

Quantitative Metabolic and Epidemiological Approaches to Fertility of the Dairy Cow

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INTRODUCTION

Declines in dairy cattle fertility, whether measured as time to conception, retention of conceptus, or percentage pregnant in a given time have been well documented in North America (NA) and elsewhere. However, there are herds within those environments that perform well above measures of fertility considered typical in NA. The Holstein genotype is arguably singular, with minor strain differences, and estimated heritability of fertility measures is low. Further, Macmillan et al. (1996) noted that declines in fertility are observed in the lactating cow rather than for the heifer. Consequently, environmental (especially nutritional influences) should explain much of the differences in reproductive performance between countries and within production environments. This paper briefly provides comparative data on different diets and production environments, from different regions of NA and Australasia, highlighting substrate and production differences for cows from these regions. Results of studies pertinent to these different environmental and nutritional challenges will be examined in the context of quantitative nutrition and the epidemiology of reproduction, with a particular focus on factors influencing pregnancy at service. It is critical to recognize that incidence density of pregnancy (i.e., getting cows pregnant quickly) is the key goal. We have more control over time to mating, but relatively little knowledge of how to influence conception and pregnancy. Areas of management that may provide opportunities to improve lactational sub-fertility will be identified and discussed. Some statistical considerations in regard to study evaluation are discussed throughout.

COMPARISONS AMONG SYSTEMS

Production and Reproductive Measures

Table 1 provides a starting point to assess differences among production systems. These estimates are not all definitive, because such data are not available; therefore assumptions have necessarily been made. Table 1, however, suggests that substantial differences between systems exist in production and reproduction. These crude observations on comparisons of body weights, milk production and composition, and some reproductive measures provide the potential to generate hypotheses about differences in reproduction among and within systems that are explored in this paper.

Table 1. Production and reproduction parameters, dietary components, and incidence of puerperal disease in USA, Canada, Australia, and New Zealand.

PRODUCTION/ REPRODUCTION MEASURES	USA^a	Canada^b	Australia^{cd}	New Zealand (HF)^e
Milk production (kg or l 305d)	8978	9624	5306	4184
Milk fat content (%)	3.29	3.7	4.05	4.3
Milk protein (true) (%)	3.07	3.19	3.31	3.54
Body weight pre-calving (kg) 5- year old estimated	675	670	600	530
Anestrus incidence (% cows calved)	23 – 28	25	20-30 (Seasonal)	17 – 20
First service conception (%)	40		49	52 – 56
Estrous detection rate (%)	50	48	90 - 94 (seasonal)	95

			73 (year round)	
Average time to pregnancy (d)	150	-	Year-round herds: 120 d - median; 53 % preg by 100d; 12 % not preg at 200d Seasonal herds: 42 d - mean	-
DIETARY DATA*	North Americaⁱ		Australia^g	New Zealand^h
Dry matter intake (DMI) (kg/d)	25		22	19.5
Estimated energy intake (mcals/d)	68.0		59.3	49.5
Crude Protein (%)	17.1		22	22.7
MP Balance (g/d)	-256.5		+403	+309
NSC (%)	39.1		28.5	20.9
NFC (%)	46.0		35.1	28.3
Lipid Intake (g/d)	697		645	617
C18:3 and C18:2 estimated duodenal flux (g/d)	32		44	45
DISEASE INCIDENCE	USAⁱ	Canada^j	Australia^k	New Zealand^l
Milk Fever (%)	5.2	7.9	3.1	3.5
Mastitis (%)	14.7	14.7	9	9-12.6
Clinical Ketosis (%)	3.7	1.5	1.5	1
Lameness (%)	11.6	20.4	15	4-5.6
Retained afterbirth (%)	9.0	8.1	6.5	1.7
Uterine infections (%)	-	4.1	3.7	0.5
Dystocia (%)	-	3.0	12.7	
Displaced abomasums (%)	3.5	4.2	<1	<1
Downer cows (%)	1.1	1	-	-
Other diseases (%)	-	-	-	1
All disease (%)	-	31	-	-
Inductions (%)	-	-	-	8

Sources

^a National DHIA, USA; ^b Duffield personal communication (2007); ^c Morton, 2004; ^d Australian Dairy Industry in Focus 2006; ^e Xu and Burton, 2004; Anon LIC, 2005-6

^f Body weight (630 kg); milk (45 l); fat (3.5 %); protein (3.19 %); Diet DM – 8.5 kg maize silage 5.5 kg alfalfa silage 1.2 kg grass hay, 5.0 kg high moisture corn, 0.15 kg soybean (whole roast), 0.7 kg soybean meal (48 % CP), 0.25 kg fish meal, 0.25 kg blood meal, 0.9 kg canola meal, 0.1 kg soy hulls, 0.1 kg protected fat, 0.1 kg beet pulp, 0.25 kg dried molasses, 0.7 kg corn gluten feed (60 % CP), 0.7 kg wheat shorts – based on Canadian research data and comparable US diets.

^g Body weight (600 kg); milk (32 l); fat (4.05 %); protein (3.31 %); Diet DM – 15 kg ryegrass, pasture 6 kg wheat or triticale, 1 kg oat hay (based on data from Bramley, 2004);

^h Body weight (525 kg); milk (28 l); fat (4.3 %); protein (3.54 %); Diet DM – 16 kg ryegrass, pasture 2.5 kg maize silage, 1.5 kg molasses (based on recent trial data)

(*Assumed that minerals and vitamins are fed to meet NRC recommendations)

ⁱ USDA-APHIS, Mike Socha, personal communication (2007)

^j Todd Duffield, personal communication (2007)

^k Curtis and Lean, 1998; Moss, 2001; Lean, 1997; Stevenson, 2000; Morton, 2004

^l Xu and Burton (2004) and B.J. Mackay, personal communication (2007)

Dietary comparisons (typical diets 70 days in milk)

Similar comparisons can be made in regard to dietary differences among countries, again with the same caveats on the quality of the observations. Despite these qualifications, some aspects are self-evident; cows in North America, in general, have higher dry matter intakes (**DMI**) and are likely to be fed diets lower in crude protein (**CP**), and higher in non-structural carbohydrates (**NSC**) and non-fiber carbohydrates (**NFC**). Further, these cows have a much lower estimated metabolizable protein (**MP**) balance. The North American cows are also likely to have lower butter fat content, an outcome reflected in a lower ratio of fat (lipid) intake to milk output and a lower estimated flow of the major essential fats (C18:2 and C18:3) to the duodenum (Table 1). However, periods in which Australian and New Zealand herds have positive MP balances and substantial amounts of pasture in the diet can be limited to periods of maximal pasture growth that coincides with seasonal mating in spring.

Disease Incidence Comparisons

The comparative data suggest that rates of calving related disorders are generally higher in the North American herds.

RELATIONSHIPS BETWEEN PRODUCTIVITY OF DAIRY COWS AND REPRODUCTIVE PERFORMANCE

There are logical hypotheses that flow from these comparisons and have been the subject of numerous studies. Specifically, observational studies highlight a negative association between increased milk production over time and lower reproductive performance. Despite many good controlled studies of intermediary metabolism and hormone concentrations; fewer studies systematically examine associations among nutrient fluxes stemming from dietary factors that influence milk production, milk composition, and reproduction. The focus of this paper is to explore relationships between nutrition and reproductive performance in the context of increasing the probability of pregnancy at breeding and examining some attempts to achieve this.

Few studies report the amount of variance in reproduction that can be explained by the factors studied, because there have been relatively few multivariable studies of fertility. Curtis and Lean (1998) were able to explain 81 % of the variance in calving to conception interval, however only 7 % of the variance in conception at first service. Similarly, Moss (2001) explained 5.8 % of the variance in conception at first service and Morton (2004) 6.7%. Westwood et al. (2002) with extremely tight control over insemination methods, estrous detection, and semen allocation explained 13.6 % of the variance in conception at first insemination. These studies all examined a wide range of risk factors including antioxidant and metabolite concentrations in blood, disease, production level, body condition score, body weight, feed intake and many others at the epidemiologic and metabolic level. Time related reproductive events are more readily explained using the variables examined to date and are more readily influenced by hormonal treatments, e.g. prostaglandins, progesterone releasing devices, etc. Conception or pregnancy to service is more difficult to explain using variables examined to date, but is also more difficult to explain inherently on a statistical basis. As shown later, conception is also difficult to influence using hormonal or dietary treatments.

Figure 1 outlines a hierarchy of events leading to reproductive outcomes. It is critical to recognize that the primary inputs to reproduction are those to the top and left, those on the bottom right are outcomes and those below and in the middle are the intermediary pathways that are influenced by those on the left and may represent mechanisms that influence the outcomes. All of these paths are influenced by disease or metabolic disorders that influence DMI, ovarian function, or fetal survival.

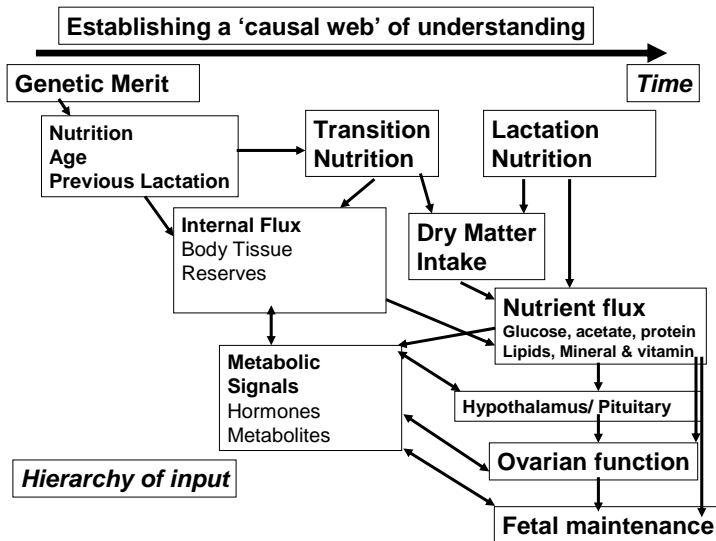


Figure 1. A causal web to develop a model of metabolic subfertility in dairy cattle

These time-ordered concepts can also be applied to practical reproductive management on farms using hazards analysis critical control point methods (Lean, 2000; Lean et al., 2003). Unless time ordered relationships are considered in statistical analyses there is a substantial risk that spurious relationships will be identified, because many of the changes in metabolism occurring in the transition period are strongly related to two driving forces, specifically DMI and increasing milk production with associated utilization of nutrients. Multivariable analyses that account for confounding factors, including disease, are therefore crucial for population-based or observational studies. For serial sampling of metabolites and hormones, time related analyses need to account for strong trends in data; otherwise, there is a very real risk of wrongly associating study variables. These are important considerations that influence advice that flows to producers from evaluating the reproductive literature.

Factors Associated with Fertility in Dairy Cows

Genetic Merit and Milk Production

Genotype by environment interactions for milk production in most dairy production regions are very small, with genetic correlations between national evaluations of Holstein bulls used being above 95 % between many countries and above 90 % for most InterBull participants. New Zealand and to a lesser extent Australia are exceptions with genetic correlations of 75 % to 80%. However, a correlation of 75 % is still high, indicating that largely there is one Holstein genotype with modest strain differences. For example Macdonald et al. (2007) reported small differences in growth parameters and puberty among strains of Holsteins of different genetic origin. Therefore, milk production (and composition) differences in Table 1 are very substantially influenced by environment, especially nutrition. This finding was confirmed in the Canadian and New Zealand studies of Peterson (1988) and more recently in Ireland (Horan et al., 2005).

In terms of the hierarchy of events leading to reproductive failure, genetic merit for milk production is a fixed input. Early studies that identified declines in reproduction over time were based on phenotypic evaluations. Spalding et al. (1975) found a difference of 20.5 % in first service conception rate between cows in the highest and lowest milk producing quadrants (a difference of 1018 kg milk over a 305-d lactation). Subsequent studies (Ferguson, 1991; Nebel and McGilliard, 1993; Royal et al., 2000; Stevenson, 2001; Washburn et al., 2002) found similar effects of increasing total lactational milk yield on conception rates. There are, however, three potential sources of bias in estimates of genetic or phenotypic associations of milk production associations with fertility measures that are time related. Some producers deliberately delay breeding some high producing or high genetic merit cows, cows that are culled can be lost from some analyses, and milk production is higher for cows that are slow to conceive. Lean et al. (1989) explored the fundamental flaw in studies that fail to account for the impact of extended days open (**DO**) on milk production. By dividing the lactation into a peak milk yield factor and a persistency factor we found that both peak milk yield and yield persistency were associated significantly with decreased reproductive performance. The probability of conception after 2 inseminations for these Californian cows was calculated taking account of dairy origin, peak daily milk production above or below the median of 38.2 kg, parity (heifer or cow), and persistency of lactation. Cows with higher than the median peak milk production were 27 % less likely to conceive in 2 inseminations than those with lower peak milk yield; and those with greater persistency of lactation were 61 % less likely to have conceived in 2 inseminations. Higher milk production had more influence on fertility than parity.

Consequently, time related associations, such as between genetic merit and DO should be treated with some caution. Olds et al. (1979) in a US study of some 60,000 cow records of mixed breeds found a 6.8 % difference in conception rates between the upper and lower quartiles of milk production. Morton (2004) in a very large Australian observational study found no large phenotypic differences in reproductive performance between daughters of sires of varying genetic merit. Interestingly, he found a small decrease of 2 % in pregnancy (pregnant by 100 d after calving or 6 wk after mating start date) for cows with highest milk fat production in the first 120 d of lactation. This resulted in population attributable risks for non-pregnancy by 100 d after calving or 6 wk after mating start date of 0.7 to 1 % (Morton, 2004). Mackey et al. (2007) in an Irish study concluded that calving rate to first service had a positive association with genetic merit for milk production and that other studies found similar or small negative effects.

Smaller controlled, randomized, trials often lack sufficient statistical power to detect modest differences in conception rate or time to pregnancy, but have the advantage of being able to provide more information on underlying mechanisms than field-based epidemiologic studies. In early studies, Fonseca et al. (1983) found no evidence, in a small controlled trial using Holsteins, of adverse impact on reproductive performance for cows that produced more than 1000kg above average and those that produced 1500kg below average. Stevenson et al. (1983), in a similar trial, found that cows with higher milk yields had better reproductive performance.

Long-term trials using genetically separated lines have been used to explore relationships between production and fertility (Hageman et al., 1991; McGowan et al., 1996; Pryce et al., 1999; Veerkamp et al., 2002). Most of these have found small increases (e.g. 5-10 d) in interval to pregnancy measures with an increase of 1000 kg of 305-d milk production. In a tightly controlled study, Westwood et al. (2002) found that reproductive performance (time to pregnancy, conception to first service, percentage pregnant at d 150 after calving) did not differ significantly between high and low genetic merit cows. The only outcome significantly influenced by genetic merit in the Westwood et al. (2002) study was display of estrus at first ovulation. Jonsson et al. (1999) replicated the Westwood study using cows on pasture and found that intervals from calving to first insemination and to conception were similar between high and low genetic merit cows.

Overall, estimates of heritability of fertility are low 0-0.03 and even if there is a considerable genetic antagonism identified between yield and fertility, this may be of limited consequence because estimates of heritability of fertility are near zero (Hansen et al., 1983). Mao (1984) and Seykora and MacDaniel (1983) estimated a genetic increase of 100 kg milk yield would result in an additional 5-10 DO. Such findings suggest that continued genetic selection for milk yield without associated selection for fertility will cause unfavorable genetic changes for fertility. Higher heritabilities have been reported for some reproductive outcomes (0.05-0.12), particularly those variables associated with time to breeding inherent, e.g. DO and inter-calving interval. Despite limitations in these measures, these and many subsequent findings reflect the pattern of subtle long-term declines in fertility observed (Spalding, 1975). However, it has been well noted that these declines in fertility are not merely associated with increased milk production; as herd sizes have increased, methods of production have changed and staff to cow ratios have declined markedly over time. Irrespective of any trend to a long term decline in fertility, genetic factors will likely explain only a small proportion of variation in fertility within a population of cows at any point in time. For an individual dairy farm, changes in genetic merit have very little immediate impact on fertility. Because the heritability estimates for reproductive traits associated with conception are very low, environmental influences will dominate. Ways will be found to improve reproductive performance, providing that there is no substantial genetic effect on time to first estrus, service, or return rate.

Internal Nutrient Flux – Contributions of Body Tissue

Macmillan et al. (1996) noted that differences between the conception rates of heifers and adult cattle were much greater in NA than in the pasture-based dairy systems of Australasia. This supported observations of Spalding et al. (1975) who noted that heifer fertility in the USA had not declined concurrently as cow fertility declined. Studies showing phenotypic and genetic antagonism between milk production and fertility suggest that milk production per se is a major risk for fertility and reinforces a view that the genotype is still inherently fertile. This is supported by differences in conception rates evident between NA and the southern hemisphere. The challenge for us is to identify the means by which high milk production may be achieved without lowering fertility. Most likely this challenge will be met by considering in detail the fluxes of nutrients required for lactation and in the recognition that the higher producing genotype requires more critical attention to detail regarding diet presentation and delivery.

It is likely that specific nutrient deficiencies either directly or indirectly influence fertility. Sources of nutrients that either directly or indirectly influence fertility are internal (body tissue reserves) or external (feed, including dirt, or water). Hormonal (luteinizing hormone (LH), follicle stimulating hormone (**FSH**), prostaglandins (**PG**), growth hormone (**GH**), insulin-like growth factor (**IGF**), insulin, leptin) or metabolite changes (glucose, non-esterified fatty acids (**NEFA**), cholesterol, urea nitrogen) are influenced by nutrient fluxes and may indicate pathways through which effects are mediated. Low body condition at calving, high body condition at calving and excessive body condition loss in early lactation have all been consistently, but not invariably, associated with reduced reproductive performance.

There is consistent evidence that an optimal body condition score before calving exists (Garnsworthy and Topps, 1982; Garnsworthy, 1989; Curtis and Lean, 1998; Morton, 2004). A low body condition score at calving is associated with not only a prolonged interval to first estrus (Jolly et al., 1995), but lower conception probability (Garnsworthy, 1989; Curtis and Lean, 1998; Morton, 2004). Cows with higher body condition scores have shorter intervals to re-establishment of cyclicity after calving. However, over-conditioned cows (body condition score > 3.5 on a 5-point scale) have other problems that delay conception. These may be partially mediated through metabolic disorders associated with excessive weight loss (Ruegg et al., 1992; Moss, 2001; Mackey et al., 2007) or increased predisposition to postpartum disease (Ruegg and Milton, 1995; Curtis and Lean, 1998). Higher genetic merit cows (milk production) mobilized more body weight than cows of lower merit after calving (Buckley et al., 2000; Snijders et al., 2001; Westwood et al., 2002). Many studies identified similar reductions in fertility measures with excessive body tissue mobilization to those observed by Butler and Smith (1989). The weight losses observed in early lactation are associated with increased reliance on lipids, ketones and acetate and decreases in tissue availability of cholesterol (Grummer and Carroll, 1988; Lean et al., 1992). There has been an outstanding research program into the effects of excessive tissue mobilization and effects on hepatic and other functions that are well summarized (Grummer, 1995).

There has been a focus on the concept of negative energy balance (**NEB**) influencing fertility, subsequent to the pivotal work of Butler and Smith (1989). This concept is based on the observation that DMI lags the milk production curve and is associated with body tissue mobilization. Many workers have found associations between NEB, metabolites and hormones in blood and measures of fertility. The concept has served us well, but should now be modified to allow a more careful consideration of the factors that comprise a negative nutrient balance (**NNB**). Westwood et al. (2002), who had the advantage of weekly body weight and daily milk data, deconstructed the term NNB into its two components: milk production and DMI. Increased yield of fat corrected milk (**FCM**) during early lactation was associated negatively with expression of estrus at first ovulation and probability of pregnancy by d 150 of lactation. However, cows that ate 6 kg of dry matter/d more than herd mates were 26 times more likely to show estrus at first ovulation, and were twice as likely to be pregnant by d 150 of lactation. Dry matter intakes and milk yield are the major determinants of energy balance, but also reflect the ingestion and loss of other nutrients. It is probable that carbohydrate, protein, lipid, and mineral balances influence fertility; hence the term nutrient balance should be preferred to energy balance in general discussion of the influence of nutrition on fertility. The extent to which tissue will be mobilized is determined by genetic merit, milk production, access to feed, composition of feed, disease, and climate. Poor access to feed, feed that impairs appetite associated with spoilage, anti-nutritional factors (i.e., acidosis), diets that stimulate milk production without providing energy density or availability, and excessive heat or cold stress place cows at increased risk of disease and reproductive failure.

Cows in Southern hemisphere herds are likely to be lower in body condition, as well as body weight than those in NA herds (Table 1). In New Zealand, cows often gain weight soon after calving (eg Mackle et al., 1996). This gain is reflected in the positive MP balance for these herds (Table 1). While cows are slower to cycle and anestrus rates may be high reflecting the lower body condition, conception probability at first breeding is higher (Table 1). By contrast the NA cows are higher in body weight and cycle earlier; but have lower fertility. Body weight loss for NA cows can be profound and is associated with very high concentrations of fatty acids and ketones in blood (Lean et al., 1994; Duffield, 2000). Concentrations of NEFA and ketones appear to be lower in Australian (Abe et al., 1994; Stephenson et al., 1997; Moss, 2001) cattle.

If NA cattle are exposed to diets low in MP and low in NDF, but have higher energy intake and NFC concentrations and intake (Table 1), these may develop a carcass higher in mobilizable lipid to labile protein than those fed on diets that provide a positive MP balance through a significant part of lactation. Vandehaar and St Pierre (2006) highlighted the partitioning of energy to body weight observed by Oba and Allen (2000) when lower NDF diets were fed. It can be hypothesized that the GH axis is up-regulated in the NA cattle, as reflected in greater start-up milk, but the internal flux of nutrients provided from tissue mobilization has a higher ratio, and almost certainly greater amounts of lipid compared to protein. Estimates of body protein reserves mobilized at calving are 25 to 27 % of total body protein in a dairy cow, that is 10 to 17 kg of protein in body tissue (Belyea et al., 1978; Botts et al., 1979; Parquay et al., 1972). Belyea et al. (1978) noted that there was a significant variation in the abilities of cows to mobilize protein. The proteins and ultimately amino acids mobilized are used for mammary milk protein synthesis and for gluconeogenesis in the liver to

support lactation. It appears that rates of mobilization of fat and protein are similar (Oldham and Emmans, 1988; Baldwin et al., 1987), but there has been little recent work on amounts of labile fat and protein in body tissue, despite this being an important area of physiology. Given the amounts of body weight lost after calving, especially in cows of high body condition, we can be confident that the lipid reserves of these cows exceed the protein reserves.

High body condition score-ketotic cows produce milk that is high in fats, especially long chain fats, and low in protein content in early lactation (Lean et al., 1994; Heuer et al., 1999). In milk, high ratios of fat to protein concentrations in early lactation (Heuer et al., 1999) and around the time of first service (Moss, 2001) are associated with reduced reproductive performance. Higher milk fat concentrations in early lactation are also associated with lower reproductive performance.

Milk protein concentrations are strongly, positively associated with fertility. Morton (2004) found that the population attributable risk for failure to be pregnant at 6 wk after mating start or 100 d after calving for milk protein concentrations < 3.5 % was 3.6 % and 5.3 % for year round calving and seasonal calving herds, respectively. This was the most important single metabolic factor in Morton's study and is a finding supported by others. For cows with higher milk protein content, Miettinen and Setala (1993) found shorter intervals from calving to first service and conception interval; McGowan et al. (1996) found a shorter calving to first estrus intervals at first calving; and Buckley et al. (2003) found higher submission and increased pregnancy rates by wk 6 of the mating period. We found reduced risk of requiring multiple inseminations (Moss et al., 2002) for cows with higher milk protein concentrations. Milk protein concentrations are markedly lower in NA cows than those in Australasian cows. Milk protein concentrations were associated positively with DMI (Hristov et al., 2005) and estimated energy balance (Garvin, 1999) and negatively associated with blood ketone concentrations (Miettinen and Setala, 1993; Duffield et al., 1997) and serum NEFA concentrations (Garvin, 1999). Milk protein concentrations also are increased by supplying rate limiting amino acids in appropriate ratios. Milk protein yield is associated with a quadratic effect of rapidly fermented carbohydrate in diets, highlighting the risks associated with diets too high in carbohydrates (Hristov et al., 2005). Milk protein production reflects MP production; however, prediction of milk protein concentrations are more challenging, as competing pools for MP use include body tissue, oxidation in gluconeogenesis, and milk. It can be hypothesized that milk protein concentration increases when other pools are more replete.

External Flux

Kronfeld and Drepper (1976) and Kronfeld et al. (1983) explored a concept of an optimal mix of precursors for lactational efficiency based on the seminal work of Max Kleiber. There is no evidence that genetic selection has modified the post-ruminal efficiency of dairy cattle, and quantitative metabolic calculations of Kronfeld et al. (1983) remain valid. Based on the work of Baldwin 1995, the theoretical efficiency of milk synthesis may vary from 0.75 to 0.92, depending on the precursors used for milk synthesis. Use of fatty acids for milk fat formation is more efficient than use of acetate, and use of glucose is more efficient than use of propionate. Given the strong influence of milk production and DMI on fertility discussed above and the significance and strength of association with milk fat and milk protein concentrations, we can review the relationships between specific feed inputs and fertility.

Strangely, there has been very little focus on the effects of carbohydrate nutrition and fertility. There are reports of the relationship between ration fiber and fertility from observational field studies. Israeli workers have been prominent in these reports (Francos et al., 1977; Mayer et al., 1978; Bogin et al., 1982). Bogin et al. (1982) found a highly significant correlation between concentrate to roughage ratios and overall conception rates. A controlled trial by Ishak et al. (1983) demonstrated a statistically significant positive association between ration fiber and conception rates lending support to field reports. A possible pathogenesis for the association is that cows entering herds with low fiber rations are exposed to greater risk of subclinical acidosis and decreased DMI. If the latter is the case, then the low fiber is only indirectly responsible for fertility depression by acting through increased risk of NNB in early lactation. However, there is also the potential for subclinical acidosis to trigger prostaglandin release, luteolysis, and loss of the conceptus. There is a negative impact of highly fermentable carbohydrates on protein metabolism (Hristov et al., 2005). Calculations derived from Table 1 show that intakes of starch and sugar (NSC) are substantially higher for the 'NA cow', by 5.6 kg over the 'NZ cow' and by 3.5 kg over the 'Australian' cow. The proportion of lactose in the milk compared to other milk solids is certainly higher for NA cattle, indicating a relative abundance of glucogenic precursors to

aminogenic and lipogenic precursors. The impacts of an increased glucogenic flux on reproductive metabolism require further investigation in terms of acidosis risk, impact on body tissue gain, and effects on metabolic and hormonal intermediary metabolism.

Protein

Dairy cattle have two specific dietary requirements for N and protein;

- ammonia is required in combination with energy sources, carbon skeletons, peptides, minerals and vitamins by rumen microbes for growth and production of microbial protein; and
- body tissues require amino acids supplied from microbial protein or from undegraded dietary protein (**UDP**) for growth, body tissue maintenance and milk production.

Cows capable of milk yields exceeding approximately 20 l have tissue requirements for amino acids that exceed rumen microbial output. Feeding more dietary protein has been negatively associated with dairy cow fertility in some, but not all studies. It appears possible that a focus on the negative effects of higher protein diets may have diverted attention from the need to meet MP requirements for high producing cows.

Westwood et al. (1998) used a series of meta-analyses to examine relationships between dietary CP and conception probability. While a higher intake of dietary CP significantly lowered conception probability (RR = 0.84 to 0.86), the potential for feeding less degradable dietary protein to modify this relationship was not demonstrated. We found that changes in urea concentration in body fluids explained 25 % (p = 0.08) of the variance in conception rate after conducting a meta-analysis of available studies. We concluded that high intakes of dietary protein may induce adaptations in urea metabolism, and the negative relationship identified between high intakes of dietary protein and fertility for NA dairy herds may not necessarily apply in Australasian dairy herds. Protein is only one of many factors that influence fertility. It is, therefore, highly unlikely that single or perhaps even serial determinations of milk urea in single cows or bulk tanks will have a high predictive value for determining the probability of pregnancy in a cow or herd. Further, given the potential for cows to adapt to high protein diets, use of a single milk urea determination on a herd will have limited value as an indicator of nutritional status and little value as a predictor of fertility.

Subsequently, Westwood et al. (2002) found that the interaction between degradability of protein and body weight loss was highly significant in explaining the interval to conception. Figure 2 shows these associations – cows that lost > 76 kg after calving and were fed a highly degradable diet were 0.55 times as likely to conceive after the voluntary waiting period (**VWP**) as cows fed on an iso-energetic, iso-nitrogenous, but less degradable diet that lost < 76 kg. Cows on the less degradable diet, but lost >76 kg were 0.6 times and cows that less weight (< 76 kg) and fed the more degradable diet 0.76 times as likely to conceive after the VWP than cows with less weight loss and fed the diet with less degradable protein. These findings support observations in the field that cows in Australasian herds maintain higher conception probability and mobilize less body weight than those in NA that produce substantially more milk. Findings are consistent with a higher MP balance of the Australian cows. Despite impacts of diet degradability on most measures of fertility, the overall contribution of protein excess appears most likely to have marked effects on reproductive performance when weight loss after calving is substantial.

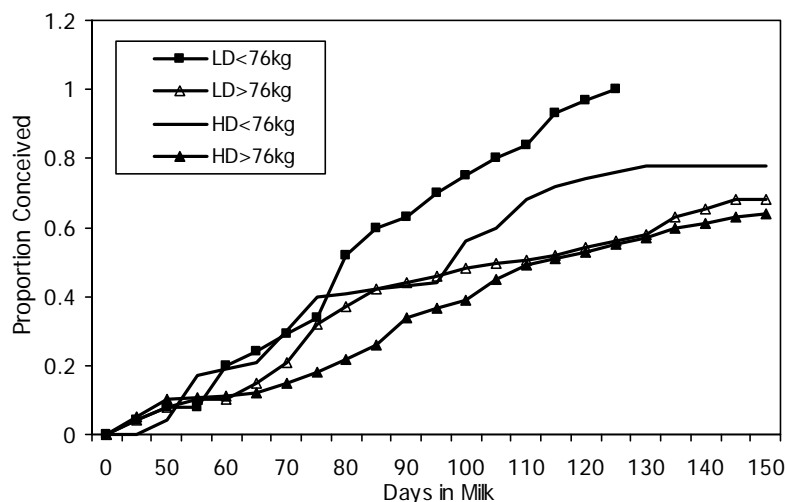


Figure 2. Proportion of cows conceiving showing interactions between weight loss after calving and dietary protein degradability – LD is low degradability protein in diet; HD is high degradability protein in diet (Westwood et al., 2002).

Lipids

There is a striking difference in lipid intake to milk yield ratio for NA cows compared to Australasian cows. From Table 1, it can be estimated that lipid intake for NA cattle is about 15.5 g/l as opposed to 20 and 22 g/liter for the Australasian cows. Furthermore, essential fatty acid (C18:2 and C18:3) intake at the duodenum, to milk yield ratios are 0.7 against 1.4 and 1.6 g/l, respectively or, approximately half. These findings suggest support for the numerous pivotal roles identified for lipids in reproductive metabolism (Staples et al., 1998; Thatcher et al., 2006). Linoleic (C18:2) and linolenic fatty acids (C18:3) are classified as essential fatty acids and must be supplied in the diet because the double bonds between the Δ -9-carbon and terminal methyl group of the fatty acids cannot be produced by cattle. Roles for fatty acids include precursors for reproductive hormones e.g., prostaglandins, membrane structures as phospholipids, and regulators of immune function. The optimal requirement for 15 to 25 % of energy being supplied as lipogenic precursors (or about 8 % long chain fatty acids in the diet) for efficient milk production was described by Kronfeld and Drepper (1976). Fat supplements can improve energy balance, reduce the risk of metabolic diseases such as ketosis, and crucially allow energy density to be maintained in diets without increased dependence on rapidly fermentable carbohydrates.

Inclusion of fat in the diet may increase serum cholesterol concentrations (Grummer and Carroll, 1988; Chilliard, 1993) and has a variable impact on reproductive performance of lactating dairy cows with some positive (Burke et al., 1996; Carroll et al., 1994; Ferguson et al., 1990; Sklan et al., 1989; Son et al., 1996) and some negative reports (Sklan et al., 1994, Carroll et al., 1994, Lucy et al., 1992). Reviews by Thatcher et al. (2002, 2006) indicated that the effect of supplemental C18:2 from oil seeds and CaLCFA on fertility varied significantly, but suggest that supply of C18:2, C18:3 and eicosapentanoic acid (C20:5) and docosahexanoic acid (C22:6) in forms that reach the lower gut, may have more profound effects on fertility.

The fat and fertility data require a meta-analytic evaluation that will include an evaluation of fat sources used, as well as amounts and ratios of specific fats fed to elucidate the optimal approaches. Linolenic acid (C18:3) predominates in forage lipids (Palmquist and Jenkins, 1980). However, concentrations of linoleic acid (C18:2) are also high in some pastures. It is possible that this and high digesta flow rates for cows on high quality pasture diets may, in part, explain some of the difference in reproductive performance achieved in well fed and managed pastured herds compared with NA herds.

Ovarian Metabolism

Nutrient Demands of the Ovary

One of the questions that is essential to address if we consider the impact of metabolites flux on fertility, is whether the ovary receives an appropriate amount of precursors for efficient reproduction. The potential for limits in metabolite availability to impair ovarian function has been explored. McClure et al. (1978) treated heifers with 2-deoxy-D-glucose, an inhibitor of glucose metabolism. The drug, which was administered for three d in late diestrus following an observed estrus suppressed the anticipated estrus and ovulation. *In vitro* studies (Armstrong and Black, 1966; Chase et al., 1992) indicated that rates of uptake and metabolism of glucose by ovarian tissue change significantly with the stage of the estrous cycle. Plasma cholesterol concentrations also undergo cyclic changes during the estrous cycle in dairy cattle.

We conducted a series of studies to examine what metabolites the ovary used and relationships between metabolites and hormones at the ovarian level to gain a better understanding of the links between dietary

inputs and ovarian function (Rabiee et al., 1997 a,b; Rabiee et al., 2000). Arterio-venous difference techniques and time series analytic methods allowed us to evaluate the influence of nutrients on ovarian responses in the cow. The ovarian environment is subject to the local effect of blood flow and interactions with hormones produced by the uterus and other glands. Our study showed that the bovine ovary utilizes significant amounts of glucose, and that glucose was the primary fuel used. The significant output of lactate suggested that anaerobic pathways were mainly used for glucose oxidation. Furthermore significant cross-correlations between the uptake of glucose and oxygen by the ovary at lag 0 and -3, for samples taken at 10 minute intervals, indicated both immediate oxidation and an approximately 30 min delay between glucose uptake and oxidation. This strongly indicated that glucose is a major source of energy for the ovary. Uptakes of 3-hydroxy-butyrate (**3-OHB**) indicated that the ovary is also able to utilize this ketone for energy. The very high metabolic rate of the luteal phase ovary uses perhaps up to 3 - 9 % of energy available to the cow.

When time series analytic methods were used to evaluate the relationship between uptake of glucose and cholesterol by the ovary in cattle, we found that uptakes of these metabolites were temporally and strongly associated. Uptake of both metabolites was pulsatile, but not significantly associated with arterial concentrations of luteinizing hormone. Control of the estrous cycle and fertility may be achieved by seeking a common regulator of uptake of these metabolites, or possibly by uncoupling the association between glucose and cholesterol. Lack of significant uptake of cholesterol by the ovary and lack of significant correlation between cholesterol uptake and progesterone output indicated that cholesterol uptake was not a rate limiting factor for steroid hormone production in the ovary. Despite this finding, other studies indicate that cholesterol is either a strong metabolic indicator of fertility or is perhaps involved in a hormonal signaling role. Westwood et al. (2002) found that higher concentrations of plasma cholesterol were associated with a shorter interval from calving to conception, with greater probabilities of conception and successful pregnancy by d 150 of lactation; a finding consistent with those of Kappel et al. (1984) and Ruegg et al. (1992), who found associations between cholesterol concentrations and fertility measures. Similarly, Moss (2001) found that low blood cholesterol concentrations at mating were strongly associated with conception failure. Lean et al. (1992) found significant negative cross-correlations between milk yield and plasma cholesterol on the same day, suggesting that cholesterol may be removed in milk or, more probably, that alterations in metabolism required for increased milk yield result in lower blood cholesterol. We concluded that as cows reach a positive energy balance and are less dependent on mobilized tissue, impetus for ketone production decreases allowing increased cholesterol synthesis.

Most of the research that has addressed the regulation of circulating concentrations of progesterone and estradiol has focused on secretion from the ovary. Our studies (Rabiee et al., 1997a,b) and others (Wise et al., 1982; Hooper et al., 1986) show that progesterone concentrations in the ovarian vein are 1000 to 10,000 times higher than peripheral blood. It appears, therefore, unlikely that progesterone production by the ovary will be a rate limiting factor for reproductive performance of cattle. Rabiee et al. (2001a,b,c; 2002a,b) found that level of feeding may have a key role in controlling progesterone concentrations in blood and also progesterone metabolism in dairy cattle. They also found that neither plasma nor milk progesterone concentrations nor the concentrations and daily yields of fecal progesterone metabolites differed in cows with differing daily milk yield. Given marked differences in DMI between NA and Australasian cows (Table 1), the possibility that progesterone excretion rates may play a role in lactational subfertility should be considered.

NUTRITIONAL MANIPULATION OF THE TRANSITION

Transition Diets

Recently there has been a strong focus on the nutritional management of the transition period. Surprisingly, there are relatively few randomized controlled trials of substantial scale examining the influence of pre-calving diets on fertility; however, in recent years these are increasing. Exposure studies provide substantial value, especially for discrete interventions e.g., minerals, fats or additives; but ultimately lack detail on nutrient flux. One of the major challenges with nutritional studies is in fully defining the diet to allow an interpretation of flux of nutrients. Given that this requires individual daily feed intake, numbers of cattle in studies are likely to be modest.

While the physiological research on the impact of manipulating various fractions of the pre-calving diet is substantial, studies of the effects of integrating dietary strategies and providing an *optimal* pre-calving diet are rare. We used a prospective cohort study to examine the effect of increasing d of exposure to ‘optimally’ formulated pre-calving diets on subsequent production (DeGaris et al., 2004a; DeGaris et al., 2007); reproduction (DeGaris et al., 2004b); and health (DeGaris et al., 2004c). These data are yet to be published in full and this report of these data should be considered in that context.

The pre-calving diets contained on a DM basis, 16.0 % CP, 4.2 % rumen undegradable protein (**RUP**), and 10.7 MJ of metabolizable energy (**ME**). The diet provided an average MP balance of 286 g/d, ME balance of 18.9 MJ, and a dietary cation-anion difference of -15.0 meq/100 g DM. The macro-mineral concentrations of the diet were consistent with those required for milk fever prevention as evidenced by the extremely low rate of milk fever across the study period (< 0.5 %). The diets consisted of ryegrass pasture, ryegrass silage or cereal hay, grain or grain by-product, canola or cottonseed meals, BioChlor[®], sodium monensin, virginiamycin or tylosin, MgSO₄, trace elements, and vitamins.

Increasing exposure to the pre-calving diet significantly increased 4.0 % fat and 3.2 % protein corrected milk (FPCM) yield and milk protein yield as a linear and quadratic effect. The increase in production found between minimal exposure (3 d or less) and optimal exposure (22 d for FPCM and 25 d for milk protein yield) was approximately 3.75 liters FPCM/d and 100 g of milk protein/d. Increasing exposure to the pre-calving diet also significantly improved reproductive measures and lowered the risk of removal from the herd. The risk of breeding/d exposed to the pre-calving diet significantly increased the risk of breeding by 1.015 (95 % CI = 1.004 to 1.027). The risk of conception/d of exposure also significantly increased by 1.019 (95 % CI = 1.008 to 1.030). This effect is large and is well demonstrated in Figure 8, showing the cumulative pregnancy rate for cows exposed to the diet for less than 10 d, those exposed for 10-20 d and those cows exposed for more than 20 d. The risk of being removed from the herd by d 150 of lactation due to voluntary culling or death/d decreased significantly RR 0.953 (95 % CI = 0.913 – 0.981) with each day exposed to the transition diets. Only 5 cases of milk fever resulted in more than 1000 calving. The magnitude of this effect on milk production is similar to that measured in a similar, but retrospective, study (Corbett, 2002) and a randomized controlled trial (DeGroot, 2004). Reproductive performance has also been improved for cows fed low DCAD diets before calving. For example, two intervention studies (Beede et al., 1992; Kim et al., 1997), and a study by Morton (2004) found that herds using anionic diets before calving had higher 6 wk or 100 d pregnancies than herds not using these strategies. In New Zealand studies, supplementation with calcium enriched molasses for the first 5 d of lactation increased proportions of inseminations resulting in conception in a study in three herds (McKay, 1994), and oral administration of calcium chloride just after calving reduced time to conception in one of four herds (Stevenson et al., 1999).

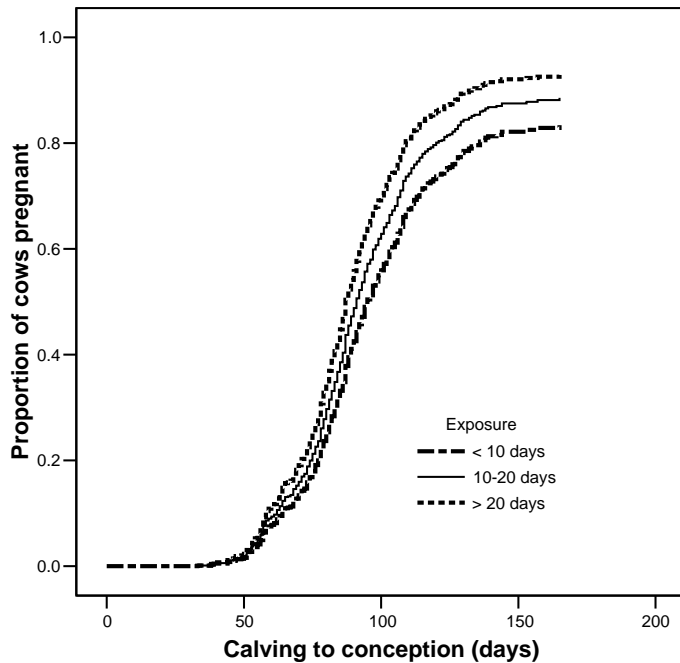


Figure 8. Proportion of pregnant cows with 3 different exposure periods in lactating dairy cows (DeGaris et al., 2004b)

Given the magnitude of the reproductive responses to the exposure of optimized transition diets, one should ask “Are there mechanisms that would support such effects?” A recent paper provided information on a comprehensive and elegant series of studies showing a pivotal role for bone in regulation of carbohydrate and lipid metabolism in mice (Lee et al., 2007). This is a possible mechanism by which a transition diet increases calcium mobilization which could profoundly influence metabolism. The statistical models developed by Lean et al. (2006), using meta-analytic methods, demonstrated that the practice of feeding prepartum diets high in calcium (1.1 % to 1.5%) cannot be recommended, as this dietary concentration range equated to peak milk fever risk. Higher concentrations of calcium (> 1%) suppress bone mobilization compared to lower inclusions of 0.5 % Ca in the diet. Table 1 shows that disease incidence is much lower in Australasia. Pre-calving calcium intake is one of several areas of disease management that needs careful review in NA.

Similarly, two papers indicate that there is a *window* in which metabolic rates can be upregulated through pre-calving nutrition. Sheehy et al. (2004) demonstrated that expression of casein and whey protein genes on exposure to prolactin was time sensitive and only responded when cows were less than 23 d from calving. Park (2005) suggests that late gestational pregnancy imprinting, an *epigenetic* effect, persists; exerting a long-term increase in milk yield, which is a finding that strongly supports the transition dietary findings of our study.

CONCLUSIONS

While it is probable that there has been a decline in fertility with increased genetic selection for milk production, the impact on inherent fertility is likely to be modest. This suggests that means of achieving good reproductive performance will be found. However, a concept that cows of higher milk production potential require more attention to detail in ensuring DMI and appropriate dietary precursors to achieve good

reproductive performance is supportable. Lactational subfertility can be considered to be largely a metabolic disorder.

From a nutritional flux perspective, it is clear that despite the extraordinarily high metabolic demands of the ovary on a mass basis, availability of substrate to the ovary will not limit performance. Responses to nutrition are mediated; therefore, through intermediary signaling with hormones or metabolites. Major differences appear likely between NA and Australasian diets in DMI, CP concentrations, MP balance, NSC intake, ratios of fat intake to milk production, and essential fatty acid intake. Pre-calving recommendations for high intakes of calcium have been widely adopted in NA, but not in Australasia. Postpartum disease incidence appears to be higher in NA and may, in part, be influenced by prepartum tissue reserves and diets. There are substantial studies that indicate that all these differences may be significant in determining the lower fertility of NA cattle.

Integrating nutritional approaches into an effective transition diet produced profound effects on production and reproduction. Similar production responses have been found in NA studies, but there has been no examination of reproductive responses in similar NA studies. If these studies are repeatable elsewhere, they provide important direction in improving future nutritional management of cattle. There is a body of evidence supporting the potential for nutrition in the period before calving to markedly influence production, health and fertility. Large randomized controlled clinical trials that report nutrient flux in detail are needed to further examine the role of nutrition in fertility. Such data will be suitable for inclusion in models that may be used to predict fertility responses.

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REFERENCES

- Abe, N., I.J. Lean, A.R. Rabiee, J. Porter, and C. Graham. 1994. Effects of sodium monensin on the reproductive performance of dairy cattle. II. Effects on metabolites in plasma, resumption of ovarian cyclicity and oestrus in lactating cows. *Aust.Vet. J.* 71:277-283.
- Anon Dairy Statistics. 2005-6. LIC, Hamilton, NZ.
- Armstrong, D.T., and D.L. Black. 1966. Influence of Luteinizing Hormones on corpus luteum metabolism and progesterone biosynthesis throughout the bovine oestrous cycle. *Endocrinology* 8: 937-944.
- Australian Dairy Industry in Focus. 2006. www.dairyaustralia.com.au.
- Baldwin, R.L. 1995. Development of model inputs and initial parameters. In *Modelling Ruminant Digestion and Metabolism*. Chapman and Hall, London, UK, pp 382-387.
- Baldwin, R.L., J. France, and M. Gill. 1987. Metabolism of the lactating cow. I. Animal elements of a mechanistic model. *J. Dairy Res.* 54:77-105.
- Beede, D.K., W.K. Sanchez, and C. Wang. 1992. Macrominerals. In: Van Horn HH and Wilcox CJ (Editors), *Large Dairy Herd Management*. American Dairy Science Association, Champaign, Illinois, USA, pp: 272-286.
- Belyea, R.L., G.R. Frost, F.A. Martz, J.L. Clark, and L.G. Forkner. 1978. Body composition of dairy cattle by Potassium-40 scintillation detection. *J. Dairy Sci.* 61:206-211.

- Bogin, E., Y. Avidar, M. Davidson, and S. Gordin. B. Israeli. 1982. Effect of nutrition on fertility and blood composition in the milk cow. *J. Dairy Res.* 49:13-23.
- Botts, R.L., R.W. Hemken, and L.S. Bull. 1979. Protein reserves in the lactating cow. *J. Dairy Sci.* 62:433-440.
- Bramley, E. 2004. Ruminal acidosis in Southern Australian dairy herds. University of Sydney PhD Thesis.
- Buckley, F., P. Dillon, M. Rath, and RF. Veerkamp. 2000. The relationship between genetic merit for yield and live weight, condition score, and energy balance of spring calving Holstein Friesian dairy cows on grass based systems of milk production. *J. Dairy Sci.* 83:1878-86.
- Buckley, F., K. O'Sullivan, J.F. Mee, R.D. Evans, and P. Dillon. 2003. Relationships among milk yield, body condition, cow weight, and reproduction in spring-calved Holstein-Friesians. *J. Dairy Sci.* 86:2308-2319.
- Burke, J.M., C.R. Staples, C.A. Risco, R.L. De La Sota, and W.W. Thatcher. 1996. Effect of ruminant grade menhaden fish meal on reproductive and productive performance of lactating dairy cows. *J. Dairy Sci.* 80:3386-3398.
- Butler, W.R., and R.D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *J. Dairy Sci.* 72:767-783.
- Carroll, D.J., F.R. Hossain, and M.R. Keller. 1994. Effect of supplemental fish meal on the lactation and reproductive performance of dairy cows. *J. Dairy Sci.* 77:3058-3072.
- Chase, C.C. Jr., R.P. Del Vecchio, S.B. Smith, and R.D. Randel. 1992. In vitro metabolism of glucose by bovine reproductive tissues obtained during the oestrous cycle after calving. *J. Anim. Sci.* 70:1496-1508.
- Chilliard, Y. 1993. Dietary fat and adipose tissue metabolism in ruminants, pigs, and rodents: A review. *J. Dairy Sci.* 76:389-73931.
- Corbett, R.B. 2002. Influence of days fed a close-up dry cow ration and heat stress on subsequent milk production in western dairy herds. *J. Dairy Sci.* 85(Suppl. 1):191-192. (Abstr.)
- Curtis, M.A., and I.J. Lean. 1998. Path analysis of metabolic and antioxidant risk factors for periparturient and postparturient conditions and reproductive performance in dairy cows. XX World Buiatrics Congress, Sydney. 2:809-818.
- DeGaris, P.J., I.J. Lean, D.M. McNeill, and A.R. Rabiee. 2004a. Effects of increased exposure to pre-calving diets containing BioChlor: Milk production. *J. Dairy Sci.* 87(Suppl. 1): 439. (Abstr).
- DeGaris, P.J., I.J. Lean, D.M. McNeill, and A.R. Rabiee. 2004b. Effects of increased exposure to pre-calving diets containing BioChlor: Reproductive performance. *J. Dairy Sci.* 87(Suppl. 1): 439. (Abstr).
- DeGaris, P.J., I.J. Lean, D.M. McNeill, and A.R. Rabiee. 2004c. Effects of increased exposure to pre-calving diets containing BioChlor: Cow health. *J. Dairy Sci.* 87(Suppl. 1): 440. (Abstr).
- DeGaris, P.J., I.J. Lean, A.R. Rabiee, and C. Heuer. 2007. Effects of Increasing Exposure to a Pre-partum Diet Containing BioChlor®. 1. Effects on Milk Production and Milk Composition in Dairy Cows. *Aust. Vet.* . Under Review.
- DeGroot, M.A. 2004. Effect of Prepartum Anionic Supplementation on Periparturient Feed Intake, Behavior, Health and Milk Production. PhD Thesis, Oregon State University.
- Duffield, T. 2000. Subclinical ketosis in lactating dairy cattle. Review. *Vet. Clin. North Am. Food Anim. Pract.* 16:231-53.

- Duffield, T.F., D.F. Kelton, K.E. Leslie, K.D. Lissemore, and J.H. Lumsden. 1997. Use of test day milk fat and milk protein to detect subclinical ketosis in dairy cattle in Ontario. *Can. Vet. J.* 38:713-8.
- Ferguson, J.D., D. Sklan, W.V. Chalupa, and D.S. Kronfeld. 1990. Effects of hard fats on in vitro and in vivo rumen fermentation, milk production and reproduction in dairy cows. *J. Dairy Sci.* 73:2864-2879.
- Ferguson, J.D. 1991. Nutrition and reproduction in dairy cows. *Vet. Clin. North Am. Food Anim. Pract.* 7:483-507.
- Fonseca, F.A., J.H. Britt, B.T. McDaniel, J.C. Wilk, and A.H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. *J. Dairy Sci.* 66:1128-1147.
- Franco, G., M. Davidson, and E. Mayer. 1977. The influence of some nutritional factors on the incidence of the repeat breeder syndrome in high producing dairy herds. *Theriogenology* 7: 105-111.
- Garnsworthy, P.C. 1989. The effect of energy reserves at calving on performance of dairy cows. In *Nutrition and Lactation in the dairy cow*. Proc. 46th University of Nottingham Easter School in Agricultural science. P.C. Garnsworthy, Ed. Butterworths, London, UK.
- Garnsworthy, P.C., and J.H. Topps. 1982. The effect of body condition of dairy cows at calving on their food intake and performance when given complete diets. *Animal Production*. 35:113-119.
- Garvin, J.K. 1999. The effect of dietary protein degradability and genetics on the protein quality of milk for cheese manufacture. Ph D thesis, Department of Animal Science, Faculty of Veterinary Science, University of Sydney, Sydney.
- Grummer, R.R., and D.J. Carroll. 1988. A review of lipoprotein cholesterol metabolism: importance to ovarian function. *J Anim Sci.* 66:3160-73.
- Grummer, R.R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. Review. *J. Anim. Sci.* 73:2820-33.
- Hageman, W.H., II, G.E. Shook, and W.J. Tyler. 1991 Reproductive performance in genetic lines selected for high or average milk yield. *J. Dairy Sci.* 74:4366-4376.
- Hansen, L.B., A.E. Freeman, and P.J. Berger. 1983. Variances, repeatabilities, and age adjustments of yield and fertility in dairy cattle. *J. Dairy Sci.* 66:281-292.
- Heuer, C., Y.H. Schukken, and P. Dobbelaar. 1999. Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds. *J. Dairy Sci.* 82:295-304.
- Hooper, S.B., D.W. Walker, and G.D. Thorburn. 1986. Cannulation of the utero-ovarian vein in intact ewes: hormone concentration and blood levels during the oestrous cycle and early pregnancy. *Acta Endocrinol.* 112:253-262.
- Horan, B., J.F. Mee, P. O'Connor, M. Rath, and P. Dillon. 2005. The effect of strain of Holstein-Friesian cow and feeding system on postpartum ovarian function, animal production and conception rate to first service. *Theriogenology* 63:950-971.
- Hristov, A.N., W. J. Price, and B. Shafii. 2005. A meta-analysis on the relationship between intake of nutrients and body weight with milk volume and milk protein yield in dairy cows. *J. Dairy Sci.* 88:2860-286.
- Ishak, M.A., L.L., Larson, F.G., Owen, E.R., Lowry, and E.D. Erickson. 1983. Effects of selenium, vitamins, and ration fiber on placental retention and performance of dairy cattle. *J. Dairy Sci.* 66: 99-106.

- Jolly, P.D., S. McDougall, L.A. Fitzpatrick, K.L. Macmillan, and K.W. Entwistle. 1995. Physiological effects of under nutrition on postpartum anoestrus in cows. *J. Reprod. Fert.* 49:477-492.
- Jonsson, N.N., W.J. Fulkerson, P.M. Pepper, and M.R. McGowan. 1999. Effect of genetic merit and concentrate feeding on reproduction of grazing dairy cows in a subtropical environment. *J. Dairy Sci.* 82:2756-2765.
- Kappel, L.C., R.H. Ingraham, E.B. Morgan, L. Zeringue, D. Wilson, and D.K. Babcock. 1984. Relationship between fertility and blood glucose and cholesterol concentrations in Holstein cows. *Am. J. Vet. Res.* 45:2607-12.
- Kim, H., S. Yoon, U. Kweon, S. Park, J. Kim, H. Jeong and J. Ha. 1997. Effect of dietary ion balance (DIB) on incidence of milk fever, milk yield and reproductive efficiency in dry cows. *RDA J. Livest. Sci.* 39:34-43.
- Kronfeld, D., W. Chalupa, and D. Sklan. 1983. Ketosis, lactational efficiency, and feeding fat. *Anim. Nutr. Health.* 38:28-33.
- Kronfeld, D., and K. Drepper. 1976. Ketosis in high-yielding dairy cows. *Adv. Anim. Phys. Anim. Nutr.* 7:1-26.
- Lean, I.J., M.L. Bruss, H.F. Troutt, J.C. Galland, T.B. Farver, J. Rostami, C.A. Holmberg, and L.D. Weaver. 1994. Bovine ketosis and somatotropin: Risk factors for ketosis and effects of ketosis on health and production. *Res. Vet. Sci.* 57:200-209.
- Lean, I.J., P.J. DeGaris, D.M. McNeil, and E. Block. 2006. Hypocalcemia in dairy cows: Meta-analysis and dietary cation anion difference theory revisited. *J. Dairy Sci.* 89:669-684.
- Lean, I.J., J.C. Galland, and J. Scott. 1989. Relationships between peak milk yield, lactational persistency and fertility in dairy cows. *Theriogenology* 31:1093-1103.
- Lean, I.J., T.B. Farver, H.F. Troutt, M.L. Bruss, R.L. Baldwin, J.C. Galland, D. Kratzer, C.A. Holmberg, and L.D. Weaver. 1992. Time series cross-correlation analysis of post-parturient relationships between serum metabolites. *J. Dairy Sci.* 75:1891-1900.
- Lean, I.J. 2000. A hazard analysis critical control point approach to improving reproductive performance in lactating dairy cows. *Asian-Australasian J. Anim. Sci.* 13 (Suppl):266-269.
- Lean, I.J., A.R. Rabiee, and N. Moss. 2003a. A hazard analysis critical control point approach to improving reproductive performance in lactating dairy cows. *Aust. NZ Comb. Dairy Vet. Conf.* pp: 419-444.
- Baldwin, R.L Lee, N.K., H. Sowa, E. Hinoi, M. Ferron, J.D. Ahn, C. Confavreux, R. Dacquin, P.J. Mee, M.D. McKee, D.Y. Jung, Z. Zhang, J.K. Kim, F. Mauvais-Jarvis, P. Ducy, and G. Karsenty. 2007. Endocrine regulation of energy metabolism by the skeleton. *Cell.* 132:456-469.
- Lucy, MC., C.R. Staples, W.W. Thatcher, P.S. Erickson, R.M. Cleale, J.L. Firkins, J.H. Clark, M.R. Murphy, and B.O. Brodie. 1992. Influence of diet composition, dry matter intake, milk production and energy balance on time of postpartum ovulation and fertility in dairy cows. *Anim. Prod.* 54:323-331.
- Macdonald, K.A., L.R. McNaughton, G.A. Verkerk, J.W. Penno, L.J. Burton, D.P. Berry, P.J.S. Gore, J.A.S. Lancaster, and C.W. Holmes. 2007. A Comparison of Three Strains of Holstein-Friesian Cows Grazed on Pasture: Growth, Development, and Puberty. *J. Dairy Sci.* 90:3993-4003.
- Macmillan, K.L., I.J. Lean, and C.T. Westwood. 1996. The effects of lactation on the fertility of dairy cows. *Aust. Vet. J.* 73:141-147.

- Mackey, D.R., A.W. Gordon, M.A. McCoy, M. Verner, and C.S. Mayne. 2007. Associations between genetic merit for milk production and animal parameters and the fertility performance of dairy cows. *Animal*. 1:29-43.
- Mackle, T.R., C.R. Parr, G.K. Stakelum, A.M. Bryant, K.L. MacMillan, and M.J. Auldist. 1996. Effect of calving liveweight on milk yield and composition and daily dry matter intake in Friesian and Jersey heifers. *Proc. NZ Soc. Anim. Prod.* 56:260-262.
- Mao, I.L. 1984. Variations in dairy cattle population: causes and consequences. *Proc. Nat'l. Invit. Workshop Gen. Improve. Dairy Cattle, Wisconsin* p. 34.
- Mayer, E., G. Fransco, and M. Davidson. 1978. Nutritional influence on fertility in Israeli dairy herds with an average 305-day production per cow of 8000 kg of fat-corrected milk. *Theriogenology* 9: 393-400.
- McClure, T.J., C.D. Nancarrow, and H.M. Radford. 1978. The effect of 2-deoxy- β glucose on ovarian function of cattle. *Aust. J. Biol. Sci.* 31:183-186.
- McGowan, M.R., R.F. Veerkamp, and L. Anderson. 1996. Effects of genotype and feeding system on the reproductive performance of dairy cattle. *Livest. Prod. Sci.* 46:33-40.
- McKay, B. 1994. Subclinical hypocalcaemia: a possible affect on fertility. In: *Proc. 11th Sem. Soc. Dairy Cattle Vet. NZ Vet. Assoc., Queenstown and Palmerston North; NZ*, pp: 89-98.
- Miettinen, P.V.A., and J.J. Setala. 1993. Relationships between subclinical ketosis, milk production and fertility in Finnish dairy cattle. *Prev. Vet. Med.* 17:1-8.
- Morton, J. 2004. Determinants of reproductive performance of dairy cows in commercial herds in Australia. Thesis, University of Melbourne, Australia.
- Moss, N. 2001. The epidemiology of subfertility in Australian dairy cows. Thesis, University of Sydney, Australia.
- Moss, N., I.J. Lean, S.W. Reid, and D.R. Hodgson. 2002. Risk factors for repeat-breeder syndrome in New South Wales dairy cows. *Prev. Vet. Med.* 54:91-103.
- Nebel, R.L., and M.L. McGilliard. 1993. Interactions of high milk yield and reproductive performance in dairy cows. *J. Dairy Sci.* 76:3257-3268.
- Oba, M., and M.S. Allen. 2000. Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 1. Feeding behavior and nutrient utilization. *J. Dairy Sci.* 83:1333-1341.
- Oldham, J.D., and G.C. Emmans. 1988. Prediction of responses to protein and energy-yielding nutrients. Pages 76-96 in *Nutrition and lactation in the dairy cow*. P.C. Garnsworthy, ed. Butterworths. London. UK.
- Olds, D., T. Cooper, and F.A. Thrift. 1979. Relationships between milk yield and fertility in dairy cattle. *J. Dairy Sci.* 62:1140-1144.
- Palmquist, D.L., and T.C. Jenkins. 1980. Fat in lactation rations: review. *J. Dairy Sci.* 63:1-14.
- Park, C.S. 2005. Role of compensatory mammary growth in epigenetic control of gene expression. *FASEB J.* 19:1586-1591.
- Parquay, R., R. De Baere, and A. Lousse. 1972. The capacity of the mature cow to lose and recover nitrogen and the significance of protein reserves. *Br. J. Nutr.* 27:27-37.

- Peterson, R., 1988. Comparison of Canadian and New Zealand sires in New Zealand for production, weight and conformation traits. *Res. Bull.*, vol. 5. Livest. Improvement. Corp., Hamilton, New Zealand.
- Pryce, J.E, B.L. Nielsen, R.F. Veerkamp, and G. Simm. 1999. Genotype and feeding system effects and interactions for health and fertility traits in dairy cattle. *Livest. Prod. Sci.* 57:193-201.
- Rabiee, A.R., I.J. Lean, J.G. Gooden, B.G. Miller, and R.J. Scaramuzzi. 1997a. An evaluation of transovarian uptake of metabolites using arterio-venous difference methods in dairy cows. *Anim. Reprod. Sci.* 48:9-25.
- Rabiee, A.R., I.J. Lean, J.G. Gooden, and B.G. Miller. 1997b. Short-term studies on ovarian metabolism in the ewe. *Anim. Reprod. Sci.* 47:43-58.
- Rabiee, A.R., I.J. Lean, J.M. Gooden, and B.G. Miller. 2000. Uptake of Glucose and Cholesterol by the Ovary of Sheep and Cattle and the Influence of Arterial LH Concentrations. *Anim. Reprod. Sci.* 64: 199-209.
- Rabiee, A.R., K.L. Macmillan, and F. Schwarzenberger. 2001a. Evaluating progesterone metabolism in ovariectomised non-lactating Holstein-Friesian cows treated with progesterone with two levels offered intake. *Anim. Reprod. Sci.* 66:35-46.
- Rabiee, A.R., K.L. Macmillan, and F. Schwarzenberger. 2001b. Affects of dry matter and metabolisable energy intakes on progesterone metabolism in lactating dairy cows. *Anim. Reprod. Sci.* 72:11-25.
- Rabiee, A.R., K.L. Macmillan, and F. Schwarzenberger. 2001c. Excretion rate of progesterone in milk and faeces in lactating dairy cow with two levels of milk yield. *Reprod. Nutr. Dev.* 41:309-19.
- Rabiee, A.R., K.L. Macmillan, and F. Schwarzenberger. 2002a. The effect of level of milk yield on plasma progesterone concentrations during the oestrous cycle of lactating dairy cows. *Anim. Reprod. Sci.* 74:121-131.
- Rabiee, A.R., K.L. Macmillan, and F. Schwarzenberger. 2002b. Effects of level of feed intake and dose of progesterone administered on plasma and faecal progesterone in ovariectomised cows. *Anim. Reprod. Sci.* 73:185-195.
- Royal, M.D., A.O. Darwash, A.P.F. Flint, R. Webb, J.A. Woolliams, and G.E. Lamming. 2000. Declining fertility in dairy cattle: Changes in traditional and endocrine parameters of fertility. *Anim. Sci.* 70:487-501.
- Ruegg, P.L., W.J. Goodger, C.A. Holmberg, L.D. Weaver, and E.M. Huffman. 1992. Relation among body condition score, serum urea nitrogen and cholesterol concentrations, and reproductive performance in high-producing Holstein dairy cows in early lactation. *Am. J. Vet. Res.* 53:10-14.
- Ruegg, P.L., and R.L. Milton. 1995. Body condition scores of Holstein cows on Prince Edward Island, Canada: relationships with yield, reproductive performance, and disease. *J. Dairy Sci.* 78: 552-564.
- Seykora, A.J., and B.T. McDaniel. 1983. Heritabilities and correlations of lactation yields and fertility for Holsteins. *J. Dairy Sci.* 66:1486-1493.
- Sheehy, P.A., J.J. Della-Vedova, K.R. Nicholas, and P. C. Wynn. 2004. Hormone-dependent milk protein gene expression in bovine mammary explants from biopsies at different stages of pregnancy. *J. Dairy Res.* 71:135-140.
- Sklan, D., E. Bogin, Y. Avidar, and S. Gur-arie. 1989. Feeding calcium soaps of fatty acids to lactating cows: effect on production, body condition, and blood lipids. *J. Dairy Res.* 56:675-681.
- Sklan, D., M. Kaim, U. Moallem, and Y. Folman. 1994. Effect of dietary calcium soaps on milk yield, body weight, reproductive hormones, and fertility in first parity and older cows. *J. Dairy Sci.* 77:1652-1660.

- Snijders, S.E.M., P.G. Dillon, K.J. O'Farrell, M. Diskin, A.R.G. Wylie, D. O'Callaghan, M. Rath, and M.P. Boland. 2001. Genetic merit for milk production and reproductive success in dairy cows. *Anim. Reprod. Sci.* 65:17-31.
- Son, J., R.J. Grant, and L.L. Larson. 1996. Effects of tallow and escape protein on lactational and reproductive performance of dairy cows. *J. Dairy Sci.* 79:822-830.
- Spalding, R.W., R.W. Everett, and R.H. Foote. 1975. Fertility in New York artificially inseminated Holstein herds in dairy herd improvement. *J. Dairy Sci.* 58:718-723.
- Staples, C.R., J.M. Burke, and W.W. Thatcher. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. Review. *J. Dairy Sci.* 81:856-71.
- Stevenson, J.S., M.K. Schmidt, and E.P. Call. 1983. Factors affecting reproductive performance of dairy cows first inseminated after five weeks postpartum. *J. Dairy Sci.* 66:1148-1154.
- Stephenson, K.A., I.J. Lean, M.L. Hyde, M.A. Curtis, J.K. Garvin, and L.B. Lowe. 1997. Effects of monensin on the metabolism of periparturient dairy cows. *J. Dairy Sci.* 80:830-837.
- Stevenson, J.S. 2001. Reproductive management of dairy cows in high milk-producing herds. *J. Dairy Sci.* 84(Suppl.): E128-E143.
- Stevenson, M.A., N.B. Williamson, and D.W. Hanlon. 1999. The effects of calcium supplementation of dairy cattle after calving on milk, milk fat and protein production, and fertility. *NZ. Vet. J.* 47:53-60.
- Thatcher, W.W., T.R. Bilby, J.A. Bartolome, F. Silvestre, C.R. Staples, and J.E. Santos. 2006. Strategies for improving fertility in the modern dairy cow. Review. *Theriogenology* 65:30-44.
- Thatcher, W.W., J. Santos, C.R. Staples, and R. Mattos. 2002. The science of Omega-3 fatty acids on dairy cows reproduction. *J. Dairy Sci.* ADSA series. Québec, Canada.
- VandeHaar, M.J., and N. St-Pierre. 2006. Major advances in nutrition: relevance to the sustainability of the dairy industry. *J. Dairy Sci.* 89:1280-91.
- van Dijk, C.J., and D.C. Lourens. 2002. Effects of anionic salts in a pre-partum dairy ration on calcium metabolism. *J. S. Afr. Vet. Assoc.* 72:76-80.
- Veerkamp, R.F., P. Dillon, E. Kelly E, A.R. Cromie, and A.F. Green. 2002. Dairy cattle breeding objectives combining yield, survival and calving interval for pasture based systems in Ireland under different milk quota scenarios. *Livest. Prod. Sci.* 76:137-151.
- Washburn, S.P., W.J. Silvia, G.H. Brown, B.T. McDaniel, and A.J. McAllister. 2002. Trends in reproductive performance in Southeastern Holstein and Jersey DHI herds. *J. Dairy Sci.* 85:244-251.
- Westwood, C.T., I.J. Lean, and J.K. Garvin. 2002. Factors influencing fertility of Holstein dairy cows: A multivariate description. *J. Dairy Sci.* 85:3225-3237.
- Westwood, C.T., I.J. Lean, and R.C. Kellaway. 1998. Indications and implications for testing of milk urea in dairy cattle: A quantitative review. (2) Effect of dietary protein on reproductive performance. *NZ. Vet. J.* 46:123-130.
- Wise, T.H., D. Caton, W.W. Thatcher, D.H. Barron, and M.J. Fields. 1982. Ovarian function during the estrous cycle of the cow: ovarian blood flow and progesterone release rate. *J. Anim. Sci.* 55:627-638.
- Xu, Z.Z., and L. Burton. 2004. Reproductive performance of dairy cows in New Zealand. www.aeu.co.nz.