

Nutritional influences on metabolic hormones and reproductive function in dairy cows

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Introduction

Production responses to nutrient supply are well established in dairy cows. Energy intake is usually the most limiting factor and milk yield increases with energy intake, following a diminishing response curve as increasing proportions of energy are partitioned towards body fat. Protein is not normally limiting because high protein ingredients can be substituted for energy-yielding ingredients; in fact, excess protein is usually more common than protein limitation because least-cost diet formulation packages always show that energy is a bigger constraint than protein. Mineral and vitamin supply can be adjusted easily with high-density supplements; the difficulty comes from defining the band between deficiency and excess.

Different sources of dietary energy and protein modify responses in milk yield and composition. In particular, the proportions of energy supplied as starch, fibre and fat have major effects on milk fat and protein yield (Sutton, 1985). These effects are mediated through changes in rumen fermentation, end products of digestion and substrate supply to the mammary gland, which are accompanied by metabolic hormonal responses that alter nutrient partitioning.

Although there are strong negative genetic and phenotypic relationships between milk yield and fertility, poor fertility is not an inevitable consequence of high milk yields. There are high-yielding herds with good fertility and low-yielding herds with poor fertility. One of the key determinants of fertility is the balance between energy output in milk and energy intake. Thus, any attempt to reduce milk yield by restricting nutritional inputs would be disastrous for cow health and fertility.

Nutrition can affect reproduction on both short-term (days) and medium-term (months) timescales. Physiologically, changes in nutrient supply alter metabolic signals to the brain and ovary, thereby coordinating ovarian function with metabolic

status (Garnsworthy *et al.* 2008a). The objective of this paper is to illustrate some of the interactions between nutrition and fertility in cattle that suggest nutritional management strategies to improve reproductive success.

The **first nutritional strategy** to aid fertility is to control BCS at calving. In dairy cows, the target should be 2.5 to 3.0 (1 to 5 scale), so that dry matter intake is not impaired by the negative feedback effect of body fat, and BCS loss is restricted to less than 0.5 units (Garnsworthy, 2007).

The **second nutritional strategy** to aid fertility is to provide high-quality diets during early lactation so that energy and nutrient intakes are not limited by dietary characteristics. A palatable, high-energy diet is required, but avoid excessive dietary fat (reduces rumen fibre digestion, decreases insulin, and stimulates body fat mobilisation), excessive starch (reduces rumen fibre digestion and encourages acidosis), and excessive protein (stimulates body fat mobilisation).

When the priorities of minimising negative energy balance, controlling body condition score and meeting nutrient requirements for milk production have been addressed, diet composition can be altered to manipulate metabolic hormones. Different sources of dietary energy and protein modify partition of nutrients and responses in milk yield and composition. Starch is a glucogenic energy source, so it stimulates synthesis of milk lactose and protein. Fibre and fat are ketogenic energy sources, so they stimulate synthesis of milk fat. These effects are mediated through changes in rumen fermentation, end products of digestion and substrate supply to the mammary gland, which are accompanied by changes in metabolic hormones that alter nutrient partitioning. Changes in metabolic hormones during early lactation alter the pattern of ovarian follicle growth and development, thereby influencing reproductive function (See reviews by Webb *et al.* 2004; Garnsworthy *et al.* 2008a). Therefore, a **third nutritional strategy** to aid fertility is to alter diet composition so as to manipulate metabolic hormones at key stages of the reproductive cycle. Features of this strategy are the main focus for the rest of this paper.

Hormonal responses to diet composition

We conducted a ten-year programme to examine dietary effects on metabolic hormones and fertility. Diets provided structured variation in total starch, site of starch digestion, fat, fibre, metabolisable protein, amino acid profile and forage type, within a narrow range of energy concentration (11.9-12.1 MJ ME/kg DM). Diets were based on mixtures of corn silage, grass silage, wheat, corn, sugar beet pulp, full-fat and extracted oilseed meals, and calcium salts of palm acid oil. In each experiment, cows were fed on a standard diet for the first 35 days of lactation and treatment diets between days 40 and 70.

Plasma concentrations of growth hormone (GH), insulin-like growth factor-I (IGF-I) and leptin were not related to diet composition, although they were affected by animal factors, such as milk yield, BCS and live weight. Plasma concentrations of insulin were positively related to dietary starch concentration, and results indicated that to maintain adequate insulin to glucagon ratio in cows at the start of the breeding period, dietary starch concentration should be above 160 g /kg DM (Garnsworthy *et al.* 2008b). Starch from wheat, corn and corn silage produced similar responses, however, so diets can be formulated to total starch content rather than rumen digestible or bypass starch (Garnsworthy *et al.* 2009a). Insulin was negatively related to dietary fat content, and results indicated that dietary total fat concentration should be below 50 g /kg DM to avoid depressing plasma insulin concentration in cows at the start of the breeding period (Garnsworthy *et al.* 2008c). Insulin was increased also by branch-chain amino acids, particularly leucine (corn gluten is a good source), which is an insulin secretagogue (Docherty & Clark, 1994). However, responses to leucine depended on total dietary protein content: for low protein diets, the insulin to glucagon ratio was greater with high leucine; for high protein diets, the insulin to glucagon ratio was greater with low leucine because the high-protein, high-leucine diet stimulated milk yield (Garnsworthy *et al.* 2008d).

Metabolic hormones and reproduction

Nutritional status is a key factor influencing reproduction, and a number of reviews discuss various aspects of nutrition on fertility in ruminants (Beam and Butler, 1999; Garnsworthy and Webb, 1999; Webb *et al.*, 1999a,b; Butler, 2000; Lucy, 2000, 2003; Webb *et al.*, 2004; Garnsworthy *et al.*, 2008a). Nutrient intake acts at various levels

within the hypothalamus-pituitary-ovarian axis to control ovarian activity, and nutritional status has also been correlated with embryo survival. In dairy cows, increased milk yields and metabolic demand have been associated with longer postpartum anoestrous intervals, abnormal oestrous cycles and reduced conception rates (Royal et al., 2000; Lucy, 2003). However, the detailed physiological mechanisms through which nutrition exerts many of these effects remain to be fully characterized.

Postpartum anoestrus

Metabolic hormones and reproductive function were measured in dairy cows of High and Low genetic merit during early lactation (Gutierrez, et al., 2006). Resumption of normal oestrous cycles postpartum occurred approximately 8 days later in High-merit cows, and this was associated with lower plasma insulin concentrations. A further study investigated if feeding diets designed to increase circulating insulin concentrations during the early postpartum period can overcome the delay in first ovulation postpartum in animals selected for increased milk yield (Gong et al. 2002). Two isoenergetic diets were formulated to either stimulate or depress plasma insulin concentrations, and these were fed to cows of High or Low genetic merit. Again, the initiation of first ovulation and resumption of normal oestrous cycles postpartum were delayed in the cows of high genetic merit. This was associated with lower circulating insulin concentrations, but did not involve an alteration in basal plasma gonadotrophin concentrations or patterns of ovarian follicular development during the early postpartum period. Feeding the diet designed to increase circulating insulin concentrations advanced the initiation of the first ovulation postpartum so that more cows ovulated within the first 50 days of lactation (Table 1).

The responses observed in the study of Gong *et al.* (2002) were independent of milk yield and energy balance. It appears that insulin acts as a metabolic signal to the reproductive system, signalling that energy status is good. The exact mechanism for this signalling is still unclear, but it probably involves interactions between insulin, IGFs, and gonadotrophins (Garnsworthy *et al.* 2008a). The roles of GH, IGF and leptin appear to be more concerned with milk yield, energy balance and body condition than with nutritional status because these hormones were not affected by

changes in diet composition that induced large differences in insulin (Garnsworthy *et al.* 2008b,c,d; 2009a).

Table 1. Percentage of cows that ovulated within 50 days of calving in High and Low genetic merit dairy cows fed on diets that induced low or high concentrations of plasma insulin (Gong *et al.* 2002)

<i>Diet</i>	<i>Genetic merit</i>	
	High	Low
Low Insulin	50%	60%
High Insulin	80%	100%

Follicle growth and oocyte quality

In our nutritional studies, we found that circulating insulin concentrations were associated with changes in numbers of follicles. Increasing dietary starch concentration was associated with greater numbers of small follicles and reduced numbers of medium-sized follicles (Figure 1), suggesting that although recruitment of follicles may be increased by higher insulin, follicular development might be impaired (Garnsworthy *et al.* 2008b). Fat supplementation of a high-starch (180 g/kg DM) diet increased the number of small follicles, but did not affect the number of medium-sized follicles and there was no benefit from supplementary fat above 8 g/kg DM (Figure 2). Taken together, these studies suggest that both fatty acid supply and insulin have minimum thresholds, so increasing either factor stimulates follicle development only when the other is adequate (Garnsworthy *et al.* 2008c). In a study of amino acids and protein levels, there was no effect of dietary treatment on numbers of follicles in different size classes; we concluded that altering metabolic hormones through manipulation of amino acid supply and balance is unlikely to have a significant impact on ovarian function in dairy cows (Garnsworthy *et al.* 2008d).

Some of our studies showed that whilst high-insulin status may encourage early resumption of oestrous cycles, high insulin might not be beneficial for oocyte quality. For example, in the study of Fouladi-Nashta *et al.* (2005) cows were fed diets with either low or high starch content in order to induce differences in plasma insulin.

Oocytes were collected for in vitro fertilisation and cultured to examine developmental potential. The high starch diet produced a significantly higher number of poor quality oocytes and a lower proportion of cleaved embryos that developed to the blastocyst stage. In another experiment, (Fouladi-Nashta *et al.* 2007), addition of fat to a high-starch diet reduced plasma insulin concentrations and improved developmental competence of oocytes. These studies indicate that high insulin status could adversely affect oocyte quality, which is consistent with our studies in beef cattle.

Optimum dietary strategy

There appears to be a potential conflict between nutritional strategies designed to start cows cycling and production of good quality oocytes. Diets designed to increase plasma insulin concentration stimulate resumption of oestrous cycles, but have detrimental effects on oocyte competence. This presents an interesting challenge for researchers and nutritionists in the field.

Our recent studies (Garnsworthy, *et al.* (2009b) addressed these differential responses by examining nutritional strategies designed to enhance or impair cyclicity and oocytes at different stages of the reproductive cycle. In an initial experiment, strategies designed, to improve only one factor, or to impair both factors, resulted in pregnancy rates of 27% at 120 days in milk; a strategy designed to improve both factors resulted in a pregnancy rate of 60% ($P=0.03$). These strategies have been tested under commercial conditions with success often reported.

Conclusions

Poor fertility is not an inevitable consequence of high genetic merit, but may be due to a combination of factors that include genetic susceptibility, management, disease, milk yield, energy balance, body reserves and specific nutritional circumstances. In general, nutritional strategies for efficient milk production are commensurate with strategies for achieving good fertility.

The absolute requirements of an oocyte or developing embryo for energy and protein are very small, compared with the cow's requirements for milk production.

Reproductive responses to nutrition are due to metabolic signals of nutrient status

rather than availability of nutrients *per se*. It seems likely that the major influence of nutrition is through metabolic hormones, such as GH and insulin, which act through the IGF system to have repercussions at the tissue or cellular level.

Because so many factors influence reproductive function in dairy cows, it is difficult to predict whether an individual cow will conceive under a given set of circumstances. However, it is possible to identify certain risk factors that may predispose cows to infertility. These include excesses or deficiencies of energy and protein, severe negative energy balance and excessive weight loss. Even when these risk factors are minimised, there still appears to be differential responses to nutrition at the ovarian level. Research is being conducted to predict these responses so that optimal nutritional strategies can be developed that improve fertility without compromising milk production. Preliminary studies suggest that this goal can be achieved.

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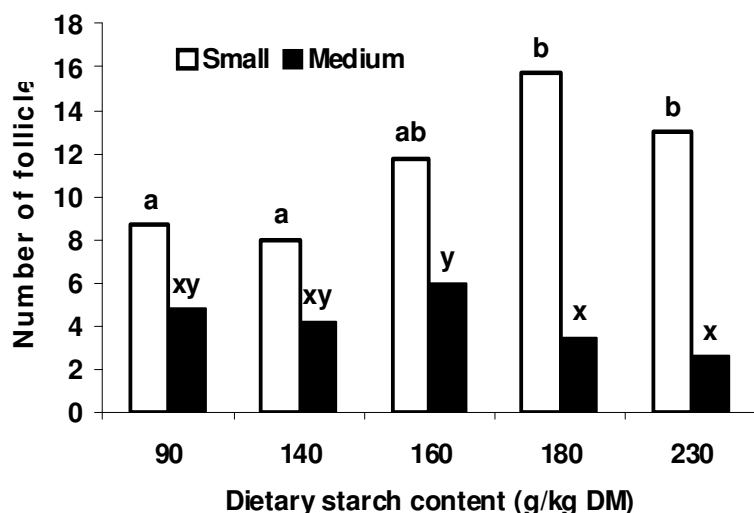


Figure 1. Numbers of small (<5 mm) and medium-sized (5-10 mm) ovarian follicles after a synchronised oestrus at 60 days in milk in high-yielding dairy cows fed on diets differing in starch content. Letters denote differences at $P<0.05$. (Data from Garnsworthy *et al.* 2008b).

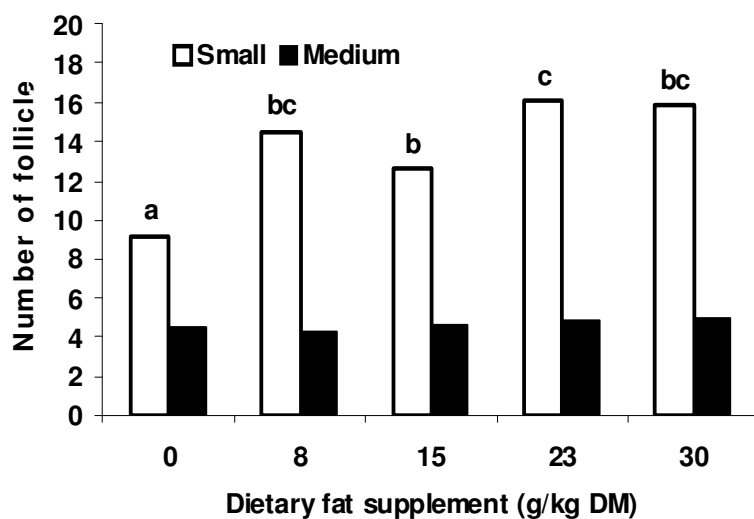


Figure 2. Numbers of small (<5 mm) and medium-sized (5-10 mm) ovarian follicles after a synchronised oestrus at 60 days in milk in high-yielding dairy cows fed on diets containing different levels of fat supplementation. Letters denote differences at $P<0.05$. (Data from Garnsworthy *et al.* 2008c).

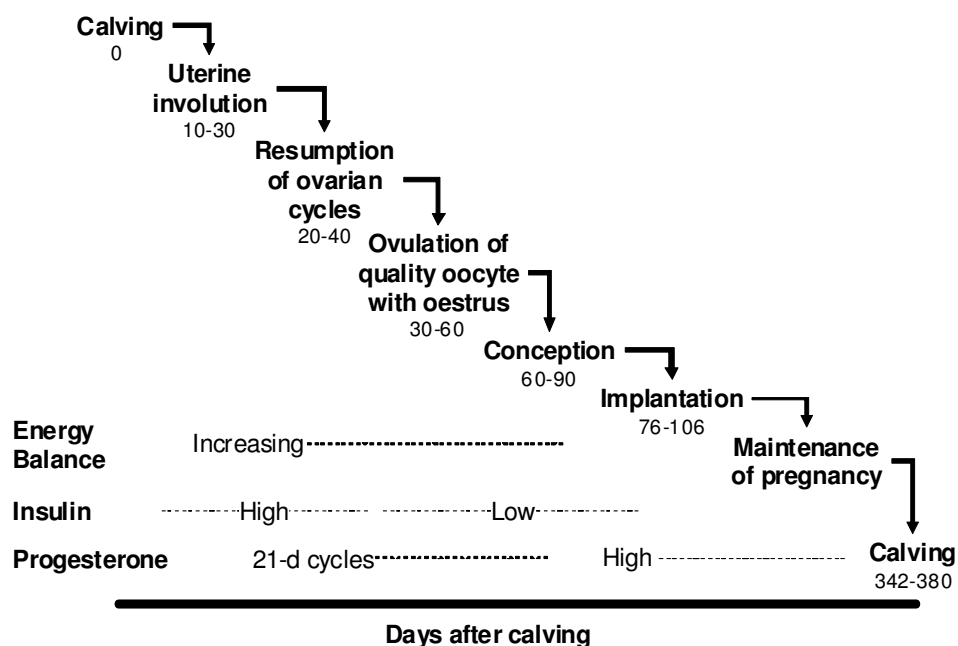


Figure 3. Sequence of reproductive events in the dairy cow. Each event depends on the success of preceding events. Numbers indicate optimum range (days after calving) to achieve an average calving interval of 365 days. Major temporal factors that influence success are: energy balance, which should start to increase early in lactation; insulin, which stimulates resumption of oestrous cycles, but may reduce oocyte quality; and progesterone, which is low during anoestrus, high during luteal phases of cycles, and low during follicular phases of cycles. (After Garnsworthy *et al.* 2008a).