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Effect of dairy cow breed on the metabolic adaptation to lactation

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ABSTRACT

Three breeds (20 Friesian, 20 Jersey and 20 Jersey/Friesian crossbred multiparous cows) were used to determine the effect of breed on indicators of metabolic status and the post partum anovulatory interval (PPAI). Effect of breed on circulating concentrations of metabolites and hormones, liver mRNA levels and PPAI was established. Weekly blood samples were taken for the first 6 weeks of lactation. Liver biopsy samples were taken from a sub-sample of cows of each breed between 23-33 days post partum, to determine levels of expression of T-GHR, GHR-1A and IGF-1 receptors using RT-PCR. Milk progesterone analysis was used to determine PPAI. There was a significant breed effect on circulating concentrations of β-hydroxy butyrate (P<0.01), non-esterfied fatty acids (P<0.001), triacylglycerols (P<0.001) and leptin (P<0.01), with Jersey cows having higher concentrations of these hormones and metabolites than Friesian or crossbred cows. Despite the concentration differences between breeds, only leptin showed a significant difference (P<0.01) in the rate of change in concentration across time between the breeds. Jersey cows were observed to have shorter PPAI (34 vs 42 and 44 days for Friesian and crossbred cows respectively: SED 6 days) but this was not statistically significant (P=0.18). There was no difference in the expression of mRNA levels of T-GHR, GHR-1A and IGF-1 receptors in the liver. It appears that grazing Jersey cows have a different fat metabolism, which affects the regulation of circulating leptin concentrations and this may have contributed to their apparent ability to return to cyclicity earlier.

Keywords: breed, pasture, leptin, IGF-1, liver mRNA receptors

INTRODUCTION

Dairy cows undergo large metabolic adaptations to meet the demands of milk production and rebreeding in early lactation. After parturition, a dairy cows' feed intake lags behind milk yield which results in a period of negative energy balance. Tissue mobilisation of body reserves in terms of lipids and amino acids is a key factor to help meet energy demands during this period. Fat stores are first used for lactation, maintenance and growth with reproductive processes receiving the lowest priority (Mwaanga & Janowski, 2000).

It is known that different breeds not only produce milk with different characteristics but also vary with their reproductive efficiency within a given management system. The Jersey breed has a higher fat concentration in milk, with a higher proportion of *de novo* synthesised fatty acids present (Beaulieu & Palmquist, 1995) and also returns to reproductive cyclicity more quickly after calving compared to Friesian cows (Fonseca *et al.,* 1983, Burke *et al.,* 1995). Reproductive efficiency may be altered by the resulting differences in long term accumulation or decline of body reserves. There is anecdotal evidence that suggests there is a difference in fat metabolism that may link to differences in energy partitioning (via changes in leptin or the somatotrophic axis) and reproduction between breeds.

Leptin may play an important role during this time by coordinating feed intake, energy expenditure and tissue nutrient use (Ingvartsen and Boisclair 2001). The negative energy balance suppresses the luteinizing hormone (LH) pulse frequency, resulting in a delayed first ovulation (Jolly *et al.,* 1995). As leptin affects fat deposition and LH concentrations (Nagatani *et al.,* 2000) it could play an important role in the processes occurring during early lactation.

The somatotrophic axis also plays an important role in the changes that occur during the metabolic adaptations as cows' transition into lactation. The hormones involved in this process include both growth hormone (GH) and insulin-like growth factor-1 (IGF-1), where IGF-1 provides the primary negative feedback for GH release (Lucy *et al.,* 2001). At the initiation of lactation, liver growth hormone receptor-1A (GHR-1A) decreases reducing the action of GH at the liver, resulting in a decrease in liver IGF-1 synthesis and release. The negative feedback loop between IGF-1 and GH ensures that as IGF-1 decreases, plasma GH will increase. Work with beef breeds has shown different breeds have differential regulation of metabolic pathways and the somatotrophic axis,

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with IGF-1 concentrations being associated with body condition (Spicer *et al.,* 2002). In dairy cows, low IGF-1 concentrations have been associated with extended post partum anovulatory interval (PPAI, Roberts *et al.,* 1997).

This study tested the hypothesis that there is a difference in regulation of fat metabolism and/or the somatotrophic axis in early lactation that influences milk production and reproduction between the three main breeds used in the New Zealand dairy industry.

METHODS AND MATERIALS

The experiment was carried out with the approval of the Ruakura Animal Ethics Committee. A herd of mixed breed dairy cows (20 Friesian, 20 Jersey and 20 crossbred cows) were calved at pasture in July-September 2003. Cows selected were: Friesian (F:J 16/0, BW 105), Jersey (F:J 0/16, BW 96), crossbred (F:J 10/6, BW 111). The cows were grazed on 2 farmlets in an all-pasture system with 10 cows of each breed per farmlet at a stocking rate of 3.3 cows/ha.

Cows were milked at approximately 0630 and 1530 h daily. Every seven days, PM and AM milk yields were measured using in-line milk meters (Westfalia Surge Metatron P21) and samples collected. Samples for all analyses were taken from a composite sample of morning and afternoon milk and analysed for milk composition (fat, protein, casein, lactose and total solids) on an infrared milk analyser (FT120, Foss Electric, Hillerød, Denmark).

Cows were weighed, condition scored and blood sampled at day 4 post partum and thereafter weekly after the AM milking (approx 0730). Blood samples were taken by venipuncture of the coccygeal vein and samples were collected into plain and EDTA vacutainers. Serum from the plain vacutainers was analysed for glucose, non-esterified fatty acids (NEFA), triacylglyerols (TAG) and β-hydroxy butyrate (β-OH). Analyses were by enzymatic and colorimetric methods using commercially available kits (Boehringer Mannheim, Germany) and a spectrophotometeric auto-analyser (Hitachi 717, Hitachi Ltd., Tokyo, Japan). Inter-assay variation was maintained at <5% (Alpha Scientific Laboratories, Hamilton, New Zealand). Plasma was harvested from the EDTA vacutainers by spinning at 3000g for 15 minutes, stored at –20°C and later analysed for insulin, IGF-1 and leptin. Plasma concentrations of insulin were measured in duplicate by double-antibody RIA method of Hales (1963) as modified by Bassett (1966) and described by Tindal (1978). Plasma concentrations of IGF-1 were measured in duplicate by the chloramine-T RIA method described by Gluckman *et al.,* (1983). Interference by binding proteins was minimised by acid-ethanol cryoprecipitation method validated for ruminants (Breier *et al.,* 1991). Plasma concentrations of leptin were measured in duplicate by a double-antibody RIA method (Blache *et al.,* 2000).

Liver biopsies were carried out between day 23-33 post partum. The procedure was carried out under local anaesthesia (Lopaine 2%, Ethical Agents Ltd, New Zealand). The biopsy punch (length 230 mm, internal diameter 3.2 mm, outside diameter 4.0 mm) was inserted on the right hand side of the cow through the tenth intercostal space (the second to last), approximately 10 cm below the line of the short ribs. Upon recovery, the biopsy sample (0.5 to 2 g) was immediately placed in a cryo-vial containing RNAlater™ (Ambion, Cat #7021, Lot # 043P65A) and stored at –20 °C until RNA isolation.

Isolation of RNA and preparation of cDNA from the liver samples was carried out using the following method. Total cellular RNA was isolated from liver using a modified guanidinium thiocyanate (GITC) method (Chomczynski and Sacchi, 1987). A cDNA copy of total RNA was prepared using the SuperScript III reverse transcriptase first strand cDNA synthesis kit (Invitrogen, Carlsbad, CA) according to the manufacturers instructions, using oligo(dT)15 (Roche Molecular Systems, Pleasanton, CA) to prime the reactions. The cDNA samples were stored at -20°C until used in reverse transcriptase polymerase chain reaction (RT-PCR). Bovine specific primers were designed for GHR-1A, total GHR [all variants (T-GHR)], IGF-1, and ubiquitin (the housekeeping gene). The GHR 1A, T-GHR, IGF-1, and ubiquitin primer pairs were based on previous publications (Radcliff *et al.,* 2003). Data are expressed as the relative expression of each target gene after treatment, normalized by the housekeeping reference gene (Pfaffl, 2001).

Concentrations of progesterone in milk were measured to determine when cows returned to oestrus. Commencement of luteal activity post partum was defined as the occurrence of 2 or more consecutive milk progesterone concentrations ≥ 3 ng/ml (Royal *et al.,* 2000). Therefore, the post partum anoestrum interval (PPAI) was defined as the time from calving until commencement of luteal activity.

Data were analysed using residual maximum likelihood (REML) in the GenStat 8 statistical package (GenStat 2005). Breed and age group (3 year old cows vs cows 4 years and older) were fitted as fixed effects. Data was analysed initially at each time point and in addition the

repeated measurements through time were modeled using spline models within the linear mixed model framework as described by Verbyla *et al.,* (1999). Breed, linear trend of time and their interaction were included as fixed effects and cow, linear trend of time within cow, spline and the interaction of breed with spline were included as random effects. Residual maximum likelihood (REML) in GenStat 8 was used to fit these models. To estimate the time to return to oestrus, data from natural cyclers and CIDR'd cows was used. The Censor procedure in GenStat was used to estimate the PPAI using the days to CIDR as a lower bound for those that had not cycled. For ease of interpretation, liveweight, body condition score (BCS) and milk data, data from the first 6 weeks of lactation was analysed to produce a mean value and associated change from week 0 (day 4 post partum) until week 6.

RESULTS

There was an effect of breed on liveweight, with Friesian cows being the heaviest (Table 1) but no difference in liveweight change over the 6 weeks. Friesian cows lost 3.6% of their live weight, whereas crossbred cows lost 1.3% and Jersey cows 1.7% of their liveweight respectively (Table 1). There was no significant difference in BCS between breeds or in the amount of change over the first 6 weeks of lactation.

Milk yield was different between breeds and increased over the first 6 weeks of lactation (Table 1). There was no difference in the size of the increase in milk yield between breeds, with the yield of Friesian cows increasing by 18%, crossbred cows by 11% and Jersey cows 15%. Jersey cows had the highest concentration of fat in milk and while over the 6 weeks, milkfat concentrations decreased in milk from all three breeds, the decrease tended to be smallest in Jersey milk. Jersey cows' milk also had the highest milk protein concentration. Milk protein concentration decreased over the first 6 weeks in all three breeds by a similar amount. Jersey cows' milk had the highest milksolids (fat + protein) concentration. There was no difference between breeds in the size of decrease in milksolids concentration over the first 6 weeks of lactation (Table 1).

TABLE 1: Effect of breed on live weight, (LWT) body condition score (BCS), milk yield and composition over the first 6 weeks of lactation.

Variable	Friesian	Crossbred	Jersey	SED	P value
Mean					
LWT (kg)	503	415	388	12	0.001
BCS	4.7	4.5	4.9	0.2	0.071
Milk yield (kg)	25.8	21.9	17.1	1.1	0.001
Fat %	4.59	4.96	5.74	0.14	0.001
Protein %	3.53	3.65	3.94	0.08	0.001
Lactose %	4.87	4.87	4.88	0.05	0.611
MS[1] %	8.12	8.62	9.69	0.18	0.001
Change over the first 6 weeks					
LWT (kg)t	-18	-5	-12	6	0.085
BCS	-0.06	-0.17	0.03	0.19	0.370
Milk yield (kg)	4.6	2.4	2.5	1.4	0.001
Fat %	-0.44	-0.45	-0.14	0.36	0.678
Protein %	-0.41	-0.65	-0.45	0.14	0.169
Lactose %	0.16	0.16	0.23	0.08	0.923
MS[1] %	-0.85	-1.10	-0.59	0.43	0.544

[1] MS – milk solids (fat + protein)

There was a breed effect on circulating concentrations of β-OH, with Jersey cows having higher β-OH concentrations ($P<0.05$, Figure 1A) from week 3 onwards. Concentrations in all 3 breeds increased from week 0 until week 2 after which concentrations decreased until week 6.

Circulating glucose concentrations were similar between the three breeds over the measurement period (Figure 1B). Concentrations decreased from week 0 until week 2 and then increased until measurements ended in week 6

Jersey cows also had higher circulating concentrations of NEFA from week 0 to week 6, with significant differences apparent at weeks 5 and 6 (Figure 1C). Concentrations in all 3 breeds decreased from week 0 until week 6 ($P<0.005$).

Circulating TAG concentrations were highest in Jersey cows throughout the measurement period. Concentrations increased from week 0 until week 6 in all breeds (Figure 1D).

There was an effect of breed on circulating concentrations of leptin from week 1 to week 4 of lactation, with Jersey cows having the highest concentrations (Figure 2A).   Concentrations of leptin increased from week 0 (F 0.83, XB 1.00, J 1.00: SED 0.09) until week 3 (F 1.02, XB 1.1, J 1.36, SED 0.12, $P<0.012$).   Circulating concentrations of leptin in Jersey cows then decreased until week 6 whereas concentrations in Friesian cows increased and concentrations in crossbred cows maintained a similar level (F 1.16, XB 1.09, J 1.15: SED 0.1). Concentrations of IGF-1 were similar between the breeds with concentrations increasing slowly in all breeds over the measurement period (Figure 2B).

FIGURE 1: Effect of breed on circulating plasma concentrations of β-hydroxy butyrate (BOH,mmol/l: A), glucose (mmol/l: B), non-esterified fatty acids (NEFA, mmol/l: C) and triacylglycerides (TAG, mmol/l: D) in Friesian (♦), Jersey (▲) and crossbred (■) cows during the first 6 weeks of lactation. Vertical bars indicate SED. * $P<0.05$.
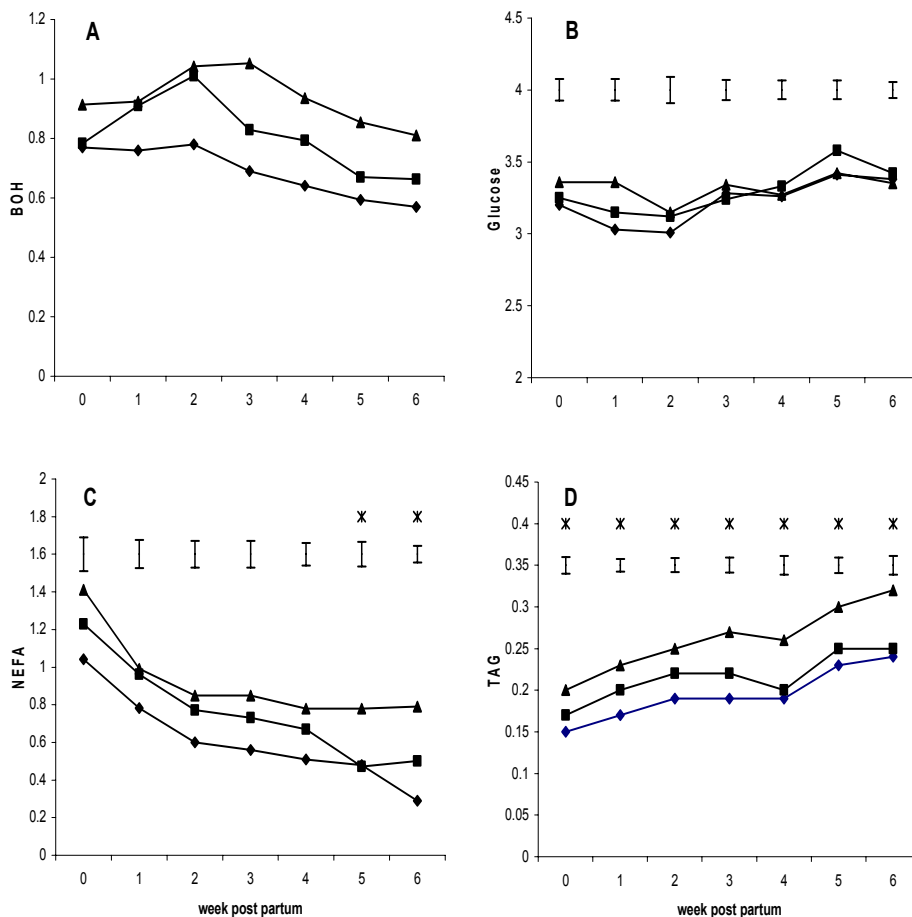
FIGURE 2: Effect of breed on circulating concentrations of leptin (ng/ml: A) and insulin-like growth factor-1 (IGF-1, ng/ml: B) in Friesian (♦), Jersey (▲) and crossbred (■) cows during the first 6 weeks of lactation. Vertical bars indicate SED. * $P<0.05$
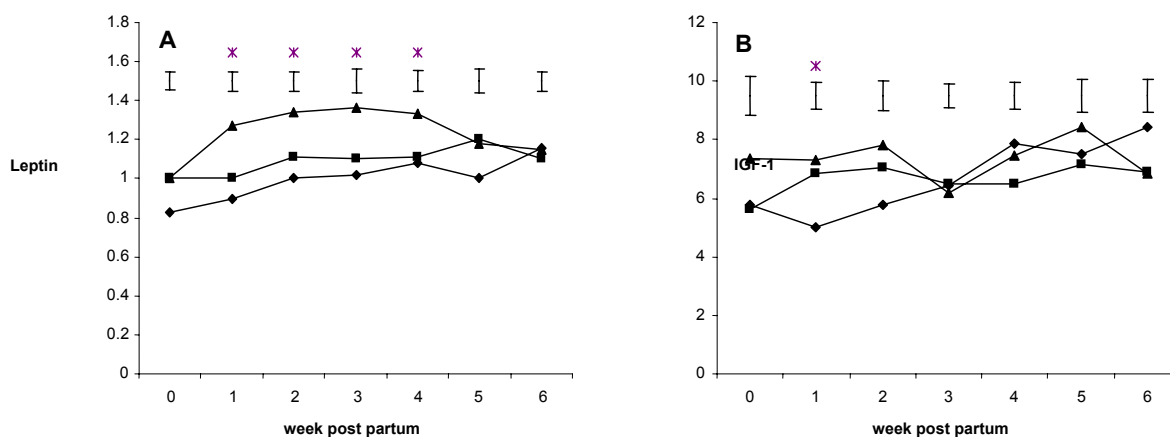
To test if blood concentrations changed at different rates between breeds, curves (using splines) were fitted to the data. There was no difference in the curvature of the splines for β-OH, glucose, NEFA, TAG, or IGF-1. There was a significant ($P<0.01$) effect of breed on the rate of change of leptin concentration, with Jersey cows having a greater rate of change than Friesian or crossbred cows.

The PPAI was not statistically different between breeds despite Jersey cows cycling on average 8-11 days earlier than Friesian or crossbred cows (Table 2). Breed had no significant effect on the relative expression of mRNA of IGF-1, T-GHR or GHR-1A receptors in the liver (Table 2).

TABLE 2: Post-partum anoestrus intervals (PPAI, days) and relative expression (arbitary units) of mRNA relative to mRNA for ubiquitin for selected genes in liver (IGF-1, T-GHR, GHR-1A) in Friesian, Jersey and crossbred cows.

	Friesian	Crossbred	Jersey	SED	P value
	n = 20	n=16	n=19		
PPAI	42	45	34	6	0.186
	n =16	n = 12	n =15		
IGF-1	1.10	1.17	1.19	0.09	0.622
T-GHR	1.04	1.03	1.07	0.08	0.818
GHR-1A	1.08	1.04	1.09	0.07	0.676

DISCUSSION

Differences in milk yield and composition between the breeds and the decrease in milk fat and protein concentration over the first 6 weeks of lactation are consistent with other published results (Thomson *et al.,* 2001, Shultz *et al.,* 1990). Jersey cows demonstrated a greater ability to maintain milk fat concentration than Friesian or crossbred cows but this effect was not seen in milk protein concentration. The ability to maintain milk fat concentration may be due to differences in fat metabolism and hence the ability to partition a greater amount of precursors for milk fat synthesis. Jersey cows had higher circulating concentrations of metabolites in blood despite similar changes in live weight and BCS. The change in BCS may be misleading as it only describes changes in sub-cutaneous fat, not internal fat deposits. Chagas *et al.,* (2006) suggested that leptin concentration does not reflect BCS when the cows have low BCS and that fat deposition was mainly occurring in internal fat depots and not subcutaneous fat depots. The higher circulating concentration of NEFA suggests that Jersey cows have a greater ability to mobilise

tissue. NEFA can be oxidised in the liver or other tissues, or incorporated directly into milk fat. The difference in fat metabolism is also supported by greater proportion of *de novo* synthesised fatty acids present in milk fat of Jersey cows (data not presented).

Leptin concentrations were similar to those reported by Chagas *et al.,* (2006) for pasture-fed cows in early lactation. However, the apparent difference in the regulation of circulating leptin concentrations seen between breeds in this study may be caused by a difference in body fatness and energy balance. Chilliard *et al.,* (2005) reviewed how leptin concentrations have been shown to be related to body fatness, both on a long term (body fatness changes according to either genetic potential of nutritional/physiological history during the previous weeks/months), mid-term (days on a given feeding level) or short-term (minutes/hours after meal intake). Given that Jersey cows replenish body reserves in mid-late lactation to a greater extent than Friesian cows (Thomson *et al.,* 2001), this may influence the long term effect on leptin expression.

The difference described in leptin concentrations in this study may be related to the internal fat and energy balance differences between breeds. The leptin relationship with body fatness is demonstrated in the difference between dairy and beef breeds e.g. leaner breeds had less leptin mRNA in adipose tissue and less plasma leptin (Chilliard *et al.,* 2005). The cause and effect relationship between body fatness and leptin expression remains to be unraveled. However, Chilliard *et al.,* (2005) found no breed difference between Charolais and Holstein plasma leptin concentrations when leptin values were corrected for individual differences in subcutaneous adipocyte size, suggesting that plasma leptin reflects primary differences in body fatness. Liefers *et al.,* (2003) reported that the recovery of leptin concentration after parturition is possibly associated with the level of negative energy balance and thus the amount of fat that has been re-accumulated during lactation. Rastani *et al.,* (2001) reported that Jersey cows remained in calculated negative energy balance for a shorter period of time relative to Holsteins. Therefore, there may be a threshold effect dependent on level of body fatness/length of the negative energy balance and leptin concentration that may have an effect on LH pulsatility and hence the apparent ability of the Jersey cow to cycle earlier. Chilliard *et al.,* (1998) reported that underfeeding decreased LH pulsatility in lean ewes but not in animals with body fatness greater than 15-20%. This is supported by Liefers *et al.,* (2003) who reported that a minimum

concentration of leptin is required to induce the first post partum LH surge. Therefore higher body fatness and leptin concentrations above given thresholds could play an important part in post partum ruminant reproductive activity.

There was no difference in IGF-1 concentrations between the 3 breeds examined in our study. Little work has been carried out comparing different dairy breeds but work examining different strains of Holstein-Friesian cows has found differences in IGF-1 and insulin concentrations (McCarthy *et al.,* 2005; M.C. Lucy, personal communication). Receptor levels for IGF-1 and GH in liver were also determined. The GHR gene in dairy cows has three mRNA variants (1A, 1B and 1C respectively, Lucy *et al.,* 2001) and in this study, total GHR (T-GHR) and GHR-1A levels were determined. The prime location for GHR-1A receptor is the liver and it is unique as the amount of mRNA is regulated by nutrition, development and physiological state (Lucy *et al.,* 2001). No difference in level of expression of receptors was determined between the three breeds in this study and this may have been expected given that the IGF-1 concentrations measured between the breeds were the same. These results indicate that in this study there was no difference in regulation of the somatotrophic axis between breeds in early lactation.

Although there was no difference in the regulation of the somatotrophic axis between breeds, there was a difference in fat metabolism which resulted in an altered leptin regulation. This preliminary study did not find a significant difference in PPAI between breeds but was conducted with a small number of cows. These results suggest that further work is required to understand the difference in leptin metabolism between breeds and the relationship with body fatness. If this difference in leptin metabolism seen in early lactation in Jersey cows is caused by the replenishing of body reserves in the previous mid-late lactation and this helps reduce PPAI, this will have implications for the feeding of pasture-fed cows in mid-late lactation.

ACKNOWLEDGEMENTS

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