors dealing with feed, animal, microbiota, farm and their interactions, as well as customer, consumer and societal demands. In this chapter relevant aspects are presented with the aim of identifying new opportunities. The focus is on feed manufacturing rather than processing of feed ingredients, both on an industrial and farm level.

Physical treatments have been studied most intensively. Particle size reduction has shown to be of importance and results in more consistent effects than particle size uniformity. Nevertheless, the best strategy remains difficult to prescribe as the results indicate an optimum between 600 and 900 μm, depending on several other factors involved. Correct mixing is also of importance but does not seem to be a critical factor in practice for at least fattening pigs and sows. Further processing of meal into pellets has proven to increase growth rate and feed efficiency under controlled pelleting conditions on average by 6 and 6-7%, respectively. The impact of other pellet characteristics on pig performance, such as diameter and the quality in terms of hardness and durability, is often unclear although the presence of fines in the pelletized feed has been proved to be undesirable.

Relatively new are studies on microbiological treatments, such as fermentation. The concept of fermented liquid feed seems to be promising and several studies have shown beneficial effects such as improved gastrointestinal health and growth performance, and reduced mortality and morbidity in both piglets and fattening

pigs. Unfortunately palatability, health and nutritional value can be affected by uncontrolled fermentation and amino acid degradation.

In conclusion, feed processing technology is important both for minimizing feed production costs and optimizing pig health and performance. Although it offers many opportunities, more research is necessary for optimization of existing and development of new technologies. A potential bottleneck for innovation is the fact that implementation in practice often requires relatively large investments. Therefore, it is necessary to have better estimates and more quantitative data of the effects of feed processing technology on pig performance.

Introduction

Crucial in the development of modern pig production is the concept of *sustainable precision livestock farming*. This concept aims to optimize productivity and efficiency by an integrated approach taking into account as many relevant factors dealing with feed, animal, microbiota, farm and their interactions, as well as customer, consumer and societal demands. Several indicators demonstrate that optimising productivity and efficiency in pig production is still potentially possible. The genetic potential is only partially utilized, the utilization of most nutrients appears to be low and there is a huge variation in pig performance among farms and within farms (Den Hartog, 2009).

As feed usually contributes to more than 0.60 of the total costs, it has received most attention. In particular nutrition in terms of nutrient requirements and nutrient supply has been given a high priority in many research groups. However, feed processing technology does play an important role in improving productivity and efficiency as well, not only with respect to minimizing feed production costs but also optimizing pig performance. Feed processing technology is therefore essential in the context of *sustainable precision livestock farming* and may offer new opportunities in optimizing the balance between nutrient requirements and supply, improving animal welfare and health status, and reducing emissions. Traditionally, the focus in research was on physical aspects, such as particle size, the type of milling, mixing and pellet quality. Curently, other aspects do receive attention as well such as chemical, biochemical and microbiological treatment of raw materials and feed. In this chapter an overview of some of the aspects is presented with the aim of identifying new opportunities from the perspective of improving pig performance. The focus is on feed manufacturing rather than processing of feed ingredients, both on an industrial and farm level.

Grinding

Most of the feed ingredients used in feed manufacturing are ground for technological and nutritional reasons. The benefits from particle size reduction are related to the handling and mixing characteristics of ingredients, pelleting efficiency and quality, and digestibility. Both hammer mills and roller mills are commonly used in the production of pig feeds. With a hammer mill, there may be a wide distribution of particle sizes around the geometric mean, whereas with a roller mill, particles tend to be more uniform in size (i.e., lower standard deviation, or s_{gw}, of the mean particle size; Koch, 2002). Therefore both particle size reduction and particle size uniformity have been studied intensively.

PARTICLE SIZE REDUCTION

Optimal particle size of pig diets seems to be dependent on several factors, including the type of ingredient, the complexity of diet composition, further processing such as pelleting and animal-related physiological factors. In theory, particle size reduction increases the surface area of ingredients, allowing for greater interaction with digestive enzymes and as a result improving performance (Goodband *et al.*, 1995). Feed efficiency rather than body weight gain were indeed improved when reducing particle size of cereal grains for piglets (Ohh *et al.*, 1983; Healy *et al.*, 1994), fattening pigs (Ivan *et al.*, 1974; Owsley *et al.*, 1981; Giesemann *et al.*, 1990; Cabrera *et al.*, 1994), and lactating sows (Wondra *et al.*, 1995a,b). On average, feed efficiency is improved by 1 to 1.5% for each 100 μm mean geometric diameter particle size reduction in the range from 400 to 1000 μm. However, there seems to be an optimal mean geometric diameter of cereal particles between 500-700 μm as below 300 μm both feed intake, body weight gain and feed efficiency tend to deteriorate (Healy *et al.*, 1994). This has been demonstrated for maize, sorghum and barley. Wheat seems to have a higher particle size optimum, i.e. between 800-900 μm. This is probably related to the tendency of this cereal to become sticky and pasty in the buccal cavity because of its high gluten content. If ground too fine, wheat can reduce feed intake. Roller mills with a differential drive produce a uniform particle size and fewer fines and may be more suitable for processing wheat in pig diets (Goodband *et al.*, 1995).

Fewer studies have been carried out with other single feed ingredients. Fastinger and Mahan (2003) recently evaluated the effects of solvent-extracted soyabean meal particle size (900 to 150 μm) on amino acid (AA) and energy digestibility. Apparent digestibility of the average of the 10 essential AA increased as particle

size decreased, but energy digestibility and the average of the non-essential AA digestibilities was not affected. The largest improvement in AA digestibility was observed when particle size of the solvent-extracted soyabean meal decreased from 900 to 600 μm. However, Lawrence *et al.* (2003) did not observe any effect of soyabean meal particle size on piglet growth performance.

Mixed grinding of ingredients resulted in similar effects as reported for single grinding of cereals, including the presence of an optimum. Although most information comes from field trials, feed efficiency seems to be improved to a similar degree as reported in studies for single grinding of cereals, i.e. by 1 to 1.5% for each 100 μm mean geometric diameter particle size reduction in the range from 600 to 1000 μm. In particular pig performance on fibre-rich feeds seems to benefit from particle size reduction. Real digestibilities of amino acids in pigs were increased when particle size was reduced in wheat-sunflower meal (Lahaye *et al.*, 2004) and in wheat-rapeseed meal diets (Lahaye *et al.*, 2008). The latter also reported that coefficients of ileal digestibility for dietary energy, OM, and DM were improved when wheat particle size in a wheat-rapeseed meal diet was reduced from 1,000 to 500 μm.

Several studies have indicated that the negative effects of too fine grinding of cereal grains are related to a higher incidence of gastric mucosal alterations, such as keratinisation of the stomach and gastric ulcers. Similar mucosal changes have been reported for pelleted diets, irrespective of the original grinding intensity before pelleting (Grosse Liesner *et al.*, 2009). Finely ground feed is more fluid when mixed with the digestive secretions of the pig's stomach compared to a more coarsely ground feed (Regina *et al.*, 1999). As a result, the acids in the stomach have a greater chance of coming into contact with and irritating the oesophageal region of the stomach. The frequency of keratinisation and ulceration increases when particle size drops below 500 μm. This was demonstrated in piglets (Healy *et al.*, 1994), fattening pigs (Cabrera *et al.*, 1994; Wondra *et al.*, 1995a,b), and lactating sows (Wondra *et al.*, 1995c). Under practical conditions, gastric ulcers seem to be more important in gestating and lactating sows than in fattening pigs. Therefore the greatest potential for fine grinding or rolling to improve feed efficiency will be for fattening pigs and to a lesser degree for piglets. It appears that piglets do a better job of chewing feed than fattening pigs. Ayles *et al.* (1996) suggested that to change occasionally (e.g., when pigs are moved or sorted) from fine-ground to coarse-ground, and then back to fine-ground diets could be an effective measure to prevent ulcers in pigs while capturing most of the benefits in efficiency of growth when fine grinding.

Other factors explaining the mechanism of action of particle size reduction may also play a role. Feed particle size directly affects the time needed to mix feed adequately, the segregation of ingredients during handling, and feed flow

ability. Moreover, Anguita *et al*. (2007) and Solà-Oriol *et al*. (2007) observed a relationship between particle size of the cereal grain fraction in the feed and voluntary feed intake which might be independent from a better digestibility due to an increase of surface area or improved homogeneity related to mixing and handling during production. In contrast to above mentioned effects, other studies failed to observe any effect of particle size on voluntary feed intake, performance or digestibility of nutrients (Gipp *et al*., 1995).

It should be noted that particle size should always be seen in relation to the size of the screen. Small kernel cereals such as sorghum may fall through the opening intact when using screens with a bigger diameter. This may affect digestibility. The latter is in particular the case for full fat rapeseed, which is hardly digestible when not ground. However, the relation between size of the screen and optimal particle size is complex as other factors, such as hammer mill revolutions per minute, tip speed and the numbers of hammers are also of importance.

PARTICLE SIZE UNIFORMITY

In contrast to the effects of particle size, effects of particle size uniformity on pig performance do not seem to be consistent. Choct *et al*. (2004) analysed processing (hammer mill vs. roller mill), particle size, and feeding method (liquid vs. dry) for wheat-based diets fed to weaned piglets. They observed that piglets fed hammer-milled diets consistently consumed more feed and grew faster. Thacker (2006) found that digestibility coefficients for DM, CP, and energy with a particle size similar in mean geometric diameter were greater for pigs fed hammer-milled oats vs. roller-milled oats. This was attributed to the larger small particle size fraction for hammer-milled oats. In contrast, Wondra *et al*. (1995b) reported positive effects of roller-milled feed on the digestibility of DM, N and GE of a maize-based diet but their results suggested an effect of mill type separate from any s_{gw} effect. Reece *et al*. (1985) described particles of hammer milled maize as more spherical in shape with more uniform edges than particles of roller milled maize. They hypothesized that a spherical shape would reduce susceptibility to attack by digestive enzymes, thus decreasing digestibility of nutrients in hammer-milled maize. However, effects on performance may also be attributed to differences in flow ability characteristics and as a consequence differences in homogeneity of the feeds. Flowability appears to be influenced more strongly by variation in particle size than by the shapes of particles (Groesbeck *et al*., 2007).

Particle size uniformity is also of relevance for preventing keratinisation of the stomach and gastric ulcers. An upper level of fine particles seems to be reasonable, as a minimum level of coarse particles is not ulceroprotective. Grosse Liesner *et*

al. (2009) showed that a higher proportion of particles < 400 μm of 0.30 resulted in increasing frequency/intensity of gastric mucosal alterations in reared piglets, i.e. higher risks for ulcerations.

Mixing

From the feed manufacturing point of view, the optimum mixing procedure would require minimal inputs of time, energy, and labour. However, for optimal pig performance adequate mix uniformity is required. Recommendations for mix uniformity, usually expressed as coefficient of variation (CV) for the distribution of some nutrient or marker within the feed, refer to values less than 10% (Beumer, 1991; Lindley, 1991). The accuracy of such recommendations is questionable both due to methodological problems when using markers and the absence of a clear relationship between mix uniformity and nutritional value of the feed. The ideal marker has not been identified yet and in practice a pragmatic choice is often made, taking into account accuracy and costs of analysis, distribution characteristics and raw material composition.

It can be argued whether improper mixing of one batch of feed would cause serious problems in pigs because of the relatively high amount of feed consumed per day and the fact that a single batch will usually be consumed in a short period of time. Traylor *et al*. (1994) demonstrated in finishing pigs that growth performance was not affected by reducing the CVs of the diet from nearly 54% (0 min. mixing time) to less than 10% (4 min. mixing time). Moreover, bone strength did not differ among pigs fed the various treatments, suggesting that minimal mixing of the diets did not create problems with Ca or P status of the pigs. In contrast, in a similar experiment with weanling piglets they did find a significantly improved body weight gain and feed efficiency. However, below a CV of 12% little response was observed. Groesbeck *et al*. (2007) also demonstrated that inadequate mixing (CV >12%) can reduce nursery pig performance. Therefore, it seems that fattening pigs are probably less sensitive to diet non-uniformity than piglets.

Thermal processing & feed form

Although mixed feed ingredients are ready for use, pig feeds are often further processed for technological, nutritional and microbiological reasons. In particular pelleting is quite common but also other processing techniques such as extrusion, expansion and pressure cooking, either or not in combination with pelleting and crumbling, are used in practice. Most of those treatments require steam

conditioning. Moreover, the temperature increases during the shaping process due to friction. Therefore shaping the feed is usually linked with effects of thermal processing and as a consequence the effects on pig performance are not easy to explain.

CONDITIONING AND PELLETING

Pelleting and other further processing techniques add considerable cost to manufacturing of pig diets. However, they may have certain advantages. For pelleting, improved rate of growth and feed efficiency are most prominent. On average, growth rate is increased by 6% and feed efficiency is improved by 6 to 7% by feeding pellets compared to meal (Hancock and Behnke, 2001). Why pelleting positively impacts growth performance has not been clearly demonstrated. Some research suggests that pelleting improves nutrient digestibility (Wondra *et al.*, 1995a). In particular starch gelatinization is often mentioned as a factor or relevance. Starch gelatinization is the rupture of starch granules, thereby allowing the linear and cyclic molecules to hydrate and become more available for enzymatic digestibility. This might be of relevance for piglets under stress conditions but is probably of less importance for older pigs. Even in studies with very young piglets, little effect of the degree of gelatinization of the cereal portion of the diet on piglet performance has been observed previously (Hongtrakul *et al.*, 1998; Medel *et al.*, 1999). Nevertheless, starch gelatinization is directly related to pellet quality and pellet quality affects the feed efficiency response. Stark *et al.* (1994) fed diets with varying levels of pellets and pellet fines, and found that as pellet fines increased in the diet, the benefits of pelleting were lost. Consequently, attention must be given to producing quality pellets in order for the benefits of pelleting to be realized.

Excessively high temperatures during pelleting or other thermal processing may be responsible for decreased performance of pigs fed diets containing specific protein sources (e.g. dried whey, fish meal, or spray-dried animal proteins) and micro-ingredients (e.g. vitamins and enzymes) compared with performance of pigs fed diets processed at lower temperatures (Traylor *et al.*, 1997; Hongtrakul *et al.*, 1998, Steidinger *et al.*, 2000). Normal pelleting conditioning temperatures of 85°C could potentially burn or scorch some of these specific ingredients and increase the potential for initiating the Maillard reaction, thereby decreasing their nutritional value.

It should be noted that pellet quality is also related to particle size and possibly particle size uniformity. Reimer (1992) indicated that fineness of grind may control 0.20 of pellet quality. Decreasing particle size from a coarse to a fine grind exposes

more surface area per unit volume for absorption of condensing steam during the conditioning process. This results in a higher feed temperature and more water absorption, which together, within the time available, increases gelatinization of raw starch. Grinding can also improve pellet quality by reducing air spaces between particles, allowing closer surface to surface contact for a given volume of feed; i.e., it increases bulk density. Large pieces of any ingredient in a feed formula result in weak spots in the pellet, especially if these are fibrous.

Pelleting also contributes to an improved microbiological status of the feed, although recontamination always remains a challenge. However, a study using a pig intestine organ model showed that *Salmonella enterica* serovar Typhimurium DT12 adhered significantly less to the ileal tissue of pigs fed non-pelleted diets than to those fed pelleted diets. This is possibly related to the secretion of mucins that are capable of binding Salmonella and allowing for colonization. These results suggest that pigs fed non-pelleted feed are better protected than pigs fed a pelleted diet (Hedemann *et al.*, 2005). Pelleting also tends to increase scores for keratinisation and (or) ulceration in pigs (Wondra *et al.*, 1995a,b; Amornthewaphat *et al.*, 1999).

PELLET DIAMETER

Few data are available on the effects of pellet size or in particular changes in pellet size during the feeding period. Although one may expect effects in the pre- and post-weaning period, in particular taking into consideration effects reported in certain choice feeding experiments, pellet diameter does not seem to be a major contributor to the low feed intakes seen in the post-weaned piglet when the pellets are presented as in a commercial situation (i.e. not choice feeding) (Edge *et al.*, 2005). Similar results were reported by Traylor *et al.* (1996), showing that pellets up to 12 mm in diameter had no influence on post-weaning performance, as did the provision of a meal-based diet.

Nevertheless, feeding behaviour seems to be affected by pellet diameter. Edge *et al.* (2005) demonstrated that the total time spent at the trough pre-weaning seems to be increased with bigger pellet diameters. However, this did not result in higher feed intake and the effect disappeared post-weaning. A'Ness *et al.* (1997) in a preliminary study found that, when suckling piglets were offered supplementary solid feed in the form of sow rolls (pellets with a very large diameter), they spent longer periods of time engaged in trough-directed behaviour than those piglets offered feed in a typical commercial pellet size (2-3 mm diameter). However, feed intake and body weight gain were not measured. It is hypothesised that, in this experiment, a larger pellet diameter will provide the incentive the piglet needs both to approach the trough and to ingest solid feed.

Liquid feed

The concept of liquid feed is widely spread in pig production. Traditionally many pigs were fed on meal mixed in a bucket with whey or water. Increasing farm size and the need for automation encouraged a move to dry feeding but recent developments in computer-driven systems have led to a revival of liquid feeding.

Piglets are fed on liquid milk by their mothers until weaning and it is logical to continue to feed them on a liquid diet provided that it can be delivered by a reliable and hygienic system. There are many advantages of using liquid feeding systems compared to dry feeding in pig production. These include improved nutrient utilization, flexibility and control of feeding programs, and improved animal performance (Jensen and Mikkelsen, 1998; Russell *et al.*, 1996; Canibe and Jensen, 2003; Brooks *et al.*, 2001; Lawlor *et al.*, 2002). Liquid feeding may also enhance gut health, reduce the need for feed medications, and improve animal welfare (Brooks *et al.*, 2001; Canibe and Jensen, 2003). Liquid feed can provide the pig with its essential daily energy and nutrients at a lower cost, because it enables the use of relatively cheap, moisture-rich co-products from the food and, more recently, biofuel industry. Excellent performance in both fattening pigs and lactating sows has been achieved on liquid feed provided that the diet is formulated correctly and proper hygiene control is maintained. In particular when highly variable, moisture-rich co-products are used, it is of utmost importance to estimate the nutritional value as precisely as possible.

Liquid feeding also creates opportunities for using digestibility-enhancing enzymes, which work more efficiently in a liquid than in a dry environment. However, effluent output can be higher when liquid feed is used, because of the extra volume of water consumed by the pigs. In addition, emissions can be higher in case of imbalances due to the variability of nutrient levels.

Fermented liquid feed

Relatively new is the concept of fermented liquid feed (FLF) either fermented with existing or introduced lactic acid bacteria. FLF is a mixture of water and starch containing feed ingredients or complete feed stored in a tank (ratio feed:water of 1: 2.5-2.75 wt/wt) at a certain temperature (20-25°C) and time (1 day) before feeding to the animals. Such compositions are characterized by a high level of lactic acid bacteria, yeast and lactic acid, low pH and low enterobacteriaceae counts. Several studies have shown beneficial effects in animals fed FLF compared to those fed with dry or liquid feed, such as improved gastrointestinal health and growth

performance, and reduced mortality and morbidity in both piglets and fattening pigs (Geary *et al*., 1999; Canibe and Jensen, 2003; Scholten *et al*., 1999). These benefits appear to be the result of enhanced nutrient availability, and reduced growth and shedding of pathogenic bacteria such as Yersinia, Salmonella, and E. coli due to low pH (Geary *et al*., 1999; Scholten *et al*., 1999; van Winsen *et al*., 2001; Demeckova *et al*., 2002). Furthermore, pepsin activity is increased due to lower pH, resulting in improved protein digestion (Scholten *et al*., 1999).

Various studies have shown that the effect of feeding FLF on growth performance and nutrient digestibility in both piglets and pigs can be inconsistent (Pedersen and Stein, 2009). Nevertheless, the use of FLF has gained recent interest also as an alternative strategy for reduction of the use of antibiotics in pig production. In addition, it has the potential of increasing the inclusion of co-products from the food and biofuel industry in animal diets to avoid wasteful disposal that can thus decrease costs and the environmental burden (Canibe and Jenssen, 2007, Canibe *et al*., 2007).

The inconsistency of effects on pig performance is partly related to the fact that spontaneous fermentation of liquid feed is unreliable. Maintaining a continuous fermentation by retaining a proportion of the feed each day can result in the development of a resident microflora dominated by yeasts. This may compromise both palatability and health, and reduce the nutritional value of the feed. Batch fermentation of the cereal portion of the diet using inoculants selected to generate high concentrations of lactic acid has the potential to produce more consistent results. This approach may enable the combination of preservative and probiotic effects of lactic acid bacteria, while also improving the availability of nutrients in the feed and reducing levels of anti-nutrients and mycotoxins (Brooks, 2008). Palatability and, as a result, feed intake are not only affected in a negative way. Both higher and lower feed intakes compared to feeding non-FLF or dry feed have been observed. With respect to reduced feed intakes, Canibe *et al*. (2009a) demonstrated that low pH and high acetic acid concentration in FLF (within the levels typically measured in FLF) do not profoundly affect feed intake in piglets by impairing palatability. In addition, Canibe *et al*. (2009b) could not demonstrate a relation between biochemical and microbiological variation in FLF samples and feed intake at farm level. Forty farms were classified in two groups, a 'low feed intake' group and a 'high feed intake' group. The biochemical characteristics and the microbiological composition to group level were determined and the characterization of lactic acid bacteria and yeasts to species level was carried out. The lactic acid bacteria isolates were identified by sequencing the 16S ribosomal DNA gene and the yeasts isolates by sequencing the D1/D2 domain of the large-subunit (26S) ribosomal DNA. In general, the results obtained indicated that there are no big differences in the biochemical characteristics measured and in

the microflora composition at group level between the two farm groups. The data on diversity of lactic acid bacteria and yeasts showed that a few species dominate in all FLF-samples.

There are indications that fermentation of only the starch containing feed ingredients and mixing them with the other ones prior to feeding, results in better pig performance than fermentation of the complete diet. In addition, Canibe and Jensen (2007) found a higher density of yeasts, a higher concentration of ethanol in the GIT, a change of the bacterial population of the stomach and a tendency for higher feed intake and body weight gain compared to feeding of the complete fermented diet.

It was suggested that feeding liquid feed containing exclusively the fermented liquid cereal grains may avoid microbial degradation of free amino acids in the feed. In particular the microbial degradation of free lysine has been suggested as an important factor that contributes to the negative impact feeding FLF may have on growth performance. Besides the fact that E. coli strains are able to degrade free lysine in FLF and large amounts of lactic acid bacteria can prevent such losses (Niven *et al*., 2006), not much is known on the factors affecting this process (Canibe and Jensen, 2009). Nevertheless, to minimize loss of synthetic amino acids, it is advised to add amino acids to liquid feeds after stable fermentation is achieved, when liquid feed contains more than 75 mMol lactic acid, or when the pH is less than 4.5 (Braun and de Lange, 2004).

Lysine can also be protected by pelleting the feed before fermentation. Canibe and Jenssen (2009) clearly showed that the disappearance of free lysine was much higher when non-pelleted, non-heated feed was fermented than when the same pelleted (83ºC) feed was used. This was the case both during the initial hours (48 h) of incubation and in a more established FLF (after back slopping had been practiced for several days). This would also indicate that the first established microflora determines the extent of free lysine degradation more than the substrate added after the system has reached steady state.

Improved digestibility by fermentation is also dependent on the level of soluble, potentially fermentable non-starch polysaccharides in the feed ingredients. This explains the bigger effects of fermentation on digestibility of barley compared with wheat (Jorgensen *et al*., 2009).

In addition to direct effects, FLF may also have indirect effects via sow milk and excreta. Demecková *et al*. (2002) showed that faeces excreted from sows fed FLF had lower numbers of coliforms, and piglets sucking from such sows excreted faeces higher in lactic acid bacteria and lower in coliforms than their counterparts sucking sows fed dry pellets. In addition, the mitogenic activity in the piglet blood lymphocytes was increased indicating a greater level of lymphocyte proliferation and possibly enhanced immune function.

Impact, opportunities & challenges

The results presented in this chapter demonstrate that feed processing technology is of crucial importance for optimizing productivity and efficiency in pig production. However, the effects on pig performance are not always clear, mainly due to complex interactions. Nevertheless, most studies indicate that pig performance can be further improved by optimization of existing and introduction of new technologies.

Particle size reduction has been shown to be of importance and results in more consistent effects than particle size uniformity. Nevertheless, the best strategy remains difficult to prescribe as the results indicate an optimum between 600 and 900 μm, depending on several other factors involved. It could be hypothesized that protein sources require a finer grinding than carbohydrate sources in order to maximize the digestion of amino acids. In particular fibre-rich protein sources such as rapeseed and sunflower meal may benefit from this approach. However, gains in pig performance must outweigh production costs. The latter usually increases when feed ingredients need to be ground separately using different screens. Moreover, grinding to particle sizes less than 600 μm sharply increase costs due to higher energy consumption and lower feed mill capacity. In addition, fine grinding without pelleting may result in increased dustiness of the feed, as well as bridging problems in bulk bins and feeders.

Correct mixing is also of importance but does not seem to be a critical factor in practice. At least this appears to be the case for fattening pigs and sows, because of the relatively high amount of feed consumed per day and the fact that a single batch will usually be consumed in a short period of time.

Further processing of meal into pellets has been proved to increase growth rate and feed efficiency under controlled pelleting conditions on average by 6 and 6-7%, respectively. However, the mechanism of action is still not fully resolved and the processing conditions used in practice are usually based on the experience of the feed mill operator. This might result in suboptimal processing conditions, in particular when taking into account the instability of certain specialty protein sources and micro-ingredients. Moreover, pelleting may abolish the preventive effect of coarse grinding on gastric mucosal alterations and could even be a risk factor for colonization of the gut with Salmonella. The impact of other pellet characteristics, such as the quality in terms of hardness, durability and pellet diameter on pig performance is often unclear. The presence of fines in the pelletized feed has proven to be undesirable. However, with respect to the effects of pellet diameter, a parameter often highlighted in sales, there is hardly any scientific basis. As a consequence, optimization of the feed form and required thermal processing conditions should receive some more attention and may still offer opportunities in optimizing productivity and efficiency.

All processing technologies discussed so far apply to dry feed and feed ingredients. However, the use of moisture-rich co-products from the food and biofuel industry offers a lot of opportunities and such ingredients can easily be fed when making use of automated liquid feeding systems. Moisture-rich co-products are usually price competitive as they do not require expensive drying and can be fed directly at the farm without interference of a feed compounder. Moreover, they may be abundantly available in the neighbourhood of pig farms. Liquid feeding has proven to result in excellent pig performance, provided the circumstances are well controlled. In particular the estimation of the nutrient content of the moisture-rich co-products, the final diet formulation and hygiene conditions are important. Inclusion of liquid co-products from the food and biofuel industry in animal diets also avoids wasteful disposal and, as a result, can decrease the environmental burden.

Liquid feeding also offers the opportunity for fermentation. The concept of fermented liquid feed (FLF) seems to be promising and several studies have shown beneficial effects in animals fed FLF compared to those fed with dry or liquid feed, such as improved gastrointestinal health and growth performance, and reduced mortality and morbidity in both piglets and fattening pigs. These benefits appear to be the result of enhanced nutrient availability, and reduced growth and shedding of pathogenic bacteria due to the formation of organic acids resulting in low pH and the presence of bioactive, often antimicrobial substances. Therefore FLF could be an alternative strategy for reduction of the use of antibiotics in pig production. However, various studies have shown that the effect of FLF on pig performance can be inconsistent. Palatability, health and nutrition value could be affected by uncontrolled fermentation. In particular the prevention of free lysine degradation has received much attention. Nevertheless, FLF seems to be manageable and large scale implementation may follow soon.

In conclusion, feed processing technology is indeed important in the concept of *sustainable precision livestock farming* and certainly offers new opportunities in pig production. However, more research is necessary for optimization of existing and developing of new technologies. A potential bottleneck for innovation is the fact that implementation in practice often requires relatively large investments. Therefore, it is necessary to have better estimates and more quantitative data of the effects of feed processing technology on pig performance.

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12

PIGLET STARTER FEEDING IN A CHANGING BUSINESS ENVIRONMENT

DR MIKE A VARLEY

BPEX, Stoneleigh Park, Kenilworth, Warwickshire CV8 2TL
E-mail: mike.varley@bpex.org.uk

One of the most significant management decisions for a pig producing business is the average weaning age or the average lactation length for the sows. This not only determines building requirements but also has a bearing on the feed inputs and health status of the weaned piglets and even growth rates to slaughter.

Many industries around the world have targeted 21 day weaning as an optimal operating value and this is normally based on the fact that physical productivity of the sow is maximized for a 3-week weaning system. This was also based on research done many years ago and mostly in Europe.

It is the purpose of this chapter to revisit this problem and explore the optimization of this management decision in the changing economic climates.

Implications for different weaning ages

Once the decision to wean at a set target age has been made, this then automatically dictates building requirements. A later weaning herd will need far more farrowing crates than a very early weaning herd because the former sows spend more time in the crates before weaning takes place. This will also alter the number of dry sow places required, that is the cheaper accommodation and there may also be implications for both weaner piglet places and for growing / finishing pig places. A 1000 sow herd, for example, weaning at 20 days with a farrowing index (litters / sow / year) of 2.3 will need (1000*2.3*30/365) farrowing crates – approximately 189 crates. The same size herd weaning at 30 days with a farrowing index of 2.1 will similarly need 224 crates. There are more expensive building costs therefore associated with the late weaning herd operating with a lower level of litter performance. This therefore has a major impact on the final decision at

the farm planning stage but also will affect on-going decisions when changes are made to the weaning age.

There are also implications on the sow feeding programme. Prolonged weaning ages tend to put more sows into acute negative energy balance and therefore they will require increased feed inputs over a longer lactation. The lactation feeds are also more expensive and hence there are overall feed cost considerations.   Shorter weaning ages put less drain on the sow's backfat reserves and can reduce feed inputs per cycle for each sow.

In terms of the inputs for piglets there are also important issues to consider. A herd weaning at 18-22 days, for example, will probably need high cost, high quality nursery units with a significant energy input for heating and ventilation, and this will require very high levels of management and stockmanship. At the other extreme, herds weaning at around 5 – 6 weeks will only need very basic semi-intensive large-group systems and this can include cheap straw yards and naturally ventilated units. This is applied in countries like Sweden, Switzerland and Denmark where very late weaning systems are popular.

The feed inputs for the piglets will also vary depending on the weaning age. A piglet weaned at 18-21 days will require a very high quality nutrition programme that has a high inclusion of milk powders, cooked cereals and specialized ingredients to sustain gut health.   A later weaned piglet weaned at 30 days of age can be weaned straight on to a starter feed with a much lower energy and nutrient concentration.

Gut health management in itself, always problematic with weaned pigs at any age, is also perhaps easier for the later weaned piglet with better immune and enzyme systems to cope with the changed environment.

In some parts of the world, both legislation and consumer pressure is also having an impact on weaning age. In the European Union, there is a minimum legal weaning age of 28 days although there are loopholes for weaning earlier under veterinary guidance. Sweden and Switzerland have a minimum of 35 days. Consumers in these regions perceive that piglets are under higher welfare environments when weaned later although any scientific proof of this has not been forthcoming.   In North and South American industries, the current situation is around 3 weeks although there are still some pig producing businesses weaning much earlier in North America. The USA in recent years has moved upwards considerably as they have realized the economic benefits associated with this.   Asian pig businesses also still operate at around 21 –25 days although some of these recently have moved upwards a little.

Physical sow productivity

One of the principal reasons back in the 1970s for producers moving to 3 week weaning was to improve sow output. The simple mathematics involved shows that

a 1000 sow herd weaning at 30 days of age has a farrowing index of around 2.1 and, if we assume 11 piglets born alive per litter, this is 23,100 piglets produced per year. If the herd moved to 20 day weaning then, with an improved farrowing index of 2.3, the herd will produce 25,300 pigs per year for costs that may well be similar.   This is a big incentive to wean earlier. Much work in this area was carried out in the UK in the 1970s and much of it was from the University of Nottingham (Cole *et al*. 1975; Varley and Cole 1976a, 1976b, 1978; 1978. Fowler and Varley 1980).

There was also much work directed at understanding the background physiological reasons for the changes seen in the sows' reproductive processes (Varley *et al*.,1981, 1984, 1986; Varley 1983)

The reproductive relationships are well established therefore but well worth reviewing again.

WEANING TO HEAT INTERVAL

The interval from weaning to oestrus is normally expected to be between 4 and 6 days but can vary considerably. Later weaning herds show less variability in this respect and will have a bigger proportion of sows showing first heat at day 4 or day 5 after weaning.   With 21 day weaning there is more variability and the average sow may show first heat at day 7 after weaning and with very early weaning this increases further with even more variability. Summarizing the research gives the following relationship:

IWO = EXP(2.16-(0.0079*WA))

where WA is weaning age (days) and IWO is Interval from Weaning To Oestrus

The relationship is illustrated in Figure 1.

CONCEPTION RATE

The ability to hold effectively to first service is also an important attribute in sow management. The evidence also points to an influence of weaning age on this and this is illustrated in Figure 2.

Clearly there is a reproductive cost when weaning earlier and this can have an important effect on piglets per sow per year.
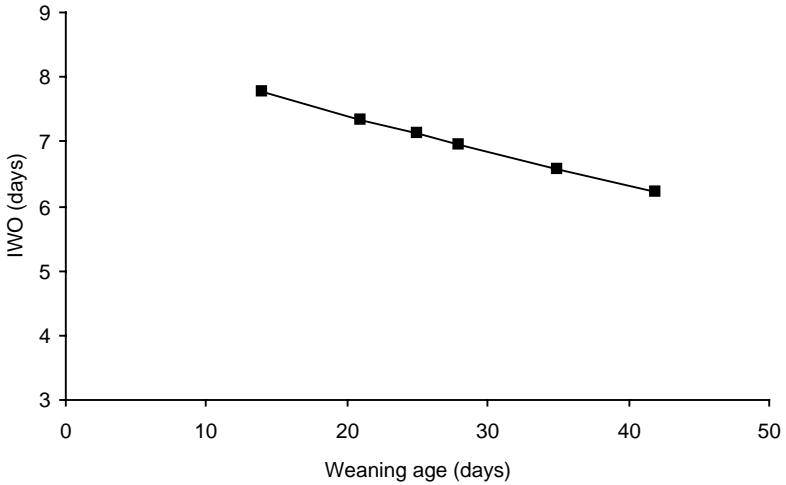
Figure 1. The effect of weaning age on interval from weaning to oestrus (IWO)
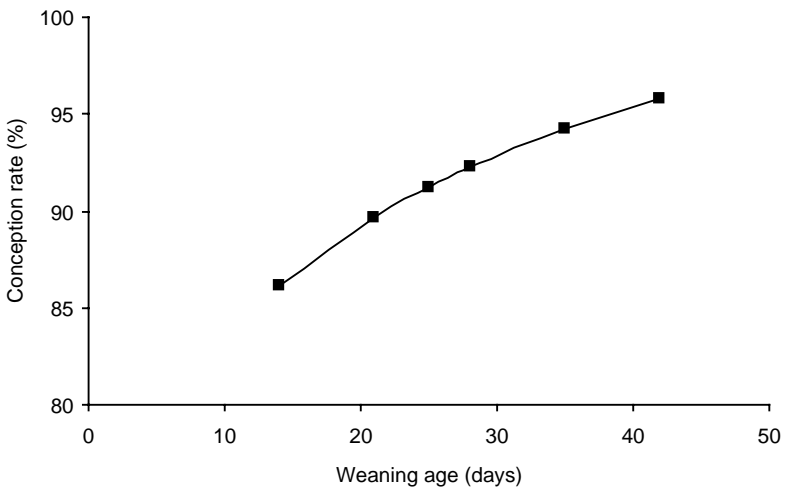
Figure 2. Effect of weaning age on conception rate%

This can be described by the following relationship :

$$CR\% = 62.7 + (8.87 * (LN(WA)))$$

where CR% is non-return rate to first service% and WA is Weaning Age (days)

LITTER SIZE

The number of piglets born alive per litter is one of the biggest determinants of profitability for any pig breeding operation and this is also profoundly affected by weaning age as can be seen in figure 3. The evidence from all the work in the 1970s showed that, in the range 21-25 days through to 35 days and above, there is little effect of weaning age on litter size; for weaning ages below 21 –25 days there is a significant drop in litter size and the magnitude of this effect is quite large.   It is likely therefore that the early North American integrators that set up businesses based on 14 – 18 days weaning were probably going to fail because of this effect. They had established high cost systems but the performance just was not there to justify the investment.
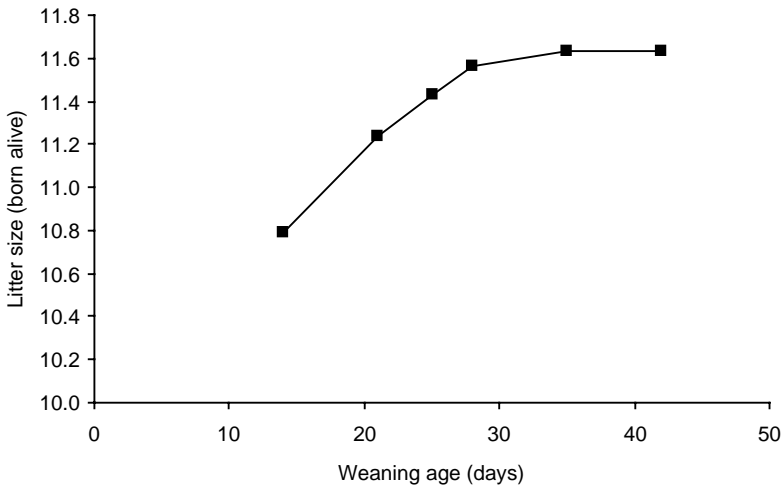
Figure 3. The effect of weaning age on litter size (born alive)

There are therefore many balancing factors in finally seeing the outcome of a specific weaning age and what the producer really needs to know is how many piglets will be produced per sow every year, often termed annual sow productivity or ASP.
    Figure 4 presents the general description of ASP for varying weaning ages.
    Figure 4 shows that, if weaning later than 3 weeks, there will be a progressive reduction in ASP; if weaning id earlier, there is no further rise but in fact ASP starts to fall.   This is because, for later weaning situations, the dominant parameter is the farrowing index and hence ASP falls. For very early weaning herds, the litter size effect is the dominant parameter and again ASP falls off.
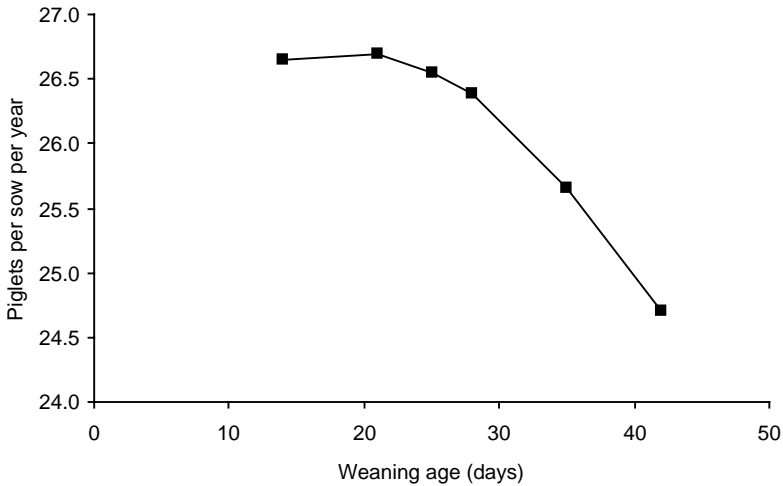
Figure 4. The Effect of weaning age (days) on Annual Sow Productivity (ASP)

It is this relationship that induced many of the industries around the world to establish businesses based on 3 week weaning making the assumption that, if they maximized ASP, they would maximize their overall profitability.

It may well be however, that this argument is seriously flawed. Each of the systems (very early, early or late weaning) is associated with different costs and revenue streams and this needs to be taken into account.

Financial productivity

The financial productivity in terms of Gross Margin Per Sow or Net Profit Per Sow or overall Net Margin for the business are much more relevant determinants of success.

The discussion above has hopefully shown that the relationships involved that finally allow lactation length / weaning age to be optimized are complex and often non-linear. In order to resolve this question, therefore, a simulation model has been constructed, including all of these factors involved, to calculate probable financial outcomes with different weaning ages and with different financial situations (Figures 5 to 8).   What becomes obvious from this exercise is that maximum physical sow productivity does not equate to maximum financial productivity and, in reality over reasonable time spans where costs and prices change, the optimum weaning age actually changes considerably.

Some of these financial drivers are given below based in a European (UK) framework and 2007 financial data (GB£) where pig and weaner prices were very

low and feed costs were high. These data highlight the likely situation where we have low pig prices coupled with very high feed prices.
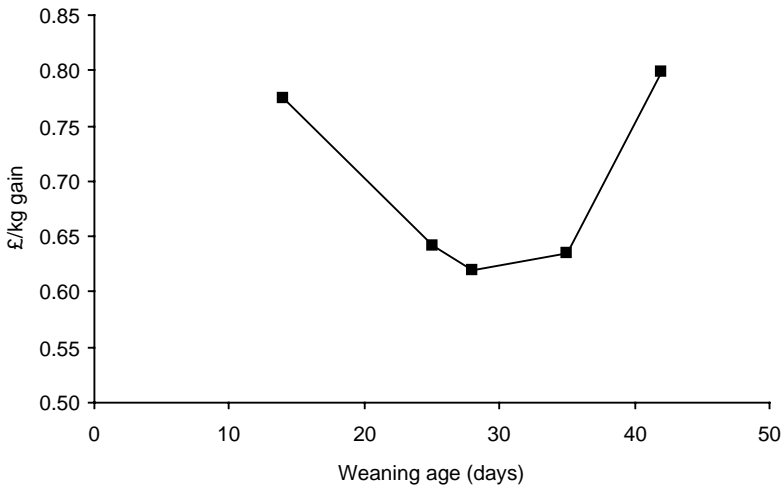
Figure 5. The effect of weaning age on cost per kg liveweight gain (£)
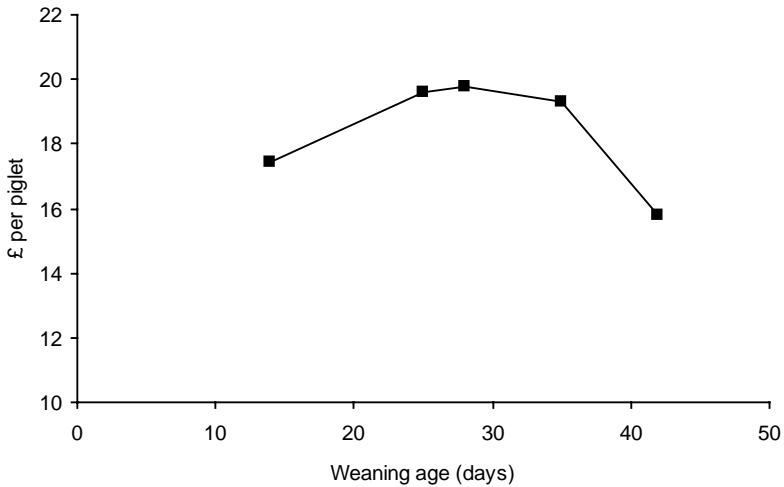
Figure 6. Effect of weaning age on gross margin per piglet (£/piglet)

The data shown in figures 5 to 8 show that far from optimizing at 3 weeks, under the UK conditions of 2007, with very high cereal and other feed prices and poor pig prices, the optimum weaning age was probably between 30 and 35 days.   It is also interesting to note that figure 12.8 shows that, whatever producers did in terms of weaning age, they could not get into a profit situation at all in 2007.
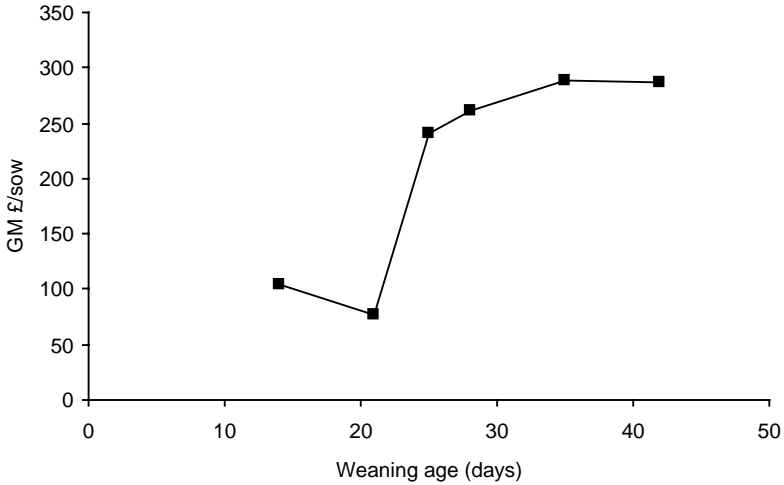
Figure 7. Effect of weaning age on gross margin per sow (£)
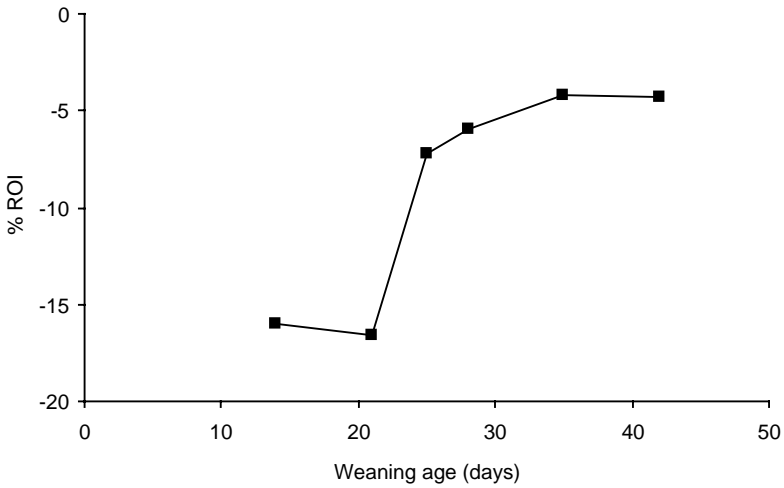
Figure 8. Effect of weaning age on return on capital invested (ROI, %)

Weaning earlier however made matters worse because of the extra costs and no increased revenue to compensate.

The lesson from this exercise is that weaning age should never be regarded as a as a fixed parameter. It cannot obviously be changed in short term business planning but, over medium and long terms, it could be managed to change. The main driver is the ratio between pig prices and general feed costs. In the 1970s in Europe, it was a situation of very low feed costs in real terms and good pig prices. At the time It was probable that the maximum physical productivity curve concurred precisely with the financial productivity curve and hence weaning at 3 weeks of age was probably correct.

In the current economic climate the situation is that weaning ages need to be higher to maximize profitability (or minimize losses).

Figures 9 through 11 illustrate the same analysis but with updated figures for 2009. Clearly there was marked change in the pig industry in this year. Pig prices returned to very high levels in the UK because of a downturn in sow numbers across Europe and also because the weak British pound against the Euro. Clearly these relationships show a much different pattern between these two years and the optimum weaning age changed. In the 2009 economic scenario it would appear that 28 day weaning was the right decision to maximize profitability.
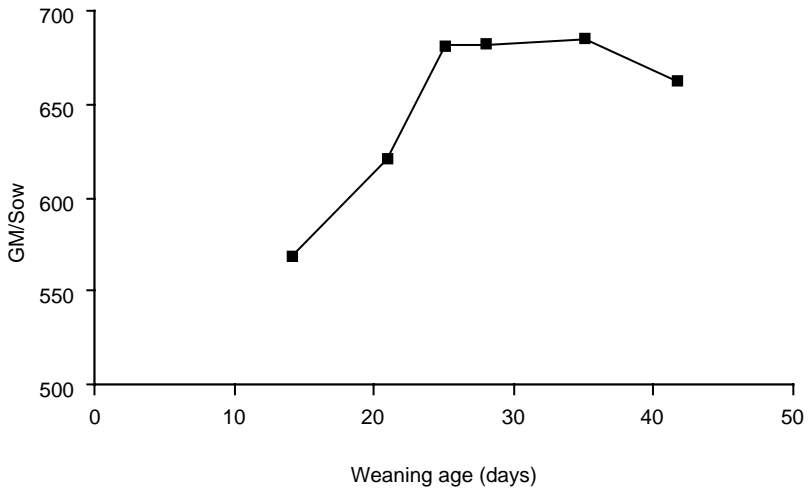
Figure 9. 2009 gross margin per sow
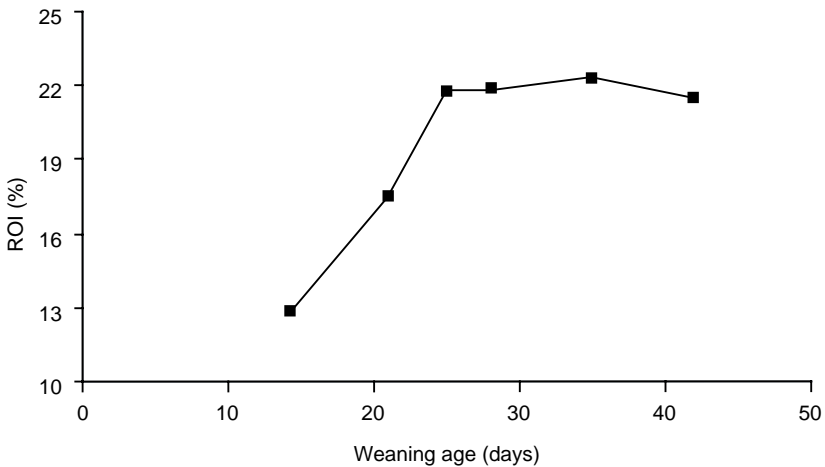
Figure 10. 2009 return on investment
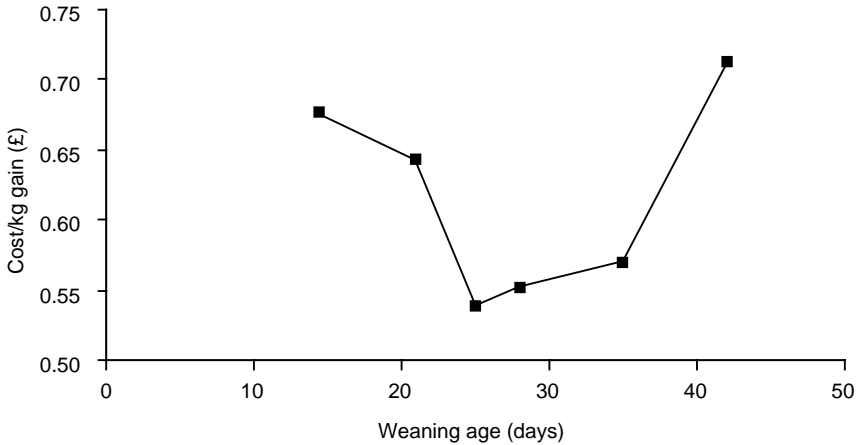
Figure 11. 2009 Cost per kg liveweight gain

These examples from what have been extreme years in the UK for pig production have therefore served to illustrate the likely variation there may be in the financial performance due to changes in weaning management when the background economic situation changes over time.

From this analysis it is also clear that every industry around the world, and also every different pig producing business, has a different cost / price structure. It has therefore been possible to compare and contrast 3 separate industries as they existed in 2007. These data are given in Table 1.

Table 1. Analysis for 3 industries using standardized data and 3 different weaning ages (2007 data)

	United Kingdom £	USA $	Brazil Riel
Cost per kg gain			
21	0.69	0.63	1.12
28	0.62	0.60	1.13
35	0.64	0.5	1.08
Gross margin/sow			
21	77	251	906
28	261	295	943
35	289	350	986
Gross margin per piglet			
21	17.5	31	71
28	19.6	31.5	70
35	19.28	34.6	73
Return on capital %			
21	-16.6	-15.4	19
28	-5.9	-13.2	22
35	-4.2	-10	27

Conclusions

From table 12.1 it can be seen that there are very large differences in the level of financial output in these different industries. The Brazilian industry in 2007 with quite low feed and other costs was at least in profit according to this whereas the USA and UK industries with much higher costs of production where not even breaking even. What is also evident, is that, for all three quite separate pig industries, the conclusion might well be in favour of the later weaning systems *in this economic time-scale*.   In another time-scale the conclusion could be different from this.

    The analysis has also shown very definitely that maximum biological productivity does not concur with maximum financial margin and the calculations for large pig businesses need computing very carefully indeed if margins are to be maximised.

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13

ORGANIC ACIDS IN PIG DIETS

KIRSI PARTANEN[1], JARKKO K. NIEMI[2], AND TIMO KARHULA[2]
[1] *MTT, Animal Production Research, Tervamäentie 179, FI-05840 Hyvinkää, Finland, kirsi.partanen@mtt.fi*
[2] *MTT, Economic Research, Luutnantintie 13, 00410 Helsinki, Finland, jarkko. niemi@mtt.fi, timo.karhula@mtt.fi*

Introduction

Organic acids and their salts have been investigated in detail as growth- and health-promoting feed additives to find effective ways to compensate for the former use of in-feed antibiotics in pig production, which has been banned in the EU (Regulation No 1831/2003). The interest in adding organic acids to piglet feed arose approximately 50 years ago following observations that *Escherichia coli* bacteria are involved in oedema disease and scouring of piglets and that the number of *E. coli* is inversely related to the number of lactic-acid-producing bacteria in the intestinal flora of piglets (Burnett and Hanna, 1963). The initial studies focused on the effects of lactic acid supplementation of feed on *E. coli* counts and on the performance and incidence of scouring in weaned piglets (Burnett and Hanna, 1963; Kershaw *et al.*, 1966); however researchers soon started to investigate various other organic acids and their salts in order to enhance growth performance and reduce scouring in piglets (Cole *et al.*, 1968; Scipioni *et al.*, 1978). Subsequently, the interest in adding acidifiers to piglet feeds extended to fattening pig feed (Kirchgessner and Roth, 1978) and most recently to sow feed (Grela and Kumek, 2002; Øverland *et al.*, 2009).

Data on the effects of organic-acid and salt additions to pig feed have accumulated over the years, and several reviews have already been published summarising both the growth-promoting effects of different organic acids and salts on pigs of different ages, and discussing the possible modes of action of these substances in the gastro-intestinal tract (e.g. Kirchgessner and Roth, 1988; Ravindran and Kornegay, 1993; Partanen and Mroz, 1999). Recently, Tung and Pettigrew (2006) calculated from the literature data that dietary organic acids

enhance the growth rate of weaned piglets on average by 6% during the first four weeks after weaning. The average growth enhancement in growing pigs is 3.5% and that of finishing pigs 2.7%. In general, the reviewers have drawn the following main conclusions about feed acidification: 1) The inclusion of acidifiers in pig diets can improve the growth performance of pigs of different ages. 2) The beneficial effects of acidifiers are most evident within the first few weeks after weaning, and the influence decreases as the pig grows. 3) Although most acidifiers lower dietary pH, they do not affect gastric pH or, more likely, this is difficult to show unless continuous measurement techniques are used. 4) Acidifiers can improve the apparent digestibility of dietary components such as protein, fat and minerals. 5) Acidifiers can modulate the gastro-intestinal microflora, influencing both beneficial and pathogenic microbes, and can reduce the incidence and severity of post-weaning diarrhoea in piglets. 6) Although organic acids and their salts have been studied extensively, the mode of action of acidifiers still remains controversial and should be studied in more detail.

The aim of this chapter is to examine some of the recent developments in the field of feed acidifiers: the development of different organic-acid and salt mixtures and carrier-absorbed and coated acidifiers. Some of these developments have already resulted in new feed acidifiers that are safe to handle and are more effective growth and health promoters than individual organic acids. The chapter will focus primarily on formic-acid-based acidifiers and benzoic acid, while aspects of other organic acids and salts are discussed to a lesser extent. The last part of this chapter presents an economic evaluation of the potential benefits of using acidifiers in sow diets, an acidifier application area in which there are still only few published studies.

Formic acid and formates as feed additives

Formic acid (E236; HCOOH) is a colourless liquid with a strong antibacterial effect. Because it is an irritant and highly corrosive, the handling and storage of formic acid in feed mills has required acid-resistant materials. However, a new ammoniation technique of formic acid and the use of carriers and coatings have reduced the corrosiveness and volatilisation of formic acid and made the handling of formic-acid-based acidifiers safe and convenient (Virtanen *et al.*, 2004). Increased organic acid additions generally reduce dietary pH in a curvilinear manner, and the pH-lowering effect of formic acid is similar to that of lactic acid when the same molar amount is added (Roth and Kirschgessner, 1989). Generally, formic acid additions of 6–8 g/kg are used and these lower the dietary pH by approximately one unit. The antimicrobial action of formic acid is partly due to

its pH-depressing effect (pK$_a$ is 3.75) but, as with all weak organic acids, the main inhibitory effect is due to the un-dissociated acid molecules (Mroz *et al.*, 2006). In their un-dissociated form, weak acids are able to penetrate the cytoplasmic membrane of micro-organisms more effectively, and the lipophilic character of the acid facilitates the penetration. The low molecular weight of formic acid could compensate its weak lipophilic activity (Martin and Maris, 2005). The bactericidal and fungicidal activity of different organic acids is strain-specific and minimum inhibitory concentrations vary with pH (Matsuda *et al.*, 1994; Martin and Maris, 2005).

The salts of formic acid that are used as feed acidifiers are sodium formate (E237; NaHCOO), calcium formate (E238; Ca(HCOO)$_2$) and potassium diformate (HCOOH HCOOK). Formates have little impact on dietary pH (Roth and Kirchgessner, 1989), except potassium diformate which lowers dietary pH although less so than formic acid (Kirchgessner *et al.*, 1997). In aqueous solutions formates dissolve readily, allowing HCOO$^-$ anions to react with H$^+$ from water, and the antimicrobial action is caused by the HCOOH molecule (Mroz *et al.*, 2006).

Formic acid, as well as sodium and calcium formates, are approved feed additives in the EU for the preservation of feedstuffs for all animal categories, whereas potassium diformate is so far the only EU-approved non-antibiotic performance enhancer that can be used for weaned piglets, fattening pigs and sows (Community Register of Feed Additives pursuant to Regulation EC No 1831/2003). In Finland, formic acid is commonly used to preserve liquid by-products, such as distiller's by-products that are used in pig feeding, and additions of 1.5–2 kg/1000 kg liquid by-product are generally used (Asko Rantanen, Altia Corporation, Finland, personal communication). Formic-acid-based preservatives are also used in liquid feed mixtures that are prepared on farms in order to prevent suboptimal fermentation in the feed. The addition level is 0.5–0.8 l/1000 kg liquid feed for continuous use. Higher levels (1–1.5 l/1000 kg liquid feed) are used for 2–3 days if there is suboptimal fermentation in the feed. According to Canibe *et al.* (2007a and b, 2008), the addition of formic-acid-based preservatives to fermented liquid feed has positive effects on the microbial characteristics of the liquid feed since it impedes the proliferation of *Enterobacteriaceae* during the first hours of fermentation. Formic acid addition can also result in enhanced pig performance (Canibe *et al.*, 2008). Recently, Taube *et al.* (2009) recommended the use of organic acids when dietary measures are required against *E. coli* and *Salmonella*.

In the animal body, formic acid is metabolically important in the transfer of one-carbon substances and is readily absorbed after ingestion and metabolised to carbon dioxide and water. However, very high intakes of formic acid or formates can be toxic, and the accumulation of formate in the blood can cause toxic symptoms similar to methanol poisoning (Makar *et al.*, 1990). Based on dose-

response studies, weaned piglets respond to high levels of dietary formic acid by decreasing voluntary feed intake (Eckel *et al.*, 1992; Laurinen and Partanen, unpublished results). Figure 1 shows the effect of high inclusions (12–60 g/kg feed) of ammoniated water-free formic acid (AMMFOR99S10; Kemira Chemicals Oy, Finland; formic acid 620 g/kg, ammonium formate 370 g/kg, and water <10 g/kg) on the feed consumption of weaned piglets that had *ad libitum* access to acidified diets for three weeks starting from day 4 after weaning. The average feed consumption decreased from 808 to 382 g/d, and the growth rate consequently decreased from 484 to 233 g/d, while the feed conversion ratio remained unaffected.
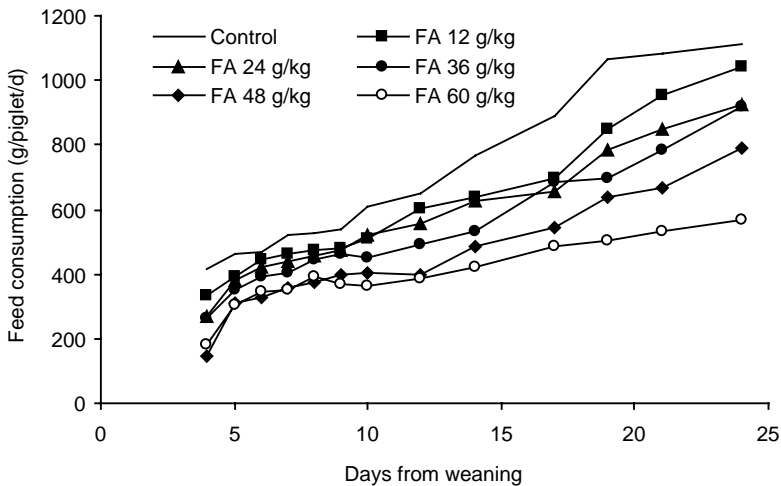
Figure 1. Effect of increased additions of ammoniated water-free formic acid (FA) on the feed consumption of weaned piglets (Laurinen and Partanen, unpublished results).

Impacts of organic acids and salts on pig performance

Different organic acids and salts have different impacts on the performance of weaned piglets and fattening pigs, and the impacts are also highly variable within specific organic acids (Partanen and Mroz, 1999; Tung and Pettigrew, 2006). This is because the acidifiers influence the performance of pigs indirectly via gastrointestinal microflora, and different organic acids have distinct differences in their antimicrobial activity (Matsuda *et al.*, 1994; Martin and Maris, 2005). Furthermore, different doses can either stimulate or inhibit microbial growth (Biagi and Piva, 2007; Apajalahti *et al.*, 2009) which may contribute to variable performance responses. According to Tung and Pettigrew (2006), citric and fumaric acid additions enhanced the growth performance of weaned piglets by

approximately 5%, and the average response of fattening pigs to formic acid and formates was 3 and 5%, respectively. However, none of the analysed effects (relative responses to the negative control) was significantly different from zero.

In the review of Partanen (2003), the response of weaned piglets to dietary organic acid additions was calculated as an effect size, which is a standardised mean difference (the difference between the experimental and control group means divided by their pooled standard deviation) and it was adjusted for sample size. Effect sizes are difficult to interpret, as they do not directly indicate how much pig performance is influenced by acidifiers. However, the estimated effect sizes can be used to quantify the degree of overlap between the distribution of observations in the experimental and control groups, and the effect size represents the proportion of control group scores that are less than the average score in the experimental group (Hedges and Olkin, 1985). Effect sizes of 0.5, 1.0, and 1.5 imply that the score of the average individual in the experimental group exceeds that of 0.69, 0.84 and 0.93 of the individuals in the control group, respectively.

The mean effect sizes of 0.54, 0.26, 0.30, and 0.62 calculated for the weight gain of weaned piglets fed diets supplemented with formic acid, fumaric acid, citric acid, and potassium diformate, respectively, imply that the weight gain of the average individual in the treatment group exceeded that of 0.71, 0.60, 0.62, and 0.73 of the pigs in the control group, respectively (Figure 2). The respective effect sizes of -0.87, -0.74, -0.92 and -0.96 obtained for feed to gain ratio imply that the average pig in the treatment group used less feed per kg of weight gain than 0.80, 0.77, 0.82, and 0.83 of the pigs in the control group. Similarly, the response of fattening pigs to different acidifiers is presented in Figure 3, and formic acid and potassium diformate are the most efficient acidifiers in this comparison.
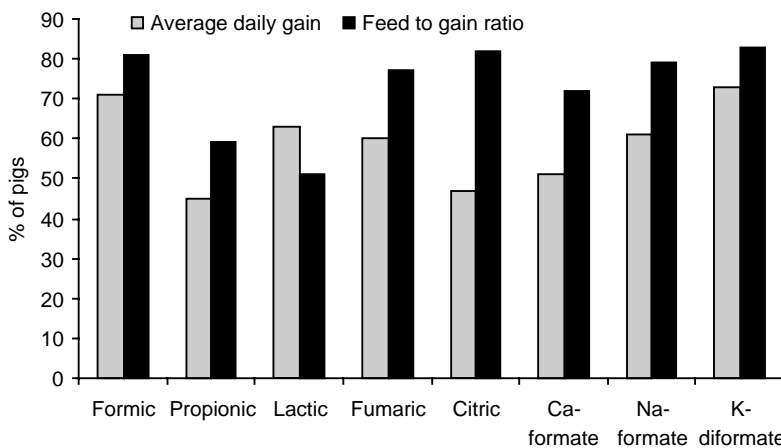
Figure 2. The response of weaned piglets to different organic acid and salt additions of ≤ 25 g/kg feed based on meta-analysis of the literature data (after Partanen, 2003). The estimated effect sizes are presented as the percentage of pigs in the experimental group whose response exceeds that of the pigs in the control group.
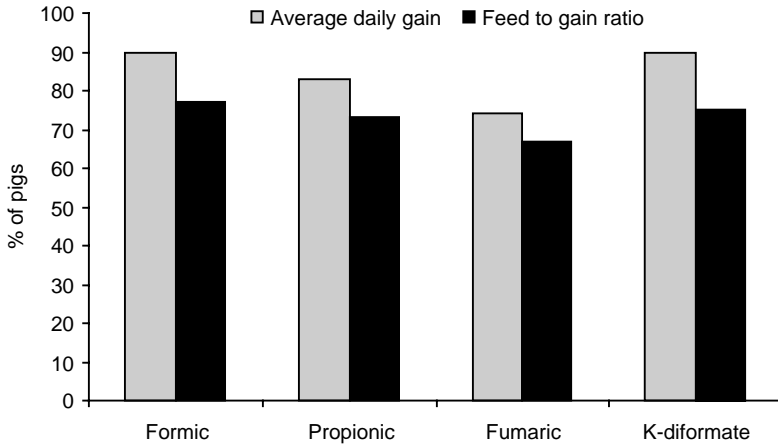
Figure 3. The response of fattening pigs to different organic acid and salt additions of ≤ 25 g/kg feed based on meta-analysis of the literature data (after Partanen, 2003). The estimated effect sizes are presented as the percentage of pigs in the experimental group whose response exceeds that of the pigs in the control group.

Formic acid is an attractive feed acidifier because it seems to enhance the growth performance of weaned piglets and fattening pigs at lower dosages (< 10 g/kg feed) than those of other organic acids and salts (generally ≥ 15 g/kg feed; Partanen, 2003). The shape of the response curve of pigs to increased acidifier additions is naturally of great interest, as this information is needed to optimise the response of pigs in relation to the cost of using the acidifier. Unfortunately, the number of dose-response studies with several incremental acidifier additions is still limited for most acidifiers, and the majority of published studies have had only 1–2 addition levels. Thus it is difficult to estimate the shape of response curve when combining the results of several studies (the meta-analysis). This is clearly seen in Figures 4 and 5, which show the measured average daily gain of weaned piglets and fattening pigs, respectively, that were fed the negative control diet and diets supplemented with increased levels of formic acid in the experiments found in the literature. The majority of studies had an addition level of <15 g/kg. When additions of <10 g/kg were used, the average growth enhancement of weaned piglets was 6%, and that of fattening pigs was 5%.

Inert carriers can have both positive and negative effects

Absorbing formic acid into an inert carrier, silica or diatomaceous earth is one possible way of making the handling of acids easier and safer (Virtanen *et al.*, 2004). These carriers can absorb acid equivalent to about 0.50 of their weight without expanding in volume. In the study of Partanen *et al.*, (2007b), the addition
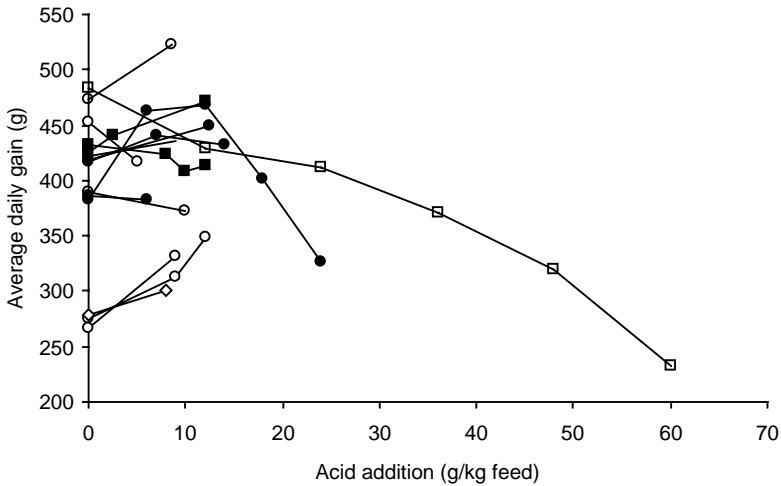
Figure 4. Average daily gain of weaned piglets fed diets supplemented with different doses of formic acid (open circle = 1-phase feeding + formic acid, solid circle = 2-phase feeding + formic acid, open square = 1-phase feeding + ammoniated water-free formic acid, and solid square = 2-phase feeding + ammoniated water-free formic acid. References: Kirschgessner and Roth, 1987a and b; Bolduan *et al.*, 1988; Baustad, 1992; Eckel *et al.,* 1992; Eidelsburger *et al.*, 1992; Maribo *et al.,* 2000a; Partanen *et al.,* 2002a; Manzanilla *et al.*, 2004; Eisemann and van Heugten, 2007; Partanen *et al.,* 2007a, Canibe *et al.,* 2008; Laurinen and Partanen, unpublished results)
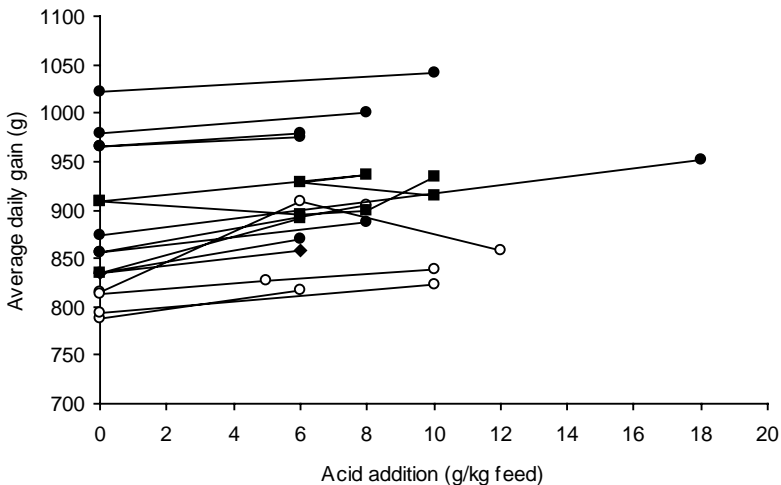
Figure 5. Average daily gains of fattening pigs fed diets supplemented with formic acid (open circle = 1-phase feeding + formic acid, solid circle = 2-phase feeding + formic acid, and solid square = 2-phase feeding + ammoniated water-free formic acid. References: Baustad, 1992; Øverland and Lyso, 1997; Siljander-Rasi *et al*., 1998; Partanen *et al*., 2002b; Øverland *et al*., 2000; Siljander-Rasi *et al*., 2006; Canibe *et al*., 2005; Eisemann and van Heugten, 2007; Partanen *et al*., 2007a).

of formic acid in liquid form or absorbed in diatomaceous earth resulted in the same average daily gain of weaned piglets (300 vs. 308 g, P > 0.05) and of fattening

pigs (863 vs. 860 g/d, P > 0.05) when the diets contained 8 and 6 g formic acid /kg feed. In the study of Siljander-Rasi *et al.*, (2006), the diets for growing and finishing pigs were supplemented with 6 g ammoniated water-free formic acid either as such or absorbed in silica /kg. The growth rates were 980 and 975 g/d (P > 0.05), but they did not differ significantly from the negative control (965 g/d). The use of carrier did however reduce the apparent faecal digestibility of dietary components. In the study of Partanen *et al.*, (2006a), the use of carrier (5 g/kg feed) resulted in reduced growth performance of the weaned piglets when blends of different inorganic and organic acids and salts were used. However, small inclusions (3–6 g/kg feed) of carrier-absorbed acidifiers have given positive performance results in some studies (Virtanen *et al.*, 2004; Partanen *et al.*, 2006b). Thus the level of carrier in the diet may influence the growth-promoting effect of carrier-absorbed acidifiers. It is not known where and how fast acids are released from the carrier in the gastrointestinal tract. It is possible that the main action site of carrier-absorbed acidifiers is not in the stomach, but further down the small intestine as it is in the coated acidifiers (Piva *et al.*, 2007).

Attempts to improve the efficacy of formic acid

Although formic acid is an effective antimicrobial and growth-promoter as such, there is an interest in increasing its efficacy by mixing it with other organic acids or salts. Small sorbate and benzoate additions are interesting since they are known to have antimicrobial effects as preservatives in relatively small dosages when they are used in strongly acidic solutions (Chipley, 1993; Sofos and Busta, 1993). The growth-promoting effect of formic acid can be improved by mixing it with a small amount of potassium sorbate or sodium benzoate (Partanen *et al.,* 2002a; Partanen *et al*., 2007b). Although the solubility of potassium sorbate and sodium benzoate is good in water, these salts are poorly soluble in formic acid, and additions < 5 g acid/kg can result in precipitation. Propionic acid addition improves the solubility of potassium sorbate and sodium benzoate to formic acid, and such acid mixtures have enhanced the growth performance of fattening pigs (Partanen *et al*. (2007b).

Another way of overcoming the problem of sorbate precipitation in formic acid is to absorb formic acid first into a solid carrier such as diatomaceous earth and then coat it with potassium sorbate. When potassium sorbate solution is sprayed onto the carrier containing formic acid, the sorbate is converted to sorbic acid forming a coating resistant to water and acidic environments. In fattening pigs, the carrier-absorbed and sorbate-coated formic acid has enhanced the growth performance of pigs in the growing and finishing periods (Sigfridson and Göransson, 2003; Partanen *et al.*, 2006; Khajarern and Khajarern, 2006). Figure 6

shows the integrated results of these three studies analysed according to St-Pierre (2001) to create dose response curves of increased additions of this acidifier. The estimated regression equations for average daily gain and feed conversion ratio are presented in Table 1. In weaned piglets, diets with 3 g sorbate-coated formic acid/kg resulted in higher growth rate compared to the negative control, whereas 6 g/kg had no benefits. In fact, this latter level may have been too high, because it resulted in a higher prevalence of gastric alterations in piglets, as became evident when they were slaughtered at the end of the experiment. The length and severity of post-weaning diarrhoea were the lowest when diets contained 3 g/kg.

Table 1. Estimated regression equations ($Y = a + bX$ or $Y = a + bX + cX^2$) for the response of growing and finishing pigs to increased additions of carrier-absorbed and sorbate-coated formic acid, based on the mixed model analysis of three studies that is presented in Figure 6.

Y	*Intercept*	*a*	*b*	*R2*
Average daily gain				
Grower period	782 (5) [***]	14.8 (2.2) [***]	-1.22 (0.19) [***]	0.86
Finisher period	872 (7) [***]	5.1 (1.4) [**]		0.61
Total fattening	830 (6) [***]	10.0 (2.7) [**]	-0.59 (0.23) [*]	0.74
Feed conversion ratio	2.79 (0.01) [***]	-0.014 (0.003) [**]		0.75
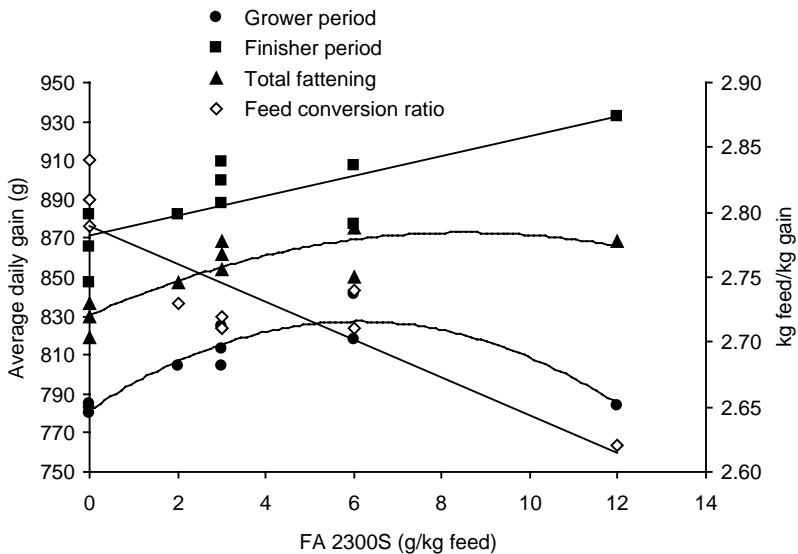
Figure 6. Effect of increased additions of carrier-absorbed and sorbate-coated acidifier (FA 2300S) on the performance of fattening pigs. Plots of adjusted observations and the mean regression line across studies, based on the mixed model analysis of three studies (Sigfridson and Göransson 2004; Partanen *et al.*, 2006b; Khajarern and Khajarern 2006a).

The additions of combinations of formic acid and lactic acid have also given promising results and enhanced the performance of weaned piglets (Maribo *et al*., 2000b; Jørgensen and Boes, 2004). Such blends of formic acid and lactic acid (1:1 and 2:1) have also been reported to reduce the number of coliforms in the small intestine of piglets more efficiently than plain formic acid (Franco *et al*. 2005). In fattening pigs, a mixture of formic and lactic acids (3:7) has given positive results already at small addition levels (3–6 g/kg feed) (Partanen *et al.,* 2006b). The mixing of organic acids with essential oils or plant extracts which also have antimicrobial properties could be another possibility to improve the growth-promoting effect of acidifiers (Manzanilla *et al*., 2004; Piva *et al*., 2007; van Dijk and Van den Hoven, 2009).

Benzoic acid and benzoates as feed additives

Benzoic acid (E210; C_6H_5COOH) is a crystalline powder with a sweet or astringent taste. It occurs naturally in many acidic fruits and berries and is widely used in the preservation of foods. As a preservative, benzoic acid is generally used in the form of sodium benzoate (E211; C_6H_5COONa) because the sodium salt dissolves better than the acid in water (550 g/l vs 3.4 g/l at 25°C). Sodium benzoate is widely used in particular for the preservation of acidic foods and juices because sodium benzoate in small concentrations effectively controls the growth of bacteria, moulds, and yeast in an acidic environment (pH < 4.5). In food preservation, benzoic acid can also be used in the form of potassium benzoate (E212; C_6H_5COONK) and calcium benzoate (E213; $(C_6H_5COO)_2Ca$). In current EU legislation, sodium benzoate is a permitted preservative in silages only. Benzoic acid is an approved feed additive as an acidifying agent, and it can be used in fattening pig diets at levels of 5–10 g/kg feed (EU 757/2007).

In the pig, most ingested benzoic acid is eliminated from the body by conjugation with glycine which occurs in the liver and kidneys, and the product benzoyl glycine (hippuric acid) is rapidly excreted in urine (Bridges *et al.*, 1970). Hippuric acid is responsible for the pH decrease observed in the urine of pigs fed diets containing benzoic acid or calcium benzoate (Mroz *et al.,* 2000; Kluge *et al*., 2006). Lower urinary pH is advantageous from the environmental point of view since it depresses the pH of slurry and consequently reduces ammonia emission from the slurry (Mroz *et al.,* 2000; Canh *et al.*, 1998). According to Canh *et al*. (1998), one unit decrease in the pH of urine and slurry decreases the ammonia emission by ca 20% and 48%, respectively.

Benzoic acid is an antimicrobial which influences the growth of micro-organisms in the gastro-intestinal tract of the pig. According to Knarreborg *et*

al. (2002), benzoic acid reduces the viability of coliforms more effectively than other organic acids. Similarly, Biagi and Piva (2007) showed that benzoic acid restricts the fermentation by pig caecal microflora more than other organic acids by using the *in vitro* gas production technique. Benzoic acid additions have been reported to restrict the numbers of total aerobic and anaerobic bacteria and lactic acid bacteria in the stomach of piglets and gram negative bacteria in the duodenum (Kluge *et al.*, 2006). In weaned piglets, benzoic acid additions of 5–10 g/kg feed have also been reported to increase the feed intake and average daily gain of weaned piglets (Kluge *et al.*, 2006; Guggenbull *et al.*, 2007; Torrallardona *et al.*, 2007). In the study of Partanen *et al.*, (2002b), the growth performance of weaned piglets fed a diet with 10 g sodium benzoate/kg did not differ significantly from the negative control.

Modes of action of feed acidifiers

Although several hypotheses have been proposed and investigated, the exact mechanisms behind the growth-promoting effect of organic acids are still unclear. According to Kirschgessner and Roth (1988), dietary organic acids act as antimicrobials in the feed and thus improve its hygienic quality. This theory is supported by the finding that the performance of weaned piglets whose liquid feed was contaminated with *E. coli* improved due to the addition of formic acid (Canibe *et al.*, 2008).

Kirschgessner and Roth (1988) also suggested that organic acids reduce gastric pH which increases pepsinogen activation in the stomach and consequently improves protein digestibility. This is perhaps the most favoured hypothesis used to explain the mode of action of dietary organic acids, but it is also controversial. The lowering of gastric pH has been difficult to show when spot samples were taken from the gastric contents of euthanized pigs. The pH of gastric contents is not constant; it fluctuates between different parts of the stomach as well as with time after feeding. Some studies in which several consecutive pH measurements have been taken from pigs with gastric cannulae or pH-measuring probe in the stomach have been able to show that acidifiers do influence gastric pH and shorten the period of high pH after feeding (Thomlinson *et al.*, 1981; Mroz *et al.*, 2006). In addition, acidified drinking water or liquid diets with fermentation or added acids could be more effective than acidified feed in lowering gastric pH (Jensen, 1998).

It is questionable whether increased pepsin activity due to the lower gastric pH is the cause of the improved apparent protein digestibility seen with diets supplemented with organic acids. Maribo *et al.* (2000b) measured enzyme activities

in different sections of the gastrointestinal tract but found no significant differences in pepsin activity in the stomach despite lowered gastric pH. There is considerable microbial activity in the stomach and the small intestine of growing pigs and bacterial nitrogen represents a considerable proportion (0.40 to 0.66) of ileal nitrogen (Bartelt *et al.*, 1999). Thus the improved apparent ileal protein and amino acid digestibilities could be due to a reduced flow of bacterial nitrogen in the ileum (Partanen *et al.*, 2001; Partanen *et al.*, 2007a). When bacterial growth is restricted by organic acids, more amino acids are absorbed rather than being incorporated into bacterial protein. This should result in improved nitrogen retention, that has been observed in some studies. However, the role of increased absorption of nutrients due additionally to feed acidification cannot be ruled out.

Ravindran and Kornegay (1993) suggested that lower gastric pH caused by dietary organic acids would influence the gastric emptying rate and thus digestibility. However, Partanen *et al.* (2007a) did not find any differences in the retention time of digesta through the upper digestive tract measured with a pulse dose marker method, but acid addition improved the apparent digestibility of several dietary components. Organic acid have also been considered to have some metabolic effects (Kirschgessner and Roth, 1988).

In growing pigs, formic acid supplementation has been shown to result in noticeable improvements in both ileal and total tract fat digestibility. Improved fat digestibility is generally associated with reduced microbial activity in the digestive tract. Lactic acid bacteria exhibit bile-salt hydrolase activity, which impairs lipid digestion in the host animal (Anderson *et al.*, 1999).

Organic acids can also improve the apparent total tract digestibility and/or retention of calcium and phosphorus (Han *et al.*, 1998; Boling *et al.*, 2000). When phosphorus-deficient diets are used, improved calcium and phosphorus digestibility can increase their retention. However, improved mineral retention has also been observed with diets containing sufficient phosphorus (Roth *et al.*, 1998b). Young piglets have high calcium and phosphorus requirements. Dietary mineral supplements act as a buffer in the stomach and can provide more favourable conditions for the proliferation of *E. coli*. Dietary calcium and phosphorus content can be reduced by microbial phytase addition. Some studies also indicate that lower gastric pH due to feed acidification could enhance the action of microbial phytase (Han *et al.*, 1998; Boling *et al.*, 2000).

Acidic gastric conditions are considered essential for the prevention of the survival of ingested pathogens in the stomach so that they do not gain access to the small intestine. Disease can also result from increased numbers of bacteria that colonise the intestines before weaning (Hampson, 1994). The outbreak of post-weaning diarrhoea is caused by the proliferation of enterotoxigenic bacteria, mainly *E. coli* in the small intestine and/or the fermentation of less digestible components of weaner diet in the large intestine (Hampson, 1994). According

to Hansen *et al*. (2007), feeding organic acids to weaned piglets enhances the biological barrier function against pathogenic bacteria in the stomach. Although coarsely ground feed resulted in more coherent gastric material with lower gastric pH finely ground feed, the supplementation of organic acid (blend of formic and lactic acids 1:1) was necessary to achieve a high enough concentration of organic acids and low pH to reduce the population of enterobacteria in the stomach, and prevent them colonizing the lower parts of the gastrointestinal tract. Several studies have reported decreased faecal *E. coli* numbers with organic acid supplemented diets (Tsiloyiannis *et al*.,2001; Kluge *et al*.; 2006; Canibe *et al*., 2008). According to Knarreborg *et al*. (2002), the effectiveness of organic acids in reducing the viability of coliforms exhibits the following order: benzoic acid > fumaric acid > lactic acid > butyric acid > formic acid > propionic acid.

Organic acids not only act on pathogenic bacteria but also modify beneficial flora. *In vitro* studies have shown that organic acids can inhibit microbial fermentation in the gastrointestinal tract, but some organic acids or their certain concentrations may stimulate fermentation (Partanen and Jalava, 2005; Biagi and Piva, 2007; Apajalahti *et al*., 2009). Organic acids may also reduce yeasts in the digesta and faeces of pigs (Canibe *et al*., 2001). Reduced microbial fermentation means that more fermentable carbohydrates are available to the animal. In the case of antibiotics, reduced microbial fermentation almost entirely accounts for the improved feed to gain ratio (Jensen, 1998). Only few studies have investigated the impact of the composition of feeds on the effects acidifiers. Partanen *et al.*, (2001, 2007a) found that formic acid addition improved the apparent digestibility of several components more in high-fibre than in medium-fibre diets. However, the performance response of growing pigs to acidified diets was not significantly different between the medium- and high-fibre diets (Partanen *et al*., 2002a).

Strongly acidic gastric conditions are not desirable because they may result in the development of gastric ulcers (Argentzio and Southworth, 1975). Dietary organic acids have not been reported to increase the prevalence of gastric lesions in weaned piglets or fattening pigs (Øverland *et al*., 2000; Canibe *et al*., 2001; Partanen *et al*., 2002). However, recent unpublished results of Partanen and Siljander-Rasi indicate that added acidifiers could contribute to the development of gastric lesions.

Economic evaluation of the possible benefits of using feed acidifiers in sow feeds

Although the effects of feed acidifiers have been studied extensively in weaned piglets and fattening pigs, the possible benefits of using them also in sows diets have so far been rarely scarcely (Grela and Kumek, 2002; Khajarern and Khajarern,

2006, Øverland *et al.*, 2009). Because sow performance studies are expensive, a preliminary economic evaluation was conducted to estimate the possible benefits of using acidifiers in sow feeds using a static farm model of piglet production (Karhula and Leppälä, 2006) and a dynamic programming model that maximises returns per sow place (Niemi, 2008).

Farm models allow the examination of issues such as single return or cost items, prices or feed characteristics while other factors are kept constant. In the study, there was focus on differences in production with and without feed acidifier but not on the level of production results. The farm model is constructed partly on the basis of data from the Finnish Farm Accountancy Data Network and various validated standards and recommendations regarding production conditions. As a result of the farm model, the production cost consists of variable and fixed costs, the desired farm family income and interest demand on capital. Variable costs include materials and supplies. Fixed costs include overheads, repair and maintenance costs of buildings and machinery, and insurance costs.

Management practices are exogenously given in a farm model, whereas they are endogenously determined in dynamic programming. This helps to gain the full benefits from the production activity and to improve the profitability of piglet production. The stochastic dynamic programming model optimises sow replacement decisions by taking into account the productivity prospects of subsequent parities and of gilts. The Bellman equation (Bellman, 1957) is of the form:

$$V_t(\mathbf{x_t}) = \max_{u_t}\left\{R_t(\mathbf{x_t}, u_t) + \beta E(V_{t+1}(\mathbf{x_{t+1}}))\right\}, \quad t = 1,...\infty \tag{1}$$

s.t. $\mathbf{xt}_{+1} = g(\mathbf{x_t}, u_t)$, and $\mathbf{x_t}$, is given,

where V_t is the value of the capacity unit, t is the time index, $\mathbf{x_t}$ is the state vector containing information on parity and litter size, and other information relevant to the replacement decision, u_t is the decision rule, $R_t(\mathbf{x_t}, u_t)$ is the instantaneous cash-flow function (i.e. production costs and returns from the current parity), E is the expectation operator, V_t+1 is the next-period value function, β is the discount rate, and $g(\mathbf{x_t}, u_t)$ is the transition equation.

The dynamics of litter size and parity are modelled in a manner similar to Kristensen and Søllested (2004). Other variables such as prices and mortality rates are kept constant throughout the simulations except when they are functions of litter size and parity number.

In both models, the average production level when not using acidifier was based on Danish herd records from 2006 (default scenario in Table 2). The summary of alternative scenarios for sow and suckling piglet performance examined in this study is presented in Table 2 and those for weaners in Table 3, respectively. In

Table 2. Default parameter values for sow reproductive performance used in the economic analysis and the magnitude of changes considered in performance improvements 1, 2, and 3 in each trait (T1 to T4) individually and in all traits simultaneously.

Trait which is improved	Default	Improvement 1					Improvement 2					Improvement 3				
		T1	T2	T3	T4	All	T1	T2	T3	T4	All	T1	T2	T3	T4	All
The number of piglets born per litter	15.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piglet mortality at birth, %	11.2	-1.0	-	-	-	-1.0	-2.0	-	-	-	-2.0	-4.0	-	-	-	-4.0
The piglets born dead per litter	1.7	-0.2	-	-	-	-0.2	-0.3	-	-	-	-0.3	-0.6	-	-	-	-0.6
The number of piglets born alive per litter	13.5	0.2	-	-	-	0.2	0.3	-	-	-	0.3	0.6	-	-	-	0.6
Piglet mortality before weaning, %	14.1	-	-1.0	-	-	-1.0	-	-2.0	-	-	-2.0	-	-4.0	-	-	-4.0
The number of piglets weaned per litter	11.6	0.1	0.1	0.0	0.0	0.3	0.3	0.3	0.0	0.0	0.5	0.5	0.5	0.0	0.0	1.1
Weaning to service interval, d	6.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Non-productive days per litter	16.0	-	-	-	-0.5	-0.5	-	-	-	-1.0	-1.0	-	-	-	2.0	2.0
The share of inseminations failed	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Farrowings per insemination	0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Interval between successive farrowings, d	164	-	-	-	-0.5	-0.5	-	-	-	-1.0	-1.0	-	-	-	-2.0	-2.0
Piglet mortality after weaning, %	3.2	-	-	-0.5	-	-0.5	-	-	-1.0	-	-1.0	-	-	-2.0	-	-2.0
The number of piglets sold per litter	11.2	0.1	0.1	0.1	-	0.3	0.3	0.3	0.1	-	0.6	0.5	0.5	0.2	-	1.3
Piglet growth, g/d	439	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piglets' age upon marketing, d	65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Litters per sow per year	2.23	-	-	-	0.01	0.01	-	-	-	0.01	0.01	-	-	-	0.03	0.03
Piglets weaned per sow per year	25.8	0.3	0.3	-	0.1	0.7	0.6	0.6	-	0.2	1.4	1.2	1.2	-	0.3	2.8
Piglets sold per sow per year	25.0	0.3	0.3	0.1	0.1	0.8	0.6	0.6	0.3	0.2	1.6	1.1	1.2	0.5	0.3	3.2

Each column corresponds to one scenario. Feed conversion ratio and average daily gain of piglets are adjusted as a percentage change from the default value.
- indicates no change when compared to the default value.
The traits that were considered to change were the percentage of stillborn piglets, piglet mortality between farrowing and weaning, and the number of non-productive days per litter (days from first oestrus to conception). The change in each parameter is measured as difference to the default value.

Table 3. Scenarios for performance improvements 1, 2, and 3 in the average weight gain and feed conversion ratio of weaned piglets.

Trait		Default	Improvement 1			Improvement 2			Improvement 3		
			TP1	*TP2*	*Both*	*TP1*	*TP2*	*Both*	*TP1*	*TP2*	*Both*
TP1	Average daily gain	100%	2.5 %	-	2.5 %	5.0 %	-	5.0 %	10.0 %	-	10.0 %
TP2	Feed conversion ratio	100%	-	-2.0 %	-2.0 %	-	-4.0 %	-4.0 %	-	-8.0 %	-8.0 %

Each column corresponds to one scenario. Feed conversion ratio and average daily gain of piglets are adjusted as a percentage change from the default value.

- indicated no change when compared to the default value.

these tables, each column represents one scenario. As studies on the measured effects of feed acidifiers on the performance of sows are scarce, it was not possible to estimate exact scenarios on how the performance of sows and piglets would respond to different doses of feed acidifier. Based on previous research, the feed acidifiers were expected to influence the following traits: piglet mortality at birth and during the suckling and post-weaning periods, the weaning to conception interval of sows, and the feed conversion ratio and daily weight gain of weaned piglets.

Effects on piglet mortality were examined at three different levels by improving one trait at a time: 1) Mortality at birth decreases 1, 2 or 4 % points compared to the average production level. 2) Mortality during the suckling period decreases 1, 2, or 4 % points compared to the average production level. 3) Mortality during the post-weaning period (up to ca 30 kg body weight) decreases 0.5, 1, or 2 % points compared to the average production level. It was assumed that both the creep feed and weaner diet contained acidifier. The average daily gain was assumed to increase by 2.5, 5, or 10% and feed conversion ratio by 2, 4 or 8%. Feed acidifiers were assumed to decrease the medication of post-weaning diarrhoea by 2.5, 5, or 10 percentage points from the baseline situation, in which 0.20 litters are medicated. Effects on medication were examined jointly with changes in the post-weaning mortality. The cost of medication was assumed to be €0.63 per piglet.

The feed intake of sows was assumed to increase by 0.2 kg per day during lactation when using feed acidifier (Khajarern and Khajarern, 2006a). Sows receiving feed acidifier were assumed to loose 2.5 kg less weight during lactation than sows not receiving feed acidifier. Based on results by Yang *et al.*, (1989), it was assumed that sows without access to feed acidifier had to eat 4.7 kg of feed per additional kilogram of weight lost in order to reach the same body condition as sows receiving feed acidifier. Smaller weight loss during the lactation period could reduce the weaning to oestrus interval and decrease the percentage of sows that return to oestrus after insemination. The investigated magnitudes of effect were decreases of 0.5, 1, or 2 days in the number of non-productive days per litter.

According to the farm model, using cost assumptions show in Table 4, improvements in sow and litter performance reduce production cost by €1.5–€6.0 per piglet in comparison to the default scenario. These results exclude the costs of the acidifier. The costs of different acidifier doses are given in Table 5. Variable costs account for about 0.40 of the production costs. The improvement scenarios result in a saving of at most €1.8 per piglet in the variable production costs in comparison to the default scenario. The desired farm family income accounts for 0.20 of production costs, and pig performance improvements result in €0.4 to €1.6 lower wage demand costs per piglet. The interest on capital represents 0.10 of production costs, and pig performance improvements reduce these costs

at most by €0.7 per piglet. Fixed costs amount to 0.30 of the production costs, and improvements in pig performance reduce them by €0.5 to €1.9 per piglet (Table 5).

Table 4. Selected prices used in the economic analysis (the use of parameters may differ between models).

Item	Price, €	Usage
Gestation feed	0.19 per fu	2.4 fu/sow/d in gestation days 1-7, 2.8 fu/sow/d in gestaion days 8-109. Thereafter the lactation feed.
Lactation feed	0.22 per fu	2.4 fu/sow/d before farrowing, 3.5 fu/d on farrowing day, and thereafter 2.5 fu/sow/d + 0.6 fu/suckling piglet
Weaning to service feed	0.22 per fu	3.5 fu/sow/d until service
Creep feed	0.53 pr kg	1 kg per piglet before weaning.
Piglet feed	0.34 per kg	34.4 kg per piglet between weaning and marketing.
Piglet price	51 per piglet	
Labour	12.3 per hour	Adjusted according to litter size using the data from Parviainen.
Interest rate	5% per year	The price of capital or the discounting of future net returns.
Diarrhoea treatment	0.63 per piglt	Default: 0.20 of litters are treated
Other variable costs	141 per litter	
Replacement sow	242 per gilt	Including income from slaughtered sow
Insemination cost	25 per event	
Investment cost per sow place	3159 € per sow	For litter size of 10 piglets sold. In the long-run, extra/lacking piglets adjust the cost by €140 per piglet.
Maintenance, insurance	1% per year	Calculated from the replacement price of production facilities.
Overhead costs	4%	Calculated on the top of business cost.

When all traits are improved simultaneously, production costs per sow place per year are €11 to €39 higher than in the default scenario. Variable costs are €9 to €30, fixed costs at most €3, the wage demands of the desired farm family income at most €2, and the interest on capital €1 to €4 higher per sow per year in the improvement scenarios than in the default scenario. This is due to the fact that input use per sow increases and input cost per piglet decreases when e.g. piglet mortality decreases (Table 6). The cost of feed acidifier must be added to these

Table 5. Production cost (€/piglet or €/sow/year) excluding the cost of feed acidifier, its distribution according to type of cost for the default scenario, and the magnitude of change considered in performance improvements 1, 2, or 3 in each trait (T1 to T4) individually and in all traits when compared to the default scenario, and the cost of feed acidifier calculated for different doses when used in both lactation feed and weaner feed.

€ per piglet

| | | Difference, € per piglet | | | | | | | | | | | | | | |
| | | Improvement 1 | | | | | Improvement 2 | | | | | Improvement 3 | | | | |
	Default	T1	T2	T3	T4	All	T1	T2	T3	T4	All	T1	T2	T3	T4	All
Variable costs	25.1	-0.1	-0.1	0.0	0.0	-0.4	-0.3	-0.3	-0.1	-0.1	-0.9	-0.6	-0.6	-0.2	-0.2	-1.8
Fixed costs	17.3	-0.2	-0.2	-0.1	0.0	-0.5	-0.4	-0.4	-0.2	-0.1	-1.0	-0.7	-0.7	-0.3	-0.2	-1.9
Wage demand of the entrepreneur family	14.8	-0.1	-0.1	0.0	0.0	-0.4	-0.3	-0.3	-0.1	-0.1	-0.8	-0.6	-0.6	-0.3	-0.1	-1.6
Interest demand on capital	7.2	-0.1	-0.1	-0.1	0.0	-0.2	-0.1	-0.1	-0.1	0.0	-0.3	-0.3	-0.3	-0.1	-0.1	-0.7
Total	64.3	-0.5	-0.5	-0.1	-0.1	-1.5	-1.0	-1.1	-0.4	-0.3	-3.1	-2.1	-2.2	-0.9	-0.6	-6.0

Cost of feed acidifier	Addition (g/kg)	€/piglet		
		0.28	0.27	0.26
		0.55	0.54	0.52
		1.10	1.08	1.03
		1.66	1.62	1.55

€ per piglet / € per sow

| | | Difference, € per sow | | | | | | | | | | | | | | |
| | | Improvement 1 | | | | | Improvement 2 | | | | | Improvement 3 | | | | |
	Default	T1	T2	T3	T4	All	T1	T2	T3	T4	All	T1	T2	T3	T4	All
Variable costs	626.8	4.5	4.6	3.1	1.8	8.5	7.2	7.4	4.3	1.8	15.4	12.5	12.9	6.7	1.7	29.6
Fixed costs	431.8	0.4	0.4	0.3	0.2	0.6	0.6	0.7	0.4	0.2	1.4	1.1	1.1	0.6	0.3	2.8
Wage demand of the entrepreneur family	369.1	0.9	0.9	0.9	0.9	1.1	1.0	1.0	0.9	0.9	1.3	1.2	1.2	1.0	0.9	1.7
Interest demand on capital	179.5	0.3	0.3	0.1	0.0	1.0	0.7	0.7	0.3	0.1	2.1	1.5	1.5	0.6	0.3	4.4
Total	1607.1	6.2	6.3	4.3	2.9	11.4	9.5	9.8	5.9	3.0	20.3	16.3	16.7	9.0	3.3	38.5

Cost of feed acidifier	Addition	€ per sow		
	0.5 %	7.1	7.2	7.3
	1.0 %	14.2	14.3	14.6
	2.0 %	28.4	28.7	29.1
	3.0 %	42.7	43.0	43.7

Table 6. The effects of improvement scenarios in specific traits of piglet production on the net return of production (€/sow place/year or € per piglet sold) excluding the cost of feed acidifier and including the effect of the reduced need to fix restore the sow's body condition after weaning (€3.9 per sow place per year or €0.3 per piglet sold).

Trait	Improvement 1		Improvement 2		Improvement 3	
	per sow place	per piglet	per sow place	per piglet	per sow place	per piglet
T1	Stillborn piglets, %	12	0.46	20	0.77	35	1.35
T2	Piglet mortality before weaning, %	13	0.50	21	0.84	39	1.49
T3	Piglet mortality after weaning, %	8	0.30	11	0.45	19	0.74
T4	Days from the first oestrus to the conception	6	0.23	8	0.30	11	0.45
T5	Traits T1 - T4 simulateneously	26	1.03	50	1.86	97	3.45
TP1	Average daily gain of weaned piglets	7	0.56	10	0.79	15	1.26
TP2	Feed conversion ratio of weaned piglets	10	0.85	16	1.37	29	2.42
TP3	Traits TP1 and TP2 simulataneously	13	1.08	22	1.84	40	3.36

results. When using 5g acidifier/kg in both lactation and weaner feed and when the acidifier costs €1300 per ton, the total acidifier cost is approximately €7 per sow place per year or €0.3 per piglet, of which 0.58 is due to the lactation feed.

The dynamic programming model produces two results that are particularly interesting, *viz.* the value of the capacity unit and the optimal decision rule. However, changes in the replacement pattern of sows are marginal. Table 5 also illustrates how changes in sow productive performance affect returns to sow place and returns per piglet sold. These returns exclude the cost of feed acidifier but include the effect of reduced need to restore sow body condition after weaning when using feed acidifier. The cost of restoring the body condition was assumed to be about €3.9 per sow place per year or €0.3 per piglet sold. The cost of using feed acidifier depends on pig feed consumption. If 5 g of feed acidifier per kg of feed is used only in the lactation feed and the acidifier price is €1200 per ton, the cost of using acidifier is €3.6 per sow place per year. If either the price or the dose of acidifier doubles, the cost of acidifier is €7.2 per sow place per year. When acidifier (€1200 per ton) is used in the weaned piglets' feed at a dose of 5 g/kg feed, the average annual cost of acidifier per sow place and year is €5.4. If either the price or the dose is doubled, the cost increases to €10.8 per sow place per year.

Of the performance improvements considered here, a decrease in piglet mortality before weaning increases the value of the capacity unit the most. A reduction in the number of stillborn piglets and an improvement in the feed conversion ratio of weaned piglets also have noticeable effects on the cost of the sow place. The smallest effect is simulated for a reduced number of non-productive days. A similar ranking was obtained when the results were normalised per piglet sold basis.

When the impact on returns per sow place is examined per unit, then results suggest the following ranking for one percentage-point change: a decrease in the post-weaning piglet mortality increases the returns per sow place by €15.7 per year, a decrease in piglet mortality before weaning by €12.7 per year, and a decrease in the number of piglets born dead by €11.7 per year. The value of one empty day less per farrow is worth €11 per sow place per year. An improvement of one percentage point in the feed conversion ratio of weaned piglets yielded €5.1 in additional annual return per sow place, whereas a one-percent increase in weaned piglet average daily gain (i.e. affects piglet place and labour requirements, but FCR remains unaffected) resulted in €2.7 in additional annual return per sow place.

There are differences between the results of the dynamic programming model and the farm model used in this chapter. When the differences in T1 to T4 are considered, the dynamic programming model generally provides a higher estimate for the total benefit. While the farm model simulates an average sow, the dynamic programming model simulates a sequence of litters and sows, which can produce varying numbers of piglets. Furthermore, farm model focuses on examining

production costs whereas the dynamic programming model maximises return per sow place. These results are therefore not comparable. A few differences are also due to model parameters and their cost structure, which are partly related to the dynamics of production.

Conclusions

Many studies have shown that the addition of feed acidifiers to pig feeds can result in enhanced pig performance. However, fairly high addition levels are used and recommended in these trials. In practical pig feeding, such high levels are not attractive and developing acidifiers that are effective in small levels should be targeted. In addition, the efficacy studies should include several addition levels so than an optimal addition level could be estimated. It seems that the growth promoting effect of organic acids is primarily due to their effects on gastrointestinal microflora. The inhibition of microbial growth causes reduced incidence of diarrhoea, improves digestibility, and means that fewer nutrients are taken away from the animal by microbes.

The profitability of using feed acidifier in sow feeds depends on how the acidifier affects sow performance. The results suggest that piglet producers have good reason to start using feed acidifier if any performance improvement scenario considered in this study can be achieved at an acidifier cost of €0.006 per kg of lactation feed while other traits remain unchanged. If the performance of sows or their piglets can be improved, the most significant effects are expected in reduced piglet mortality and improved feed conversion ratio. Although pig performance improvements reduce the per piglet production costs and increase the net return per sow place, annual production costs per sow can even increase. This is because reduced post-weaning piglet mortality, for instance, increases the number of piglets, and thus the amount of feed and production facilities needed per sow.

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14

AMINO ACID REQUIREMENTS IN PIGLETS WITH SPECIAL EMPHASIS ON TRYPTOPHAN AND VALINE

ETIENNE CORRENT[1], AUDE SIMONGIOVANNI[1], JAAP VAN MILGEN[2,3], NATHALIE LE FLOC'H[2,3]

[1]*Ajinomoto Eurolysine S.A.S., 153 rue de Courcelles, 75817 Paris cedex 17, France ; * [2]*INRA, UMR 1079, S.E.N.A.H. F-35590 Saint-Gilles, France;* [3] *Agrocampus Ouest, UMR1079, S.E.N.A.H. F-35000 Rennes, France*

Introduction

Twenty amino acids are the building blocks of proteins. Nine of these (Lys, Thr, Met, Trp, Val, Ile, Leu, His, Phe) are either not synthesised or synthesised only in small quantities by pigs (Boisen, 2003). They must therefore be supplied through the diet and are referred to as indispensable or essential amino acids (IAA). Feed must provide the IAA in sufficient quantities to cover the requirement of the animal, while avoiding an excess supply. The term "requirement" has no precise definition (Mercer *et al.,* 1989) and, for growing animals, is usually associated with the maximization of growth. Dose-response trials are frequently used to determine amino acid requirements but the reported results can be quite variable. This variability can be the result of different experimental conditions but also of different modes of expressing requirements. Consequently, a direct comparison of reported requirements is very difficult to achieve. A meta-analysis is a statistical technique used to compile and compare information from different sources and to analyse the relationships that exist within and between different sources of information. This chapter initially will discuss briefly experimental conditions that have to be ensured when carrying out dose-response trials to determine amino acid requirements. Because amino acid requirements are often expressed relative to Lys (referred to as "ideal protein"), special emphasis is given to this mode of expression. The second part of this chapter will evaluate different studies on the Trp and Val requirements in piglets using a meta-analysis, and the factors of variation of Trp and Val requirements will be discussed.

Experimental design to determine an amino acid requirement for growing animals, expressed relative to lysine

Nutrient requirements can be expressed in numerous ways. Amino acid requirements have been expressed as a quantity of amino acids in the feed, relative to (digestible, metabolisable or net) energy, relative to weight gain, or relative to Lys. They may also be expressed on a total, apparent ileal digestible or standardised ileal digestible basis. In addition to the different response criteria used (e.g., daily gain, nitrogen retention, plasma urea nitrogen), different statistical models are used to determine the requirement. Although all of these have strengths and weaknesses, the desired mode of expression will determine the experimental design that has to be used in requirement studies.

In growing animals, ideal protein is a concept where the amino acid pattern (defined as g of amino acid / 100 g Lys) maximizes nitrogen retention. In this profile, all amino acids are supposed to be equally limiting for performance, just covering the requirements for all physiological functions. Lysine has traditionally been used as a reference because it is the first limiting amino acid for growth in piglets. Although the concept of ideal protein was developed in the late 1950s and early 1960s, one of the first reference studies in pigs was carried out by Wang and Fuller (1989). It is frequently assumed that the ideal protein profile does not change for a given growing stage. In practical nutrition, this offers the advantage that the Lys requirement will vary (per kg of feed or per MJ of energy), but not the ideal protein profile expressed relative to Lys.

To estimate the requirement of an amino acid relative to Lys, the latter needs to be the second-limiting amino acid in the experiment, after the amino acid that being studied. In a dose-response study, different levels of the studied amino acid should be provided, while the Lys content in the diet should remain constant (and sub-limiting). Figure 1 illustrates this experimental constraint for Val. When Lys is second-limiting for performance (weight gain, feed intake, feed efficiency), an increasing Val supply will result in a (linear) increase in the response criterion up to the point that the Val supply is no longer limiting, which is indicated by the breakpoint. A further increase in Val supply would not result in a change in the response criterion. If Lys is actually second-limiting, the plateau value will be determined by the Lys supply. At the breakpoint, Val and Lys are co-limiting and the ratio between the two will be the Val requirement. When Lys is not the second-limiting nutrient in a Val dose-response study, an increasing Val supply will initially result in an increase in the response criterion. When the Val supply is further increased, another unknown factor (e.g., an amino acid other than Val and Lys, energy, feed intake) will become limiting (indicated by the ooo-symbols in Figure 1). The breakpoint of that line corresponds to the Val requirement relative

to the unknown factor. Interpreting this breakpoint as a Val requirement relative to Lys would underestimate the actual Val requirement.
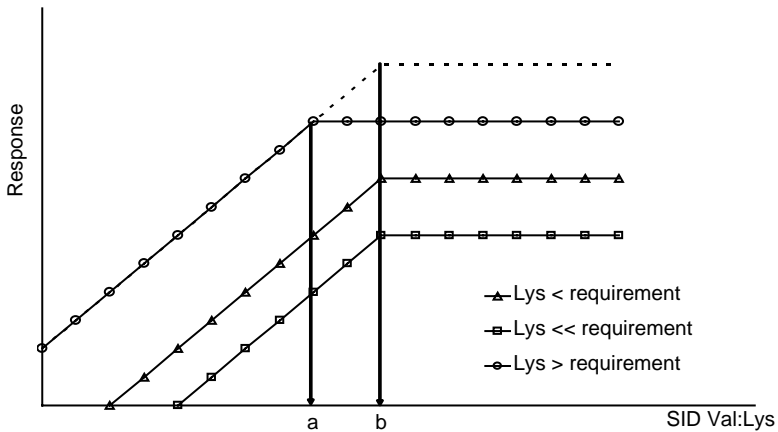
Figure 1. Theoretical response to increasing levels of dietary standardized ileal digestible (SID) Val:Lys when Lys is (or is not) the second-limiting amino acid after Val. The two arrows represent the estimated requirements. When a factor other than Lys is second-limiting for the response criterion, the calculated Val:Lys requirement ratio (a) will be underestimated in comparison to (b) where Lys is the second-limiting factor.

As shown in Figure 1, it is assumed that the optimal Val:Lys ratio is not affected by the Lys content in the diet when Lys is actually the second-limiting factor (Boisen, 2003). The constraint that Lys should be the second-limiting amino acid only applies to experimental studies in order to express the requirement relative to Lys whereas in practical nutrition, the Lys levels are higher to ensure that the piglets can fully express their growth potential.

Many studies have been carried out on amino acid requirements where Lys is not (or does not appear to be) the second-limiting amino acid. By itself, this does not invalidate these studies but the reported amino acid requirement should then be expressed relative to the second-limiting factor and not relative to Lys.

Tryptophan requirement in piglets

Tryptophan is the fourth-limiting amino acid in piglet diets in Europe. Being an IAA, it has to be supplied by the diet. In addition to its utilisation for protein deposition, Trp also plays other important roles. It is involved in feed intake regulation (Henry *et al.*, 1992; Zhang *et al.*, 2007), the immune response and the animal's defence system, and it limits the impact of an unfavourable health environment on performance (Le Floc'h *et al.*, 2007; Trevisi *et al.*, 2009).

There appears to be no consensus concerning the Trp requirement. Apart from variation that may be due to different experimental conditions, there appears to be a discrepancy in Trp recommendations originating from Europe and North America. No comprehensive studies appear to have been carried out to explain this discrepancy, although factors such as differences in raw materials used and genetics have been suggested as contributing factors. The purpose of this section is to compile and analyse experimental results on the Trp requirement using a meta-analysis. The principles of meta-analyses have been reported recently by Sauvant *et al.* (2008).

THE META-ANALYSIS METHOD

The objective of a meta-analysis is to integrate information for different sources to identify a response curve that account for variation that exists between and within experimental studies. Meta-analyses were first used in psychology, social sciences and medicine, while the use of this method in animal nutrition is more recent (Sauvant *et al.*, 2008). Meta-analyses have been used to quantify the response of ruminants to nitrogen (Rico-Gomez and Faverdin, 2001), starch (Offner and Sauvant, 2004), or fat (Schmidely *et al.*, 2008) and to understand better phosphorus utilization in growing pigs (Schulin-Zeuthen *et al.*, 2007).
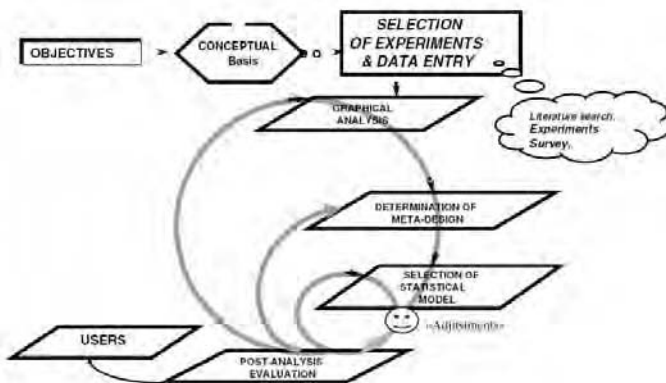
Figure 2. Schematic representation of the meta-analytic process (Sauvant et al., 2008).

The main steps of a meta-analysis are presented by Figure 2 and all steps must be carried out as rigorously as possible. Once the objectives of the study are clearly defined, a database containing experimental results is built. The trials may have been published in scientific journals, as abstracts or as trial reports. To select the trials that are the most relevant for the objectives, a graphical analysis has to be

carried out. This graphical analysis will provide information about outliers, factors of variation, and will give a first idea about the type of mathematical model that can be used to describe the variation within-studies.

CHARACTERISTICS AND SELECTION OF THE TRIALS

A database was compiled with studies on the Trp requirement in piglets. The trials in the database originated from scientific publications (articles or abstracts) and trial reports from research institutes (in collaboration with Ajinomoto Eurolysine, some of which were published). Only those trials were selected in which at least 2 different Trp levels were used and where crystalline Trp was used to provide different levels of Trp within a trial. This resulted in a total of 130 trials that were selected. One of the main challenges in a meta-analysis of nutritional studies is the evaluation of the experimental diets. Reported information must include the raw materials used and the analyzed, calculated, or anticipated chemical composition. To standardize the information, the composition of the experimental diets was calculated using the raw materials included and the nutritional values as reported in the INRA feed tables (Sauvant *et al.*, 2004). Diet composition was calculated using the EvaPig® software (Noblet *et al.*, 2008).

Among the 130 trials, 0.58 originated from Europe, 0.33 from USA and 0.09 from other countries (Australia, Canada and Taiwan). Although trials were carried out between 1970 and 2008, most trials were published after 2000. European trials were more recent than American trials (Figure 3).
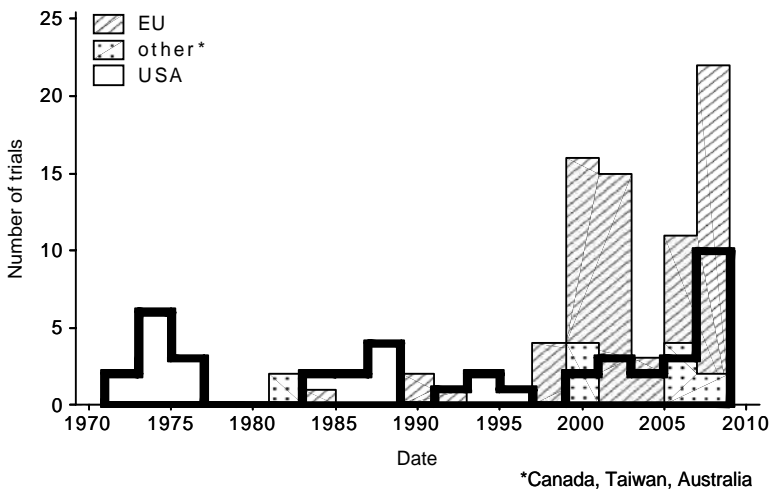
Figure 3. Trials studying the Trp requirement in piglets as a function of year and geographic origin of the study (Ajinomoto Eurolysine s.a.s. database as of July 2009).

Because the response can be different according to the weight of animals, a discriminant analysis based on age, initial and final weight of animals, and duration of the experiment, was carried out to determine three weight categories (Table 1).

Table 1. weight categories of piglets.

Weight categories	Number of trials
Prestarter : 7.2 ± 1.5 - 10.3 ± 2.5 kg	25
Starter : 11.3 ± 1.8 - 24.2 ± 4.4 kg	53
Prestarter-starter : 7.3 ± 1.1 - 22.0 ± 4.8 kg	52

To verify if there were inconsistencies in the database, the average daily gain (ADG) was expressed as a function of average daily feed intake (ADFI) (Figure 4). This figure also indicates that feed efficiency improves the more recent the publication, certainly due to the improvement in genetic and knowledge about nutrition. This correlation was not associated either with origin of the trials or with weight categories.
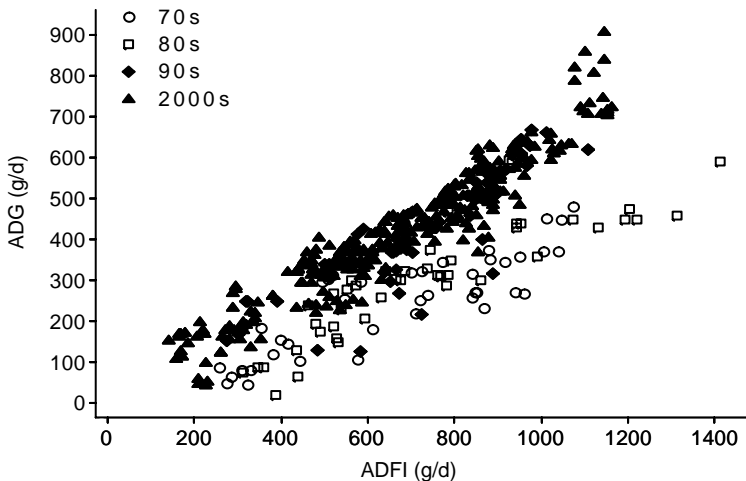
Figure 4. Average daily gain (ADG) as a function of average daily feed intake (ADFI) for different trial dates.

To find a common response for the trials, the within-study response of piglets to Trp has to be quantified and the standardized ileal digestible (SID) Trp:Lys ratio which maximizes growth performance has to be determined.

Lysine dietary levels

Two references were used to verify if Lys was limiting performance after Trp for each trial:

- NRC (1998) recommends a Lys supply that varies with the weight of piglets (from 9.2 to 13.4 SID Lys/kg diet for 3-35 kg piglets),

- Whittemore *et al.* (2003) recommends 12.0 to 14.0 SID Lys/kg diet for 6-15 kg piglets and 11.2 SID Lys/kg diet for 10-30 kg piglets.

Figure 5 presents the SID Lys content in the diets for each trial of the 'Starter' weight category using the two Lys recommendations given above. Lysine is certainly not sub-limiting in those trials where the SID Lys content is above the recommendations. These types of trials cannot be used to determine a requirement expressed as a ratio relative to Lys and were removed from the meta-analysis. This concerned 19 trials for the three weight categories.
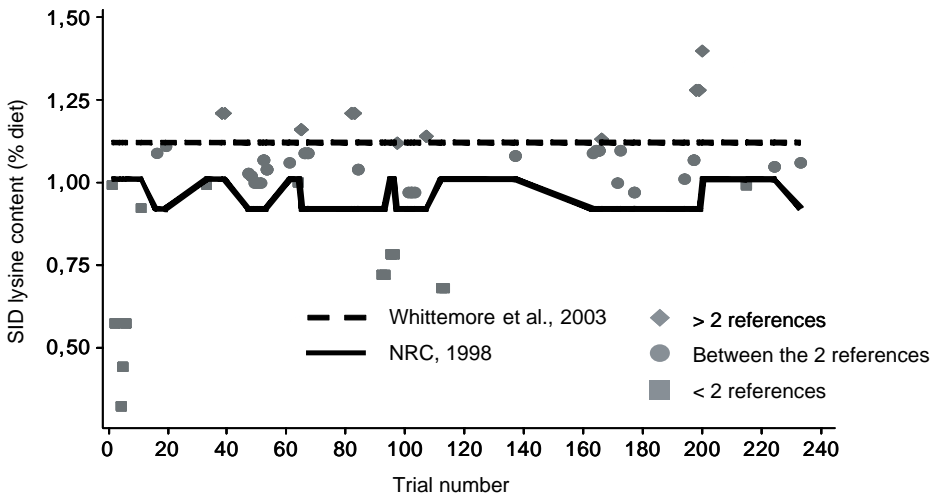
Figure 5. Standardized ileal digestible (SID) Lys content in diets used to determine the Trp requirement in piglets, relative to the Lys requirement as recommended by Whittemore et al. or the NRC. The data shown here are only for 'Starter' piglets.

Essential amino acids dietary levels

As indicated before, Lys must be second-limiting for performance after Trp when the Trp requirement is to be expressed relative to Lys. This means that it is important to ensure that no other amino acid than Lys is limiting performance

after Trp. This verification was also done using the recommendations of the NRC (1998) and Whittemore *et al.* (2003). To illustrate this procedure, Thr is used as an example. Threonine is generally considered to be the second-limiting amino acid in practical diets. Whittemore *et al.* (2003) recommended a minimum ratio of 0.65 SID Thr:Lys for piglets and the NRC (1998) recommended a minimum ratio of 0.63. Figure 6 presents the calculated SID Thr:Lys ratios for the trials of the 'Starter' weight category. It appears that the Thr content in diet was below these recommendations in several studies. In these studies, it is probable that Thr was limiting performance before Lys, thereby invalidating the calculation of a Trp:Lys requirement ratio. For example, Lougnon *et al.* (1984) carried out a Trp dose-response study in piglets while the SID Thr:Lys was only 50%. In this trial, no response was observed above 0.15 SID Trp:Lys. As indicated in Figure 1, this value for the Trp:Lys requirement may be underestimated due to the Thr deficiency. The same verification was also carried out for other amino acids, resulting in some surprising results. For example, Borg *et al.* (1987) estimated the Trp:Lys requirement ratio at 0.15, but this value was obtained with a SID Val:Lys ratio of 0.48, which is well below the recommended requirement (the SID Val:Lys requirement for piglets is discussed in the last part of this chapter).
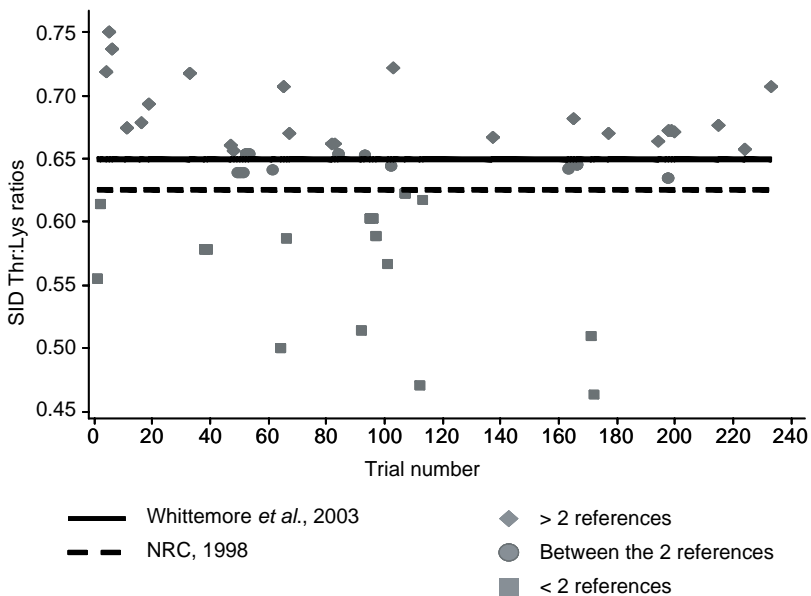
Figure 6. Standardized ileal digestible (SID) Thr:Lys content in diets used to determine the Trp requirement in piglets, relative to the Thr:Lys requirement as recommended by Whittemore et al. or the NRC. The data shown here are only for 'Starter' piglets.

The trial selection resulted in the elimination of 89 trials. The reasons for trial elimination are given in Figure 7.
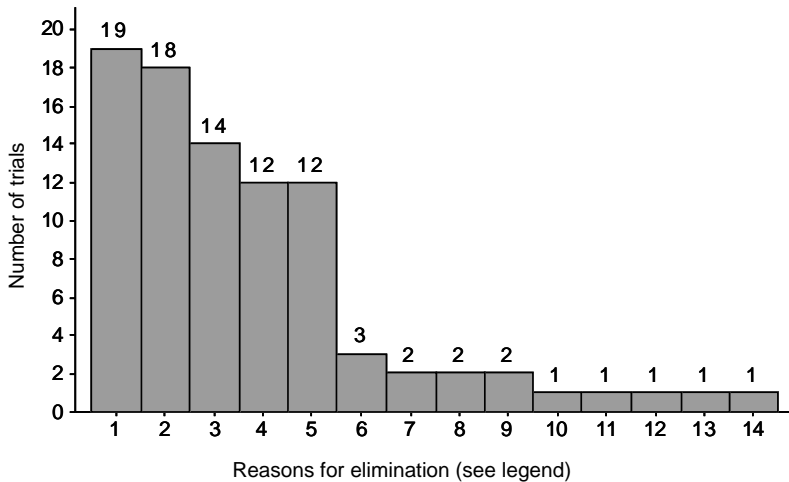
1. Non sub limiting Lys. 2. Two-levels trials which are not on a linear part (slope or plateau) of an a priori model. *Amino acid (AA) deficiencies* [3. More than three AA. 4. Thr. 5. Val. 6. Val and Ile. 7. Val and Leu. 8. Met + Cys. 10. Leu. 11. Thr and Met+Cys. 12.Leu and His]. *Other reasons* [9. Doubtful large neutral AA. 13. Doubtful net energy value. 14. Doubtful dietary crude protein value].

Figure 7. Reasons for trial elimination in Trp requirement studies. When the Trp requirement is expressed relative to Lys, the latter should be second-limiting for performance. Trials were eliminated from the analysis if this condition was not met.

Final selection

After the elimination of trials because of anomalies in diet composition or number of treatments within a study, 41 trials (out of 130; 0.315) were maintained in the meta-analysis. The SID Trp:Lys tested in the 41 trials followed a normal distribution with an average value of 0.175 (Figure 8). Most studies were carried out using a SID Trp:Lys range of 0.14 to 0.22. To study the response of piglets to Trp between and within studies, the ADG was expressed as a function of SID Trp:Lys (Figure 9). As can be seen in this figure, the variation between studies is considerably greater than the variation within a study.

Modelling the response of piglets to dietary Trp

The requirement should be determined through a regression model because a point-by-point comparison using analysis of variance does not take into account that response variables (e.g., body weight, feed intake, feed efficiency) are continuous rather than discrete (Shearer, 2000; Pesti *et al.*, 2009). Although requirements are
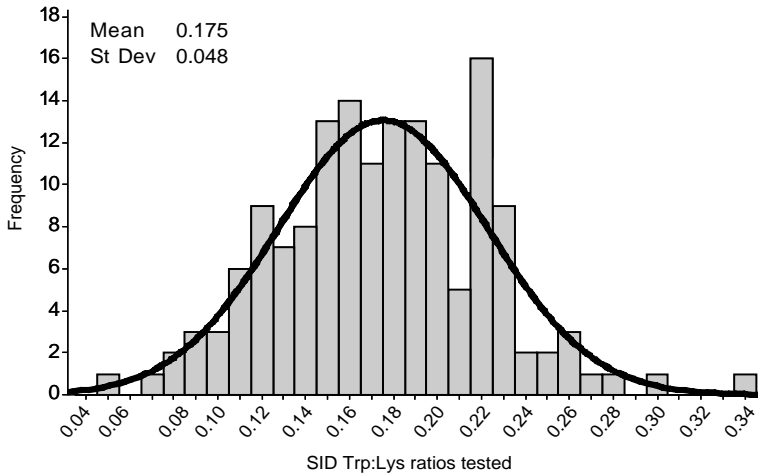
Figure 8. Distribution of standardized ileal digestible (SID) Trp:Lys of diets used in experiments designed to determine the Trp requirement in piglets.
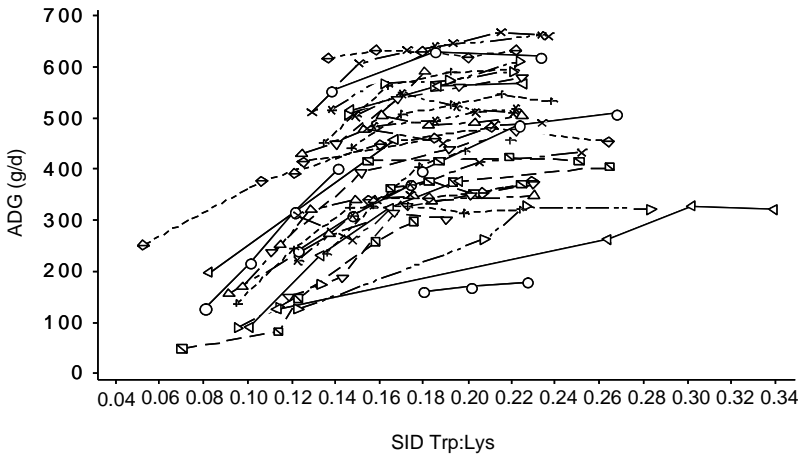
Figure 9. Average daily gain (ADG) as a function of standardized ileal digestible (SID) Trp:Lys content of diets in experiments designed to determine the Trp requirement in piglets.

sometimes estimated using linear models (e.g., through a quadratic function), these models may not be appropriate if the response criterion does not (further) respond to a high level of the amino acid. Non-linear models such as the linear-plateau (or broken-line), curvilinear-plateau and asymptotic models are frequently used to estimate amino acid requirements (Figure 10). These models differ conceptually, which will be discussed later. In a meta-analysis, data from different origin are used. As can be seen in Figure 9, the maximum differs widely between studies (e.g., because of the use of animals of different body weight). This means that a trial effect must be taken into account in the model. In Figure 10, the parameter

"A" in all three models represents the plateau. A model was used in which a trial-specific plateau was used (A_i), whereas the other model parameters were assumed to be common across trials: for both linear-plateau and curvilinear-plateau models, U as the origin of ordinate, and R as the minimum SID Trp:Lys level required to reach the plateau. For the asymptotic function, b and c are constant of the model. The part of the equation that is given in parentheses therefore indicates the general response curve to Trp, whereas A_i is indicative for differences between trials.

Linear-plateau model: $Y_{ij} = A_i (1 + U(R-x_{ij}))$ if $x_{ij} < R$

$Y_{ij} = A_i$ if $x_{ij} \geq R$

Curvilinear-plateau model: $Y_{ij} = A_{ij} (1 + U(R-x_{ij})^2)$ if $x_{ij} < R$

$Y_{ij} = A_i$ if $x_{ij} \geq R$

Asymptotic model: $Y_{ij} = A_i (1 - be^{-cxi})$

Y_{ij} : response criterion (e.g., daily gain, daily feed intake, feed efficiency) for trial "i" and observation "j"
X_{ij} : SID Trp:Lys for trial "i" and observation "j"
U : origin of ordinate
R : minimum SID Trp:Lys level required to reach the plateau
b, c: function parameters
A_i : trial 'i' specific plateau

Figure 10. Graphical and mathematical representations of the three models tested.

The requirement values estimated by the three models for each of the performance traits are shown in Table 2 and Figure 11. Compared with the curvilinear-plateau model, the linear-plateau model gives the lowest values requirement estimates

whereas the asymptotic model gives the highest estimates. This illustrates that the choice of the model is a potentially important factor of variation of reported requirements, as previously shown by Baker *et al.* (1986) and confirmed by Barea *et al.* (2009a).

Table 2. Standardized ileal digestible (SID) Trp:Lys requirement in piglets estimated by different statistical models.

Response criterion	Linear-plateau[1]	Curvilinear-plateau[1]	Asymptotic[2]
Average daily gain	16.7 (0.3)	21.6 (0.8)	26.7 (2.9)
Average daily feed intake	/	22.1 (1.1)	24.7 (3.5)
Gain to feed	16.2 (0.4)	20.3 (0.9)	20.7 (2.2)

[1]The minimum SID Trp:Lys level required to reach the plateau
[2]The SID Trp:Lys level required to reach 95% of the asymptote
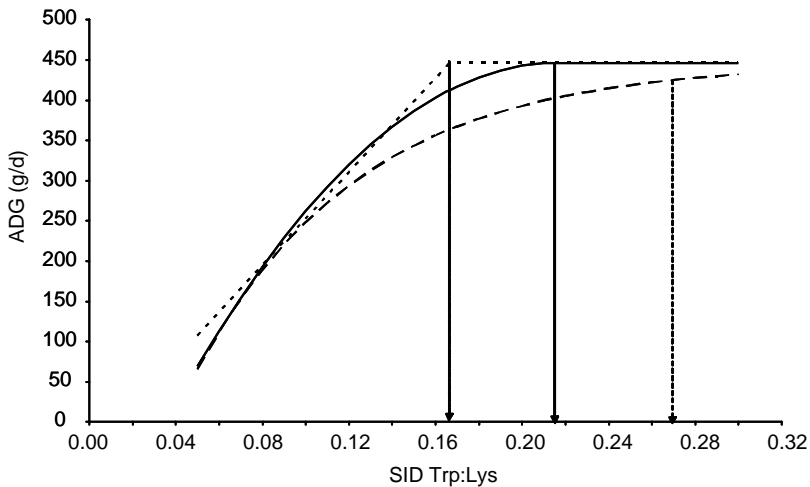
Figure 11. Graphical representation of standardized ileal digestible (SID) Trp:Lys requirement in piglets using average daily gain (ADG) as the response criterion estimated by three different models. The plateau corresponds to the average of plateau values (Ai) estimated in different trials of the meta-analysis.

The linear-plateau and curvilinear-plateau models both fit the data well and the requirement is defined as the minimum Trp:Lys required to reach the plateau. However, for the asymptotic model, the plateau is attained at an infinite level of Trp:Lys. Requirements are then often estimated as the level of Trp:Lys required to attain an arbitrary chosen percentage of the asymptote value, in this case 95%.

    The three models differ in the way they can be interpreted biologically. In the linear-plateau model, the marginal efficiency of Trp utilization is assumed to

be constant up to the requirement, to become zero thereafter. In the curvilinear-plateau model, the marginal efficiency declines linearly with increasing Trp supply to become zero at the Trp requirement. Although all the models can be used to represent the response of an animal to a limiting nutrient supply, the response of a population of animals may be different. For example, it has been shown that if the response of an individual animal is given by a linear-plateau model, the response of the population of these animals will resemble that of the curvilinear-plateau model (Pomar *et al.*, 2003). Through their structure, these models also have different practical applications. With the linear-plateau model, a supply of Trp just below the requirement will result in a rapid decrease in ADG. For the curvilinear-plateau model, a small reduction in the Trp supply will only marginally reduce ADG. The requirement value of the latter model therefore includes a safety margin, which may be more adapted for economic optimization (Pesti *et al.*, 2009).

In conclusion, the choice of the statistical model contributes to the reported variability in Trp requirements. Consequently, a comparison of studies must be done on the basis of the same model to account for this variability.

TRYPTOPHAN REQUIREMENT FOR PIGLETS: IS THERE ANY DIFFERENCE BETWEEN NORTH AMERICA AND EUROPE?

Among the 41 trials retained, 14 were carried out in North America and 21 in Europe. It is possible to take account for the geographical origin in the meta-analysis. Nevertheless, the effect of origin is partially confounded with that of time because European trials are more recent than American trials (Figure 3). This effect of time may be more apparent for feed efficiency. Table 3 gives the results of the analysis where the effect of geographical origin is accounted for in the requirement estimate of Trp.

Table 3. Effect of geographical origin on requirement estimates for standardized ileal digestible (SID) Trp:Lys in piglets. Requirements were estimated by the curvilinear-plateau model.

Response criterion	All countries (41 trials)	North America (14 trials)	Europe (21 trials)	P (effect of origin)
Average daily gain	21.6 (0.8)	21.5 (0.9)	20.8 (0.9)	> 0.10

The *P* value indicates that the Trp requirement for ADG, estimated by the curvilinear-plateau model, was not affected by geographical origin. Based on this analysis, there is no reason to assume different Trp requirement levels in

both continents. The explanation for the apparent difference reported sometimes may find its origin in the experimental design (e.g., Lys level and supply of other essential amino acids) and the statistical method used to determine the requirement. The use of a basal diet deficient in Lys in these trials is the most important point that has to be taken into consideration when designing amino acid requirement studies. Additionally, the linear-plateau model is often used in North American studies and this may explain the origin of a debate on the potential difference.

Valine requirement in piglets

Valine, like Ile and Leu, is a branched-chain amino acid (BCAA). With the decrease of dietary crude protein levels, Val appears as a limiting amino acids for piglets (Lordelo *et al.*, 2008, Barea *et al.,* 2009a). Recommendations for the Val requirement given by various national research institutes are given in Table 4, with an average value of about 0.70 SID Val:Lys. Relatively few studies on the Val requirement have been carried out and only 12 studies have been published since 1970 for piglets (Table 5). As indicated before, there is a large variability in reported requirements between studies due to the way of expression. Apart from expressing the requirement relative to Lys, the NRC (1998) also reported other modes of expression. When the results of the 12 studies are compared with the requirements reported by the NRC (1998), 9 studies reported a requirement equal or higher than that of the NRC (ranging from 1.00 to 1.28).

Table 4. Comparison between recommended dietary standardized ileal digestible (SID) Val:Lys in piglets.

Country	Reference	SID Val:Lys
FR	INRA (Sève, 1994)	0.70
USA	NRC (1998)	0.68
GB	BSAS (Whittemore et al., 2003)	0.70
BR	UFV (Rostagno et al., 2005)	0.69
ES	FEDNA (de Blas et al., 2006)	0.71
DK	DSP (Jørgensen and Tybirk, 2008)	0.70

With only 12 studies available, it is difficult to carry out a meta-analysis. The objective to express the requirement relative to Lys required a further selection. To be selected, the article had to provide sufficient information about the amino acid and energy content of the diets (or the composition of feed ingredients), and about performance and statistics. At least 4 levels of Val should have been used in the dose-response study. The trials that met these criteria and dealt with piglets are

Table 5. Published valine requirements in piglets, and comparison with NRC (1998) recommendations.

	Source	Weight range (kg)	Tested valine levels (n)	Published result	Proportion of NRC
Chung and Baker, 1992	Journal	10	-	0.68 Val:Lys	1.00
Mavromichalis *et al.*, 2001 - Exp. 4 -	Journal	5 - 10	5	0.60 g SID Val/MJ ME	1.02
Mavromichalis *et al.*, 2001 - Exp. 5 -	Journal	10 - 20	6	0.53 g SID Val/MJ ME	1.06
Warnants *et al.*, 2001	Journal	8 - 21	5	0.68 SID Val:Lys	1.00
Theil *et al.*, 2004	Journal	8 - 20	3	0.59 g AID/MJ ME	1.28
Wiltafsky *et al.*, 2009a	Journal	8 - 22	6	0.65 SID Val:Lys	0.96
Barea *et al.*, 2009a - Exp. 4 -	Journal	12 - 25	5	0.68 to 0.80 SID Val:Lys	1.00 to 1.18
James *et al.*, 2001	Abstract	9 - 15	6	0.62 to 0.67 SID Val	0.90 to 0.97 %
Kendall *et al.*, 2004	Abstract	13 - 32	5-6	0.65 SID Val:Lys	0.96
Gaines *et al.*, 2006	Abstract	8 - 12	4	0.92 SID Val	1.14
Gaines *et al.*, 2006	Abstract	12 - 20	4	0.78 SID Val	1.13
Barea *et al.*, 2009b - Exp. 4 -	Congress	12 - 25	5	0.69 to 0.74 SID Val:Lys	1.01 to 1.09

MJ: Mega Joule - ME: Metabolizable Energy - SID or AID : Standardized or Apparent Ileal Digestible
Adapted from Barea *et al.*, 2009a

listed in Table 6. In all these trials, a basal diet deficient in Val was supplemented with increasing quantities of L-Val.

To determine whether Lys was supplied at an adequate level, we compared these data with recent published results (Garcia *et al.*, 2008; Kendall *et al.*, 2008), who reported a SID Lys requirement for piglets of 1.26 to 1.29 g Lys SID/MJ NE, depending on body weight. Comparing the Lys level of the trials in Table 6 with these recommendations, it appears that Lys was probably not the second-limiting amino acid in one of the experiments reported by Mavromichalis *et al.* (2001) (1.34 g SID Lys/MJ NE).

Diet composition and amino acid profiles of the remaining trials are reported in Table 7 and ADG, ADFI and feed conversion ratio (FCR) of these given in Figures 12, 13 and14.

In most trials, ADFI and ADG were maximum, and FCR was minimum between 0.70 and 0.75 SID Val:Lys.

In one trial (Wiltafsky *et al.,* 2009a), the plateau appeared at a lower SID Val:Lys level (about 0.65) and this is probably due to the fact that Lys was not the second-limiting amino acid. Indeed, the SID Thr:Lys was 0.63 in this study whereas the SID Trp:Lys was 0.18. This may have resulted in a lower requirement estimate for Val. To avoid any interactions due to the AA profile, this trial was not considered further.

It has been shown earlier that the estimated requirement depends on the model and response criterion used. Nevertheless, experimental results appear to be rather

Table 6. Designs of the selected trials aiming at refining the Val requirement of piglets of various weights.

	Source	Country	Weight range (kg)	Tested valine levels (n)	SID Lys (%)	Net Energy (MJ/kg)	SID Lys (g/MJ of NE)	Recommended SID Lys (g/MJ of NE)
Mavromichalis et al., 2001 - Exp. 4 -	Journal	USA	5.8 - 10.0	5	1.50*	11.2	1.34	1.29*
Mavromichalis et al., 2001 - Exp. 5 -	Journal	USA	10.9 - 20.0	6	1.14*	10.4	0.96	1.26**
Barea et al., 2009a - Exp. 4 -	Journal	EU (FR)	12.0 - 25.0	5	0.92	10.3	0.89	1.26**
Wiltafsky et al., 2009a	Journal	EU (DE)	7.9 - 21.7	6	1.00	10.3	0.97	1.26**
Barea et al., 2009b - Exp. 4 -	Congress	EU (FR)	12.0 - 25.0	5	1.12	10.8	1.04	1.26**

*Assuming that Lys SID/ Lys Total = 88% - * Garcia et al., 2008 - ** Kendall et al., 2008

Table 7. Composition and nutritional values of the experimental diets used in the selected Val dose-response trials.

LW range (Kg)	Mavromichalis et al., 2001 - Exp. 5 - 10.9 - 20.0	Barea et al., 2009a - Exp. 4 - 12.0 - 25.0	Wiltafsky et al., 2009a 7.9 - 21.7	Barea et al.,2009b - Exp. 4 - 12.0 - 25.0
Feed Composition g/kg				
Wheat	-	161.8	300.0	-
Maize	230.0	485.1	240.0	776.0
Barley	-	161.8	139.9	-
Oats-	-	-	-	
Maize Gluten Meal	-	-	-	94.0
Wheat Middlings	-	-	-	-
Maize Starch	148.0	-	-	-
Soyabean Meal	50.0	138.3	-	74.0
Soyabeans Extruded	-	-	-	-
Peanut Meal	100.0	-	-	-
Peas-	-	100.0	-	
Whey Powder	-	-	100.0	-
Sweet Milk Powder	100.0	-	-	-
Lactose	100.0	-	-	-
Gelatin	50.0	-	-	-
Molasses	-	-	20.0	-
Sucrose	100	-	-	-
Vegetable/Animal Fat	40.0	10.0	15.0	10.0
Others	55.1	32.5	29	33.0
L-Lysine	7.3	5.7	6.8	7.6
DL-Methionine	4.1	1.4	2.2	0.9
L-Threonine	3.7	2.1	2.5	2.1
L-Tryptophan	1.0	0.9	0.6	1.4
L-Isoleucine	1.6	0.4	2.2	1.3
Other crystalline amino acids	10.6	-	42.9	-
Nutritional Values (as is)				
Net energy, MJ/Kg	10.4*	10.3	10.3*	10.8
Crude protein, g/kg	180*	146	174.0	168
Total Lys, g/kg	13.0*	10.0	10.8	12
SID Lys, g/kg	11.2*	9.2	10.0	11.4
SID Ratio to Lys				
Met+Cys	0.65*	0.59	0.61	0.59*
Thr	0.70*	0.65	0.625	0.65*
Trp	0.20*	0.22	0.18	0.22*
Val	0.49*	0.58	0.49	0.63*
Ile	0.55*	0.55	0.63	0.65*
Leu	0.99*	1.13	1.05	1.66*
His	0.38*	0.32	0.32	0.34*

* Estimated with EvaPig® (Noblet et al., 2008)
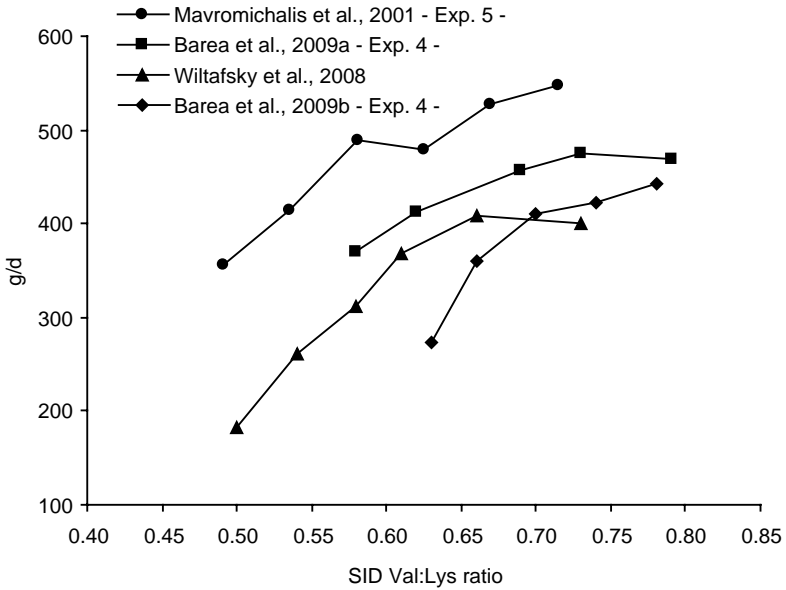
Figure 12. Standardized ileal digestible (SID) Val:Lys dose response - Effect on average daily gain (g/d).
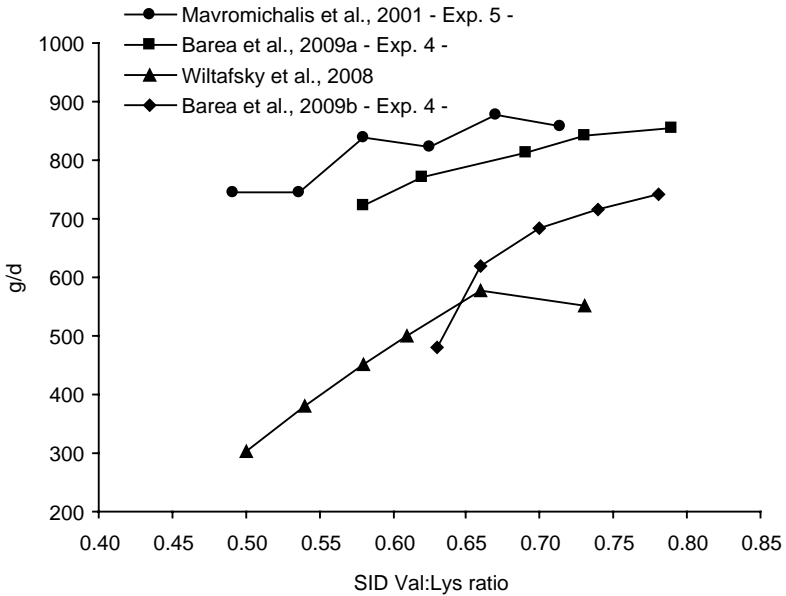
Figure 13. Standardized ileal digestible (SID) Val:Lys dose response - Effect on average daily feed intake (g/d).
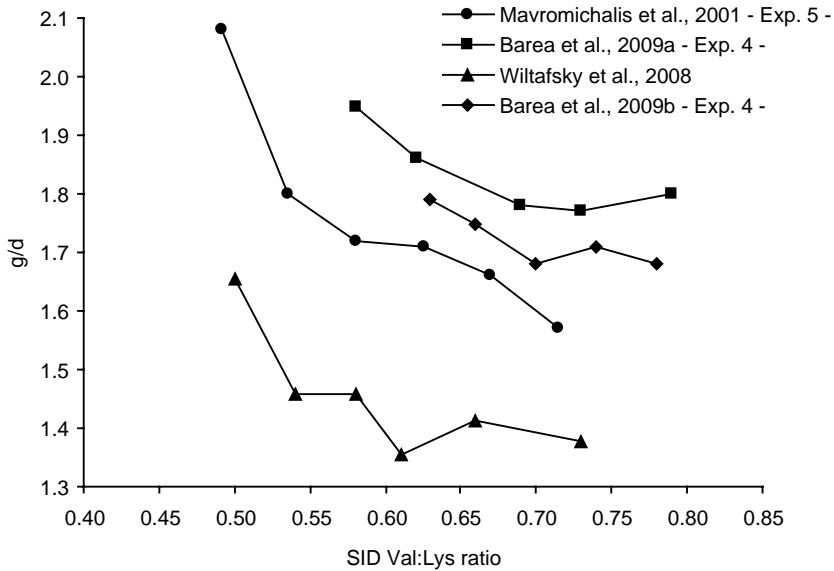
Figure 14. Standardized ileal digestible (SID) Val:Lys dose response - Effect on feed conversion ratio (g/g).

homogeneous (Table 8) with requirements estimates for ADG, ADFI and FCR of 0.72, 0.74 and 0.71 SID Val:Lys, respectively. Four additional unpublished trials (Corrent and Primot, 2009) confirmed this data. Therefore, it is concluded that 0.70 SID Val:Lys is a minimum requirement estimate for piglets.

Table 8. Standardized ileal digestible (SID) Val:Lys requirement for piglets determined by different statistical models.

	Models used	*Average daily gain*	*Average daily feed intake*	*Feed conversion ratio*
Mavromichalis *et al.*, 2001 - Exp. 5 -	Linear*	0.71	0.71	0.71
Barea *et al.*, 2009a - Exp. 4 -	Linear-plateau	0.70	0.74	0.68
	Curvilinear-plateau	0.75	0.81	0.73
Barea *et al.*, 2009b - Exp. 4 -	Linear-plateau	0.69	0.69	0.70
	Curvilinear-plateau	0.74	0.74	0.73
	Average	0.72	0.74	0.71
	Standard deviation	0.024	0.044	0.022

* The linear response indicates that the SID Val:Lys requirement was not possible to determine and is at least equal to the highest SID Val:Lys ratio tested

INTERACTIONS BETWEEN BRANCHED-CHAIN AMINO ACIDS (BCAA)

The BCAA Ile, Leu and Val share in part a common catabolic pathway. The first two steps in their catabolism are common to all three BCAA and involve BCAA aminotransferase (BCAAT) and branched-chain alpha-keto acid dehydrogenase (BCKAD). An excess supply of one of the BCAA may lead to the activation of the common enzymes complexes, stimulating the catabolism of all three BCAA (Harper *et al.,* 1984; Langer and Fuller, 2000). These interactions might be a possible factor of variation of requirement estimates.

Leucine appears to be more potent than Val and Ile in the antagonistic effect on BCAA metabolism. For instance, Wiltafsky and Roth (2008) determined that a dietary Leu supply above the requirement increased the activity of BCKAD and thereby the catabolism of Ile, Leu and Val. As a result, performance of piglets was reduced due to the lower availability of the other BCAA for protein deposition. The interaction between Leu and Val supply in piglets has been discussed by Barea *et al.* (2009b). In their study, the Val requirement was not directly affected by Leu content in the diet, but an excess of Leu amplified the negative effect of a Val deficiency.

Strong interactions have also been described between Leu supply and Ile requirement. Mavromichalis *et al.* (1998) was one of the first to suggest that the assumed ideal Ile:Lys requirement of 0.60 was too high. Lordelo *et al.* (2008) and Barea *et al.* (2009c) did not find an increase in performance for SID Ile:Lys levels greater than 50% in piglets receiving cereal and soybean meal based diets with a moderate BCAA content. Wiltafsky *et al.* (2009b) proposed a SID Ile:Lys requirement varying from 0.49 to 0.54 when there was no excess of both Val and Leu (0.70 and 0.108 relative to SID Lys, respectively), but the requirement may be higher (0.59 SID Ile:Lys) when Val and Leu were in excess (1.00 and 1.60 relative to SID Lys, respectively).

Conclusion

Experimental findings are essential to the feed industry to design more efficient formulas and to face the challenges of economy, health and environment. To make decisions, it is important to take into account how experimental results have been obtained. Indeed, the apparent variability often lies in experimental design and should not be confounded with non-controlled factors or external parameters. In addition, the different statistical models also contribute to different amino acid requirements reported in the literature. With the availability of five crystalline amino acids for use in animal nutrition, it becomes technically possible to further

reduce dietary protein content and to formulate diets in which six amino acids are co-limiting for performance. This also means that the risk of providing a diet with an insufficient amino acid supply is greater. Therefore, minimum constraints on digestible amino acids in formulas with security margins must be considered. A meta-analysis is a powerful approach to determine nutrient requirement using data obtained from different sources. In the meta-analysis we carried out for Trp, we did not observe a difference between studies originating from the United States compared with those originating from Europe. Across traits, the requirement was 0.216 SID Trp:Lys with the curvilinear-plateau model, but this value was 5 points lower when estimated with the linear-plateau model. There is less information available concerning the Val requirement in piglets, and reported requirement estimates (0.70 SID Val:Lys) appear to be less variable than those for Trp. The BCAA may mutually interfere with the response and requirement estimates for BCAA.

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The reference list of the 130 trials used for the meta-analysis about the Trp requirement is available on request.

15

FACTORS AFFECTING PORK QUALITY

J D WOOD

Division of Farm Animal Science, School of Clinical Veterinary Science, University of Bristol, Langford, Bristol, BS40 5DU

Introduction

Meat quality refers to those characteristics of meat that are important to consumers other than fatness/leanness which are part of carcass quality. Meat quality in pork comprises the colour of lean, the colour and softness of fat and, most importantly, the 'taste' of pork, which includes tenderness, juiciness and flavour. All these meat quality characteristics show variation because of the many factors during production and processing that affect them. The variation can be reduced and the overall level of meat quality can be improved by combining the appropriate genetic, feeding and processing inputs that control meat quality.

Colour/pH/water retention

PSE PORK

Pork should ideally be a red/pink colour. However sometimes the colour is too pale or too dark. Extremely pale pork which is also watery is termed pale, soft, exudative (PSE) pork. The role of genetics in this condition is shown by breed variation (Monin and Sellier, 1987). A breakthrough in our understanding of the genetic basis of PSE came with the work of Fuji, Otsu, Zorzato, De Leon, Khanna, Weiler, O'Brien and MacLennan (1991) who showed that PSE in some pig muscle was caused by a mutation in the halothane gene which controls calcium release from the sarcoplasmic reticulum in muscle cells. The increase in intracellular calcium activates muscle metabolism, causing a rapid fall in pH after slaughter due to lactic

acid accumulation. This causes denaturation of muscle proteins leading to a loss of waterholding capacity within the muscle structure (Huff-Lonergan, 2009). The pale colour comes from the structural changes to muscle fibres on the surface of pork which scatter and reflect incident light (Warriss, 2000). The Hal 1843 DNA test for this specific mutation in the halothane gene has been used by breeding companies around the world to eliminate the recessive gene (n) from at least one of the parent lines. It is sometimes retained in one of the lines because the gene increases carcass lean content and muscularity (de Vries, Sosnicki, Garnier and Plastow, 1998). However it has been shown that heterozygotes for the halothane gene have an intermediate pattern of pH fall and water-holding capacity between nn and NN parent lines (Murray, Jones and Sather, 1989) so complete elimination from breeding lines may be the best approach.

BREED EFFECTS

Historically, the Pietrain breed had the highest incidence of PSE than any other breed (Monin and Sellier, 1987). This was linked to high carcass lean content and thick muscles (high muscularity). Through use of the Hal 1843 DNA test, modern Pietrain-type breeding animals used in Britain have retained these leanness and muscularity advantages whilst losing the PSE characteristics. Results from a recent study commissioned by the British Pig Executive (BPEX) at the Universities of Leeds and Bristol are summarised in Table 1. Hampshire, Pietrain and Large White sires were used to create crossbred progeny with Large White x Landrace dams. The pigs were slaughtered at the Tulip plant in Spalding and loin joints were despatched to Bristol. Pietrain loins had deeper 'eye' muscles than those from the other breeds. Muscle pH at 24hr post mortem was marginally lower in Hampshire and Pietrain pigs causing a slightly higher drip loss (non significant differences). The lightness of muscle was similar in the 3 breeds although Hampshire and Pietrain pigs had higher chroma values, indicating a brighter colour. These differences in muscle quality between Pietrains and the other breeds are insignificant compared with those between Pietrains carrying the halothane gene and other breeds (Monin and Sellier, 1987).

IMPORTANCE OF WATER RETENTION FOR MEAT QUALITY

The BPEX results on meat quality in different genotypes (Table 1) showed that modern Hampshires have tender pork, with drip loss and pH characteristics similar to Large Whites. There was no evidence of low pH causing reduced

water-holding capacity which is associated with the RN⁻ gene, a dominant gene found predominantly in the Hampshire breed. Previous research has shown that the RN⁻ gene causes a low pH_{24hr} which reduces water retention in cooked pork. However, tenderness and juiciness in fresh pork appear to be improved (Enfalt, 1997). The taste panel results for juiciness, pork flavour and overall liking shown in Table 1, although not significantly different between the breeds, were all in favour of Hampshires.

Table 1. Carcass and meat quality in pigs from 3 sire breeds (unpublished results of BPEX research at the Universities of Leeds and Bristol)

	Hampshire	*Pietrain*	*Large White*	*Significance*
Slaughter wt (kg)	102.0[a]	100.3[ab]	96.3[b]	*
Loin measurements (mm)[c]				
Width	106.6	108.7	105.4	ns
Depth	55.4[b]	60.6[a]	57.5[ab]	***
Fat thickness	4.6	3.9	3.7	ns
pH_{24hr}	5.47[b]	5.47[b]	5.55[a]	***
Drip loss[d]	0.0478	0.0484	0.0467	ns
Colour of muscle				
L Lightness	54.3	54.4	54.9	ns
Chroma	6.5[a]	7.0[a]	5.6[b]	***
Hue	35.0	35.2	34.6	ns
Taste panel scores[e]				
Tenderness	4.3[a]	4.1[b]	4.1[b]	*
Juiciness	4.4	4.3	4.3	ns
Pork flavour	4.2	4.1	4.1	ns
Overall liking	4.4	4.2	4.2	ns

[ab] Means within rows with different superscripts are significantly different (P<0.05)
[c] Last rib position
[d] as a proportion of loin muscle wt
[e] 1 to 8 scales, after griddling steaks

The importance of pH in tenderness and juiciness is because it controls water retention in pork. At low pH values, eg 5.3 to 5.5, the structural proteins have no net electrostatic charge and are close together, restricting the space within the protein lattice framework for water. At these pH values, water retention is minimal. At higher pH values, the proteins repel one another and the space for water increases, so water retention increases (Huff-Lonergan, 2009). Higher water retention is associated with greater juiciness and tenderness in many situations. The effect of pH_{24hr} on waterholding capacity and pork eating quality were illustrated by Lonergan, Stalder, Huff-Lonergan, Knight, Goodwin, Prusa and Beitz (2007).

The study involved 1535 castrates from 8 breeds participating in the US National Barrow Show Sire Progeny Tests of 1991, 1992 and 1994. Samples of loin muscle were removed at 24hr post mortem and despatched to Iowa State University where meat quality measurements were made. Steaks were aged for 7-10 days after which they were oven broiled to a central temperature of 71°C and evaluated by a trained taste panel. When the results were examined in groups representing different pH_{24hr} categories (Table 2), it was found that cooking loss gradually increased as pH declined from 5.95 to 5.5 and both tenderness and juiciness declined.

Table 2. Cooking loss and eating quality in 5 groups of pigs (1535 in total) classified according to pH_{24hr} in the *longissimus* muscle(Lonergan *et al*, 2007)

	pH_{24hr} group				
	>5.95	*5.8-5.95*	*5.65-5.8*	*5.5-5.65*	*<5.5*
Cook loss [f]	0.212[e]	0.226[d]	0.240[c]	0.252[b]	0.266[a]
Tenderness[g]	3.5[a]	3.3[b]	3.2[c]	3.1[d]	3.0[d]
Juiciness[g]	3.3[a]	3.2[a]	3.1[b]	3.0[c]	2.9[c]

[abcde] Means within rows with different superscripts are significantly different (P<0.05)
[f] Proportion of initial weight
[g] Higher values denote more tender or juicy

The overall mean pH recorded at 24hr post mortem in the study of Lonergan *et al* (2007) described in Table 2 was 5.71. This is higher than usually reported in other studies and as routinely found at Bristol (e.g. Table 1). Possible explanations for high 'ultimate' pH values are a high level of pre-slaughter stress over a prolonged period in which muscle glycogen stores are mobilised and aerobically metabolised; or perhaps extremely rapid chilling of the carcass which inhibits a complete pH decline. Care must be taken when chilling rapidly to avoid muscle contraction which toughens pork.

Pork can be injected with or immersed in solutions (marinades) which change the electrostatic charge on muscle proteins and alter muscle pH, thus increasing water-holding capacity. This in turn increases tenderness and juiciness as shown in the study of Sheard, Nute, Richardson, Perry and Taylor (1999). Loin portions were injected with 0, 3 or 5% polyphosphate solutions so that weight increased by 5 or 10%. They were stored in vacuum packs for 3 days for equilibration then 20mm thick slices were grilled to a central temperature of either 72.5 or 80ºC. The trained taste panel evaluated sensory characteristics on 1 to 8 scales. The results (Table 3) show that the 5% polyphosphate marinade greatly increased both tenderness and juiciness scores. The effect occurred at both final internal temperatures, 72.5ºC, which equates to 'medium done' and 80ºC which equates to 'well done'. The higher temperature reduced the tenderness and juiciness scores. Both of these effects, that of the marinade and that of internal temperature was achieved by changing

water retention. The loss in weight during grilling was lowest in steaks with 5% marinade cooked to 72.5ºC (33.9%) and was greatest in steaks with 0% marinade cooked to 80ºC (42.2%). These had the highest and lowest values for tenderness and juiciness respectively. The loss in weight after 3 days in vacuum packs was also lower in the 5% solution (2.2%) than the 0% solution (4.5%) when both were injected to increase in weight by 5%. The results in Table 3 also show lower pork flavour following the 5% polyphosphate injection and after grilling to 80ºC. This shows that greater water retention alters the profile of flavour precursors, an effect which can be masked by adding flavours to the marinade.

Table 3. Effects of polyphosphate injection on sensory characteristics of pork (results for male pigs, means for 50 and 100 g/kg injection level). (Sheard *et al,* **1999)**

Polyphosphate (g/kg)	*0*		*50*		*Significance*	
Internal temperature (ºC)	*72.5*	*80.0*	*72.5*	*80.0*	*72.5*	*80.0*
Tenderness[a]	4.5	4.2	5.7	5.3	*	*
Juiciness[a]	4.0	3.4	4.4	3.9	ns	*
Pork flavour[a]	3.3	3.8	3.1	3.2	ns	*

[a] 1 to 8 scales.

IMPORTANCE OF GENETICS AND HANDLING PROTOCOLS.

There is evidence that genetic factors other than the halothane and RN⁻ genes affect muscle pH, colour and water retention. Several genetic markers have been proposed (Cairns,2009). It remains the case that raising stress levels in pigs before slaughter can produce PSE pork if the stress occurs just before slaughter and anaerobic metabolism occurs. Longer term exposure to stressors causes glycogen depletion and aerobic muscle metabolism causes the pH after slaughter and beyond to remain high. The result is dark, firm and dry (DFD) pork which is tender but has a poor flavour. Good handling protocols are therefore important in achieving good muscle quality in pigs.

A recent unpublished study of pork from Gloucestershire Old Spots pigs conducted at Bristol showed that they had a much narrower range of pH_{24hr} values than pork obtained from 2 supermarkets, assumed to come from 'modern' breeds. It is impossible to say whether this is a genetic effect associated with non-intensive selection in the traditional breed or more careful pre-slaughter handling.

Tenderness

Tenderness is the most important aspect of eating quality and arguably the most important characteristic of pork. Tenderness varies because so many factors

influence it, any of which can dominate the final result. The most important factors are growth rate in the live animal, marbling fat in the pork itself and the processing factors chilling rate, period of ageing and the use of marinades (some of which operate by changing water retention as shown above).

GROWTH RATE

Growth rate was identified several years ago in work conducted by the UK Meat and Livestock Commission (MLC) at Stotfold as an important factor in tenderness variation (MLC,1989). In that work, pigs fed *ad libitum* rather than restricted grew faster to 80kg live weight (827 vs 681 g/d) and had higher tenderness in both pork and bacon. Scores for tenderness (1 to 8 scale) were 5.2 and 4.7 in pork and 5.6 and 5.2 in bacon for ad libitum and restricted groups respectively. A recent study commissioned by BPEX at the Universities of Leeds and Bristol has provided more up-to-date information. Male and female Large White cross pigs growing to 90 or 110kg slaughter weight were separated into fast and slow growing groups on the basis of growth rate from weaning. Growth rate above 600g/d was classified as fast and below this slow. A 3rd group consisted of pigs whose growth had been interrupted because of illness. The pigs were slaughtered at the Tulip plant in Spalding and loin joints were despatched to Bristol for analysis. Loins were conditioned at 1°C for 10 days before objective measurement of toughness and assessment of eating quality by the trained taste panel. The results (Table 4) show that the fast growing group had the most tender muscle, judged both by the objective 'shear force' test and the taste panel test. In the latter case, the statistical interaction was due to the fact that tenderness differed more between the groups at 110kg than at 90kg although was always highest in the fast growing pigs. Juiciness was also higher in the fast growing group and 'overall liking' also tended to be higher. Toughness was especially high in the pigs with interrupted growth. These results should encourage producers to target growth rate as a factor in meat quality as well as performance.

MUSCLE FAT (MARBLING FAT)

Many studies have shown positive correlations between carcass or muscle fat (marbling fat) content and tenderness or juiciness scores in cooked pork. However, the correlations in the majority of studies are low, averaging about 0.2, showing that overall only about 0.04 of tenderness or juiciness variation is explained by marbling fat (Wood, Jones, Francombe and Whelehan, 1986). Nevertheless, there

Table 4. Meat quality in 3 groups of pigs differing in pattern of growth (unpublished results of BPEX research at the Universities of Leeds and Bristol)

	Growth rate group			
	Fast	*Slow*	*Interrupted*	*Significance*
Growth rate (g/d)[d]	680[a]	540[b]	490[c]	*
P$_2$ fat thickness (mm)	10.1[a]	9.1[b]	8.4[b]	***
Muscle pH	5.38[b]	5.43[a]	5.47[a]	***
Drip loss[e]	0.0434	0.0445	0.0508	ns
Chroma	7.28	6.69	7.44	ns
Toughness (kg)	4.49[b]	4.81[ab]	5.35[a]	*
Tenderness[f]	4.36	4.01	3.97	+
Juiciness[f]	4.59[a]	4.34[b]	4.37[b]	*
Overall liking[f]	4.43	4.19	4.27	ns

[abc] Means within rows with different superscripts are significantly different (P<0.05)
[d] From weaning to slaughter
[e] Proportion of loin muscle wt
[f] 1 to 8 scales, after griddling steaks + Interaction with carcass weight

remains a view that marbling fat is a positive indicator of pork eating quality. Lonergan *et al* (2007) compared marbling fat and pH$_{24hr}$ as predictors of eating quality in a large population of pigs from 8 breeds. Despite their conclusion that separating into pH groups was useful in explaining tenderness variation (Table 2), correlations involving both predictors were low. Correlations for tenderness were 0.2 for pH and 0.13 for muscle lipid. Those for juiciness were 0.12 for pH and 0.02 for muscle lipid. The average values for marbling fat in this study were 0.025 to 0.030 of loin muscle weight, higher than seen in most European studies.

Bristol has examined the effects of low protein diets on marbling fat and pork eating quality. In a study by Teye, Sheard, Whittington, Nute, Stewart and Wood (2006), dietary crude protein was reduced from 210 to 180g/kg by replacing soyabean meal with wheat. No attempt was made to balance essential amino acids in the low protein diet. The pigs on this diet grew more slowly from 40 to 100kg live weight than those on the high protein diet (849 vs 955 g/d) and had slightly higher P$_2$ fat thickness values (13.6 vs 12.8 mm). They particularly had a higher concentration of marbling fat in the loin muscle (0.286 vs 0.174), a 64% difference between the diets. These relatively high values for marbling fat (for British pigs) are explained by the fact that the pigs had 0.50 Duroc genes, the Duroc being a breed which has a high ratio of muscle fat to subcutaneous fat. Loin steaks were examined by the taste panel after ageing at 1°C for 10 days and griddling to 72.5°C internal temperature. The results (Table 5) showed that steaks from pigs fed the low crude protein diet were more tender and juicy than those fed the high crude protein diet.

Table 5. Using low protein diets to increase marbling fat and improve eating quality (Teye *et al***, 2006)**

	Crude protein (g/kg)		
	210	*180*	*Significance*
Daily wt gain (g)	955	849	**
P$_2$ fat thickness (mm)	12.8	13.6	ns
Marbling fat [a]	0.0174	0.0286	***
Tenderness[b]	4.2	4.8	**
Juiciness[b]	3.9	4.4	**

[a] Total fatty acids as a proportion in *m.longissimus*   [b] 1 to 8 scales, after griddling

In an earlier study, Wood, Nute, Richardson, Whittington, Southwood, Plastow, Mansbridge, da Costa and Chang (2004) found that a low crude protein (160g/kg) diet fed to pigs of 4 pure breeds between 9 and 21 weeks of age increased marbling fat in both the *psoas* (tenderloin) and the *longissimus* (loin) muscles. The low protein diet reduced growth rate by about 24%. Values for marbling fat were higher in the *longissimus* muscle, that has a higher ratio of white glycolytic to red oxidative muscle fibres, than the *psoas,* a 'red' muscle (Table 6). The effect of diet on marbling fat was inconsistent between the 4 breeds and between the muscles. In *longissimus,* the difference between diets was much greater in Berkshire, Duroc and Large White breeds (around a 70% effect) than in Tamworth (16%). In *psoas,* the effect was much smaller overall (around 25%) and more similar between the breeds.

Table 6. Effects of crude protein (200 or 160g/kg) diets on total muscle lipid (marbling fat) (proportion of muscle wt) in *longissimus* **and** *psoas* **muscles of 4 pure breeds (Wood** *et al,* **2004)**

	Berkshire	*Duroc*	*Large White*	*Tamworth*
Longissimus				
200	0.0205	0.0177	0.0097	0.0120
160	0.0342	0.0310	0.0168	0.0140
Significance	***	***	**	ns
Psoas				
200	0.0157	0.0131	0.0102	0.0119
160	0.0178	0.0161	0.0131	0.0152
Significance	*	*	*	*

In more recent work even lower protein diets than in the 2004 and 2006 studies have been evaluated but an attempt to mitigate the reduction in growth rate was introduced by maintaining lysine and other essential amino acids as a proportion of protein as protein is reduced. This has successfully allowed similar growth

rates in the high and low protein treatments but the effect on marbling fat is less than in the earlier studies (Table 7). Fat content was increased in the low protein compared with the standard regime much more in the Large White than the Duroc genotype. Preliminary examination of the data shows a smaller effect on sensory characteristics than seen previously.

Table 7. Effect of feeding standard and 'low protein' regimes to Duroc and Large White crossbred pigs between 40 and 120 kg live weight (Stonehouse, Hughes, Hallett, Stewart and Wood, 2009)

	Duroc		*Large White*	
	Std	*LP*	*Std*	*LP*
P_2 fat thickness (mm)	18.9[b]	19.0[b]	13.7[a]	16.9[b]
Fat in body (g/kg)	157[b]	159[b]	137[a]	169[b]
Marbling fat [c]	0.0195[b]	0.0208[b]	0.0096[a]	0.0118[a]

[ab] Means within rows with different superscripts are significantly different (P<0.05).
[c] Total fatty acids as a proportion in *longissimus*

AGEING

Leaving pork to age for a period of time at 1°C after slaughter is one of the surest ways of increasing tenderness. Juiciness and flavour also often improve. The effect on tenderness is mainly caused by proteolytic enzymes, in particular calpains, degrading the filamentous protein structure of muscle (Warriss, 2000). A further factor is that the removal of key proteins creates more space in the intra fibre lattice for water and, as we have seen elsewhere in this review, this also leads to greater tenderness. Pork tenderises more quickly than beef and about 0.80 of the total effect is achieved within 5 days compared with 10 days in beef (Dransfield, Jones and MacFie, 1980). However, the remaining 0.20 is worth achieving, especially if the pork is inherently tough. Results of a Danish study are shown in Table 8. Pork steaks were aged in vacuum packs at 1°C for 1, 2, 3 and 6 days of age and evaluated for tenderness after cooking using a shear force test and a taste panel. Tenderness increased with time of ageing and juiciness and flavour tended to increase. The recommendation in the MLC Blueprint for Tender Pork is that pork loins for premium markets should be aged for 14 days before sale.

If the pork is inherently tender to begin with it will tenderise less during ageing than more standard material. This was shown in a study of breeds by Meinert, Christiansen, Kristensen, Bjergegaard and Aaslyng (2008). Pure Duroc and Duroc crosses, which had high tenderness, showed no change with ageing between 2 and 6 days whereas pure Hampshire increased in tenderness during this time. In a comparison of processing procedures, Taylor, Nute and Warkup (1995) found that pork steaks

Table 8. Effects of ageing time on eating quality. Danish study in loin steaks (Bejerholm, 1991)

	Ageing time (days)			
	1	*2*	*3*	*6*
Tenderness[c]	-0.2[a]	0.0[a]	1.0[b]	1.6[b]
Juiciness[c]	1.4[a]	1.3[a]	1.8[ab]	2.2[b]
Flavour[c]	1.9[a]	1.8[a]	2.2[ab]	2.3[b]
Shear force (units)	104[a]	99[a]	90[ab]	79[b]

[ab] Means within rows with different superscripts are significantly different (P<0.05)
[c] Hedonic (liking) scale -5 to +5

from carcasses that had been electrically stimulated after slaughter were initially more tender than those that had received standard post slaughter (no ES) treatment or a delayed chill. Loin samples from all 3 treatments were aged in vacuum pack for 4, 7 or 12 days. Tenderness increased with ageing time in all treatments but much less in the ES group (Table 9). However, these steaks were the most tender after 12 days.

Table 9. Tenderness in loin steaks aged for different times from pig carcasses undergoing a delayed chill, a standard chill or a standard chill following electrical stimulation (ES) (Taylor, Nute and Warkup, 1995)

	Ageing time (days)	*Delayed*	*Standard*	*ES+standard*
Shear force (kg)	4	7.9[a]	8.0[a]	6.4[b]
	7	7.3[a]	7.4[a]	5.8[b]
	12	6.8[a]	6.9[a]	5.5[b]
Tenderness[c]	4	3.6[a]	3.8[a]	4.3[b]
	7	4.0[a]	4.0[a]	4.6[b]
	12	4.3	4.3	4.6

[ab] Means within rows with different superscripts are significantly different (P>0.05)
[c] 1 to 8 scale

Boar taint

Boar taint, an odour/flavour of cooked pork or bacon from male pigs which some people dislike, is not a major cause for concern in UK where entire males have provided 0.50 of slaughter pigs since 1980. However, critical comments about pork flavour continue to be made by retailers and there is still widespread resistance to meat from entire males in mainland Europe. The impending European ban on surgical castration without an anaesthetic makes boar taint a 'hot' issue at present and there is great interest in ways to control it.

VACCINATION AGAINST BOAR TAINT

A major recent development is the licensing throughout Europe of the Pfizer product Improvac, a vaccine against boar taint. This suppresses testosterone production, leading to low concentrations of androstenone, one of the compounds responsible for boar taint. Because androstenone inhibits skatole clearance from the liver, low levels of androstenone also cause low levels of skatole, the other boar taint compound (Dunshea, Colantoni, Howard, McCauley, Jackson, Long, Lopaticki, Nugent, Simons, Walker and Hennessy, 2001). In the study of Dunshea *et al*. (2001), all treated males had concentrations of skatole and androstenone in backfat lower than the thresholds below which consumers cannot detect boar taint ie 0.2 and 1.0 µg/g for skatole and androstenone respectively.

Results of a recent study (Lodge, Nute, Baker, Hughes, Wood and Pearce, 2008) comparing the sensory characteristics of pork produced from Improvac-treated and control males are in Table 10. The treated pigs were vaccinated at 10 weeks and 20 weeks of age, the second vaccination being given 5 weeks before slaughter at 105 kg. Loins from 50 pigs in each group were aged at 1°C for 10 days in vacuum packs before griddling to a central temperature of 72.5°C. Subcutaneous fat and lean tissue were then evaluated by the trained taste panel. Both control and treated groups had the same tenderness and juiciness scores. The fat of treated pigs had higher pork odour and lower abnormal odour scores. The lean of treated pigs had higher pork flavour and lower abnormal flavour scores. In addition to these objective results, the 10 panellists were asked to express their preferences for flavour and for overall eating quality. These 'liking' scores were also in favour of the treated pigs. The results for treated pigs are characteristic of those found in castrated males in a genotype with low taint levels such as the Large White.

Table 10. Taste panel scores for sensory characteristics of griddled pork in control and Improvac-treated male pigs (Lodge *et al*, 2008)

	Control	*Improvac*	*Significance*
Fat			
Pork odour	4.0	4.2	**
Abnormal odour	2.9	2.4	***
Lean			
Tenderness	5.2	5.2	ns
Juiciness	5.0	5.0	ns
Pork flavour	4.5	4.7	**
Abnormal flavour	3.2	2.7	***
Flavour liking	4.3	4.8	***
Overall liking	4.2	4.7	***

1 to 8 scales

BREED EFFECTS ON BOAR TAINT

In searching for a genetic solution to the boar taint problem, workers at Bristol showed that the Meishan had much higher concentrations of skatole and androstenone in backfat than Large Whites (Doran, Whittington, Wood and McGivan,2002a). Meishans had a lower expression of the enzyme CYP 2E1 which clears skatole from the liver and this explained why the backfat level of skatole was high. In subsequent work, it was shown that high androstenone inhibits CYP 2E1 (Doran, Whittington, Wood and McGivan, 2002b) so it appears that androstenone is the key boar taint compound. The search for the specific genetic basis for these metabolic differences continues.

Recent work at Bristol has shown that the Large White breed has low concentrations of boar taint compounds. A comparison between 0.75 Duroc and 0.75 Large White males fed the same diets between 40 and 120 kg showed that Durocs had higher concentrations of both skatole and androstenone in backfat from the last rib region (Figure 1). The proportion of pigs that exceeded the nominal thresholds for both skatole and androstenone (0.2 and 1.0 µg/g respectively) was 0.028 for Large Whites and 0.14 for Durocs. In a second study, the effect of growth rate on boar taint compounds was examined in Large White x Landrace pigs. The results (Table 11) showed that pigs that grew quickly had slightly higher concentrations of both taint compounds than those growing slowly or pigs whose growth had been interrupted. These are the same pigs as shown in Table 4. The actual concentrations of skatole and androstenone are low compared with those for Durocs in Figure 1 and with Meishans in earlier studies.
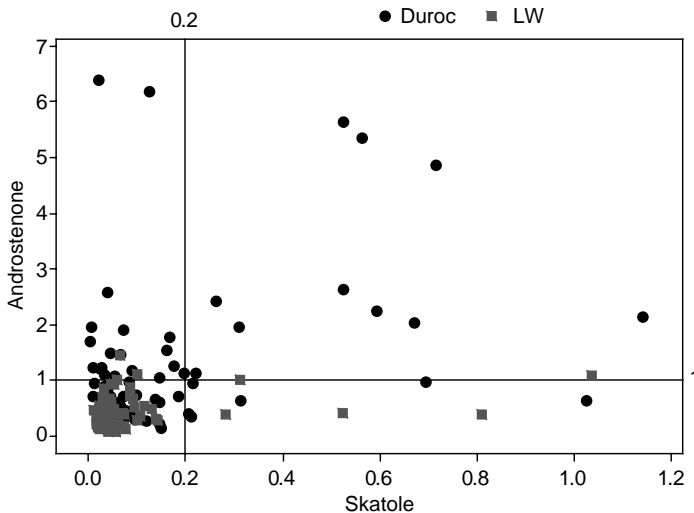
Figure 1. Concentrations of androstenone and skatole (µg/g backfat) of 0.75 Duroc and 0.75 Large White male pigs weighing 120kg live weight and fed the same diets.

Table 11. Effects of pattern of growth on skatole and androstenone concentrations in backfat of male pigs (µg/g)

	Growth rate group			sig.
	Fast	*Slow*	*Interrupted*	
Androstenone	0.500	0.383	0.397	*
Skatole	0.080[a]	0.056[b]	0.060[ab]	**

[ab] Means within rows with different superscripts are significantly different (P<0.05)

CONTROLLING BOAR TAINT BY DIET

The boar taint compounds are synergistic in their effects on the odour of cooked pork. In particular, skatole enhances the odour of androstenone (Annor-Frempong, Nute, Whittington and Wood, 1997). Unpublished evidence suggests that abnormal odours are more strongly associated with skatole than androstenone. For these reasons, treatments which reduce skatole concentrations may reduce boar taint. Unlike androstenone, skatole can be manipulated by diet because it is produced during digestion. It is a product of bacterial breakdown of tryptophan that occurs in the hind gut. Several workers have shown that feeding complex fibre sources which are not degraded until they reach the hind gut change the fermentation conditions there such that less skatole is produced and absorbed from the gut. Sugar beet feed (Wood, Longland, Enser and Nute, 1993) and chicory (Hansen, Mejer, Thamsborg, Byrne, Roepstorff, Karlsson, Hansen-Moller, Jensen and Tuomola, 2006) are such sources and both have been shown to reduce skatole concentrations in adipose tissue. In current research funded by BPEX, short-term feeding of different proportions of dried chicory before slaughter as a cost-effective way to reduce skatole and improve the eating quality of pork from entire male pigs is being investigated.

Acknowledgements

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LIST OF PARTICIPANTS

The forty-third University of Nottingham Feed Conference was organised by the following committee:

MR M. HAZZLEDINE (*Premier Nutrition*)
DR R. KIRKLAND (*Volac International*)
MR W. MORRIS (*BOCM PAULS Lt*d)
DR D. PARKER (*Novus Europe*)
DR M.A. VARLEY (*Provimi Ltd*)
DR P. WILCOCK (*AB Agri Ltd*)

DR J.M. BRAMELD
PROF P.C. GARNSWORTHY (*Secretary*)
DR T. PARR
PROF A.M. SALTER   *University of Nottingham*
DR K.D. SINCLAIR
DR W. WAPENAAR
PROF R. WEBB
DR J. WISEMAN (*Chairman*)

The conference was held at the University of Nottingham Sutton Bonington Campus, 8-10 September 2009. The following persons registered for the meeting.

Allen, Mrs D	QNM Ltd, 46A Mudford Road, Yeovil BA21 4AB, UK
Aronen, Dr I P	Raisio Feed Ltd, P O Box 101, Raisio 21201, Finland
Avantaggiato, Dr G	Institute of Sciences of Food Production, Via Amendola 122/0, Bari 70126, Italy
Blake, Dr J	Dietcheck Ltd, Highfield Office, Little London, Andover SP11 6JE, UK
Brade, Mr M	Newquip Ltd, NQ House, Coneygarth Way, Leeming Bar, Northallerton DL7 9EE, UK
Brameld, Dr J M	University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK
Bravo De Laguna, Mr F	Nutreco Swine Research Centre, Boxmeerseneg 30, Sint Anthonis 5845ET, The Netherlands
Brookes, Mr O	University of Nottingham, School of Biosciences, Sutton Bonington Campus LE12 5RD, UK
Chambers, Mr J	JC Consulting, Carmel Cottage, Parsonage Lane, Chilcompton, Radstock BA3 4JZ, UK
Chaosap, Miss C	University of Nottingham, School of Biosciences, Sutton Bonington Campus LE12 5RD, UK
Clark, Dr M	153 Battenhall Road, Worcester WR5 2BU, UK
Cole, Mr J	BFI Innovations Ltd, 1 Telford Court, Chestergates, Dunkirk Lea, Cheshire CH1 6LT, UK
Corrent, Mr E	Ajinomoto Eurolysine S.A.S, 153 rue de Courcelles, Paris 75017, France
Davies, Mr T	Kite Consulting, The Crown Building, Watling Street, Brentwood, Staffordshire ST19 9LL, UK

den Hartog, Dr L	Nutreco, P O Box 220, Boxmeer 5830AE, The Netherlands
Dillon, Mr P	TEAGASC, Moorepark Dairy Production Research Centre, Fermoy Co. Cork, Ireland
Downey, Mr N	Scanutec, Sca Mill, Dalton Airfield Industrial Estate, York YO7 3HE, UK
Falkowski, Dr J	University of Warmia and Mazury, Kortowo Bl. 37, Olsztyn 10-719, Poland
Ferris, Dr C	Agri-Food and Biosciences Institute, Large Park, Hillsborough BT26 6DR, UK
Freeston-Smith, Ms C	Premier Nutrition, The Levels, Rugeley, WS15 1RD, UK
Fullarton, Mr P	Forum Products Ltd, Betchworth House, 57-65 Station Road, Redhill RH1 1DL, UK
Garnsworthy, Prof P C	University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK
Golds, Mrs S	University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK
Gould, Mrs M	Volac International Ltd, 50 Fishers Lane, Orwell, Hertfordshire SG8 5QX, UK
Graham, Mr M	CMD Agribusiness and Five F, Tremywawr, Breidden Avenue, Arddleen, Llanymynech SY22 6SP, UK
Gregson, Miss E	University of Nottingham, School of Biosciences, Sutton Bonington Campus, Loughborough LE12 5RD, UK
Hall, Miss C	BOCM Pauls, First Avenue, Portbury Dock, Portbury, Bristol BS20 7XS, UK
Hazzledine, Mr M	Premier Nutrition, The Nutrition Centre, The Levels, Rugeley, Staffs WS15 1RD, UK
Henderson, Mr Ian	Scotmin Nutrition Ltd, 13 Whitfield Drive, Heathfield Industrial Estate, Ayr KA8 9RX, UK
Hooley, Mrs E	University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK
Houseman, Dr R	BEI Ltd, 18 Melrose Road, Bishop Monkton, Harrogate HG3 2RH, UK
Hunter, Mrs M	University of Nottingham, School of Biosciences, Sutton Bonington Campus, Loughborough LE12 5RD, UK
Hurdidge, Mr L	Brotal, Collivald House, Ocean Way, Cardiff CF24 5AD, UK
Ingham, Mr R	YARA, The Homestead, Troutbeck Bridge, Windermere LA23 1HF, UK
Issac, Mr P	Mole Valley Feed Solutions, Hollycroft, Halwill Junction, Beaworthy EX21 5XB, UK
Jacklin, Mr D	Consultant, 10 Station Close, Riding Mill, Northumberland NE44 6HR, UK
Jagger, Dr S	ABN, 64 Innovation Way, Peterborough Business Park, Peterborough PE2 6FL, UK

Johnston, Ms C	5M Publishing, 4 Haywood House, Hydra Business Park, Neither Lane, Sheffield S35 9ZX, UK
Jones, Mr H	Roquette UK, Sallow Road, Corby, Northants NN17 5JX, UK
Joseph, Mr I	Zintec Feed Solutions, Downwood, Shobdon, Hertfordshire HR6 9NH, UK
Kirkland, R	Volac International Limited, 50 Fishers Lane, Orwell, Hertfordshire SG8 5QX, UK
Leek, Dr A	Premier Nutrition, The Levels, Rugeley WS15 1RD, UK
Linden, Ms J	5M Publishing, 4 Haywood House, Hydra Business Park, Neither Lane, Sheffield S35 9ZY, UK
Lowe, Dr J	Dodson & Horrell Limited, Kettering Road, Islip NN14 3JW, UK
Lucey, Mr P	Dairygold Co Op Ltd, Lombards Town, Co. Cork , Ireland
Metzger-Petersen, Miss K	Dr. Eckel, JM Stiefel Feld 10, Niederzissen 56651, Germany
Millar, Mr C	Gain Feeds, Glanbia PLC, Bridge, Bridge Street Portlaoise, Ireland
Mitchell, Mr P	Crown Chicken Ltd, Green Farm, Edge Green, Kenninghall, Norwich NR16 2DR, UK
Morgan, Mr W	Writtle College, Writtle, Chelmsford, Essex CM1 3RR, UK
Morris, Mr W	BOCM Pauls, First Ave, Royal Portbury Docks, Portbury, Bristol BS20 7XS, UK
Mounsey, Mr A	Pentlands Publishing Ltd, Station Road, Great Longston, Bakewell, Derbyshire DE45 1TS, UK
Mul, Mr A	Business Development Feed Ingredients, De Jasmin 4 Boxmeer, 5831, SL, The Netherlands
Niewold, Dr T	Katholieke University of Leuven, Kasteelpark Arenberg 30, 3000 Heverlee Leuven, Belgium
Northover, Mrs S	University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK
Overend, Dr M	Huve Pharma N.V., Uitbreidingstraat 80, 2600 Antwerp , Belgium
Packington, Mr A	DSM Nutritional Products, Heanor Gate, Heanor, Derbyshire DE75 7SG, UK
Parker, Dr D	Novus Europe, Avenue Marcel Thiry 200, Brussels B1200, Belgium
Parr, Dr T	University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK
Partanen, Ms K	MTTAnimal Production Research, Tervamaentle 179, Hyvinkaa 05840, Finland
Pine, Dr A	Premier Nutrition Products Ltd, Brereton Business Park, The Levels, Rugeley, Staffs WS15 1RD, UK
Riley, Mr A	Danisco Animal Nutrition, PO Box 777, Marlborough, Wiltshire SN8 1XN, UK
Robinson, Mr P	Kingshay, Bridge Farm, West Bradley, Glastonbury BA6 8LU, UK
Salter, Prof A	University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

Simongiovanni, Miss A	Ajinomoto Eurolysine S.A.S, 153 rue de Courcelles, Paris 75017, France
Sinclair, Dr K	University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK
Stafford, Dr J	Dairyco, The Old School House, Bradbourne, Ashbourne, Derbyshire DE6 1PA, UK
Stokes, Dr C	CSIRO, Climate Adaptation Flagship, CSIRO Davies Lab, 1 University Road, Douglas Q4814, Australia
Taylor, Mr S	Optivite Ltd, Unit 5, Manton Wood, Enterprise Park, Worksop, S80 2RS, UK
Tucker, Prof G	University of Nottingham, School of Biosciences, Sutton Bonington Campus LE12 5RD, UK
Twigge, Dr J	Frank Wright Trouw, Belnheim Road, Ashbourne, Derbyshire DE6 1HA, UK
van der Kolk, Mr W	Lucta SA, Burg Aalberslaan, Krimpen 2923CD, The Netherlands
Van Straalen, Dr W	Schothorst Feed Research, P O Box 533, Leylstad 8700AM, The Netherlands
Vandi, Mr L S	Vetagro Spa, Via Porro 2, Reggio Emilia 42100, Italy
Varley, Dr M	The Pig Technology Co, BPEX, Whitegates, Willow Lane, Clifford, Wetherby, W Yorks LS23 6JH, UK
Wapenaar, W	University of Nottingham, School of Veterinary Science and Medicine, Sutton Bonington Campus LE12 5RD, UK
Wathes, Prof C	Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield AL9 7TA, UK
Webb, Prof R	University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK
Wellock, Dr I	ABN, 64 Innovation Way, Peterborough Business Park, Peterborough PE2 6FL, UK
Wilcock, Dr P	ABN, ABN House, Oundle Road, Peterborough PE2 9PW, UK
Wilde, Mr D	Massey Bros Feeds Ltd, Cranage Mills, Holmes Chapel, Cheshire CW4 8EE, UK
Wilkinson, Dr M	DEFRA, Nobel House, 17 Smith Square, London SW1P 3JR, UK
Wilson, Mr S	BOCM Pauls Ltd, Tucks Hill, Burston, Diss IP22 5TJ, UK
Wiseman, Prof J	University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK
Wonnacott, Dr K	Dairyco, AHDB, Stoneleigh Park, Kenilworth, Warwickshire CV8 2TL, UK
Wood, Prof J	University of Bristol, Department of Clinical Vet Science, Langford, Bristol BS23 2XP, UK
Wynn, Dr R	KW Alternative Feeds, Bishopdyke Road, Sherburn in Elmet LS25 6JZ, UK

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