Blood metabolic profiles in Uruguayan Holstein and Uruguayan Holstein x New Zealand Holstein-Friesian dairy cows

I. PEREIRA[1], D. LABORDE[2], N. LOPEZ-VILLALOBOS[3], G. RUPRECHTER[1], M. CARRIQUIRY[1] and A. MEIKLE[1]*

[1]Faculty of Veterinary Medicine and Agronomy Sciences, University of Uruguay, Montevideo, Uruguay
[2]Castiglioni 453, Trinidad, Uruguay
[3]Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11-222, Palmerston North 4442, New Zealand
*Corresponding author: anamei@adinet.com.uy

ABSTRACT

Blood metabolic profiles were determined from 40 days prepartum to 60 days postpartum in Uruguayan Holstein (UH) (n = 13) and UH x New Zealand Holstein-Friesian first cross (UH-NZHF) (n = 13) cows with seven in each group experiencing their second lactation (L2) and six experiencing their third lactation (L3). Non-esterified fatty acids increased around calving and tended to be greater in UH-L3 cows as well as beta-hydroxybutyrate concentrations for 20 days after calving, consistent with the greater body condition score losses in this group. The UH cows had greater prepartum total protein concentrations than UH-NZHF cows. Concentrations decreased around calving and increased immediately thereafter. In UH-NZHF-L2 cows, total protein levels were consistently low throughout lactation. UH-NZHF L3 cows had greater albumin concentrations than UH L3 cows. Insulin concentrations were not affected by strain or lactation number and were diminished around calving. A similar pattern was found for IGF-1, although an interaction between strain and days-in-milk was found. Compared to UH cows, UH-NZHF cows had greater levels of IGF-1 at 35 days prepartum but lower levels of IGF-1 at calving. These data suggest that UH cows, especially L3 cows, had a more pronounced negative energy balance than UH-NZHF cows that may reflect a different pattern of nutrient partitioning possibly related to different pregnancy rates.

Keywords: Holstein strains; metabolites; hormones.

INTRODUCTION

Genetic selection for milk production had been associated with a decrease in reproductive efficiency, as well as with a high negative energy balance during the transition period (Lucy, 2001). In order to supply energy for milk production, there are important losses of body condition score which are reflected in metabolic and endocrine changes that may affect fertility (Butler, 2003). A negative energy balance is characterised by high plasma non-esterified fatty acid (NEFA) concentrations which are often accompanied by increases in β-hydroxybutyrate (BHB). The physiologic adaptation mechanism that prioritises milk production is regulated among other signals by insulin and insulin-like growth factor- 1 (IGF-1). These are "indicator" signals that inform the reproductive axis regarding the metabolic status (Lucy, 2001; Butler, 2003). The genetic origin of Uruguayan dairy herds is mostly from the confined production systems of North America and Canada, where total mixed rations are fed. It has been reported that North-American Holstein (NAH) cows produce more milk which has been associated with a greater net energy balance which is in turn associated with a lower body condition score, than New Zealand Holstein-Friesian (NZHF) cows (Lucy *et al*., 2009). Even if NAH cows have a shorter postpartum anoestrus, they require more services per conception (Kolver *et al*., 2002) with a lower resulting pregnancy rate (Macdonald *et al*., 2008). Few studies have addressed the differential changes of the endocrine and metabolic signals according to strains that may explain the distinct productive and reproductive outcomes. A greater body condition score loss in NAH cows was associated with reduced blood IGF-1 concentrations when compared with NZHF cows, indicating a stronger uncoupled somatotropic axis (Lucy *et al*., 2009). Chagas *et al*. (2009) found that glucose fractional turnover rate was lower in NAH cows compared with those of NZHF origin, indicating a more severe insulin resistance. This suggested that differences in milk production between NAH and NZHF cows in early lactation may, at least in part, be explained by the greater degree of insulin resistance in NAH cows. Although these physiological mechanisms to adapt to lactation requirements should be basically similar among strains, these studies suggest that there is a different pattern of nutrient partitioning. Moreover, dry matter intake in the grazing production systems is usually lower than in confined systems. This may be insufficient to sustain the high milk production set by the genotype of the cow (Kolver & Muller 1998).

We investigated the endocrine and metabolic changes in Uruguayan Holstein (UH) and UH x NZHF (UH-NZHF) dairy cows during the peripartum period under grazing conditions, and evaluated their relationship with productive and reproductive parameters.

MATERIALS AND METHODS

Experimental design

Uruguayan Holstein (UH) (n = 13, genetic origin from North America) and UH-NZHF first cross (n = 13) cows from the experiment described in Pereira *et al.* (2010) were selected. In each strain, there were 7 and 6 cows experiencing their second or third lactations respectively. The cows were managed on native pasture as one herd, receiving 11 kg dry matter (DM)/hd/d of a diet composed of 7 kg DM of sorghum silage, 3 kg DM of sorghum grain, 1 kg DM of sunflower meal (36% crude protein), 100 g of urea and a commercial prepartum mineral supplement. After calving, cows were managed as one herd under a rotational grazing system with supplementary feed added to maintain a pasture cover of 1,200 kg of pasture DM and estimating to achieve a dry matter intake of 18 kg of total DM/cow/d. Blood samples were collected during the period from 40 days before calving to 60 days after calving, at 15 day intervals during the prepartum period and twice weekly from calving to 60 days postpartum. Samples were taken by coccygeal venopunction in heparinized tubes, centrifuged at 3,000 rpm for 15 minutes and the plasma frozen at -20°C.

A concentrated calving period was achieved by imposing a breeding period of three months from September to November. During the first two months artificial insemination was used with natural mating used during the last month. Oestrus was detected twice a day, and animals inseminated 12 hours after heat detection. Pregnancy diagnosis was performed by rectal palpation 60 days after the mating period had finished (Pereira *et al.*, 2010).

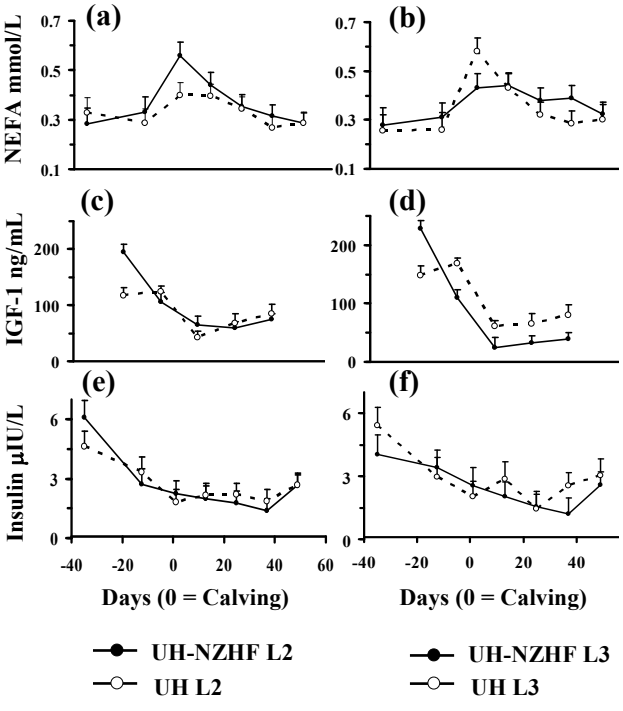
Metabolites and hormonal determination

Hormone determinations were performed by Nuclear Techniques Lab., Faculty of Veterinary Medicine, Uruguay. Insulin was determined by a ^{125}I-Insulin RIA kit (Diagnostic Products Co., Los Angeles, California, USA). The sensitivity of the assay was 2.2 µIU/mL and the intra-assay coefficient of variation were 8.2% and 9.4% for Control 1 (2.2 µIU/mL) and Control 2 (12.6 µIU/mL) respectively. IGF-1 concentrations were determined using inmunoradiometric assay with a commercial kit (IGF1 RIACT Cis Bio International, GIF SUR YVETTE CEDEX, France). The sensitivity of the assay was 0.7 ng/mL and intra-assay coefficient of variation for Control 1 (74 ng/mL) and Control 2 (535 ng/mL) were 6.9 and 7.2%, respectively. Metabolic determinations were assayed in two assays at the laboratory of Miguel C. Rubino, DILAVE, Pando, Uruguay. BHB and NEFA were determined by spectophotometry using D-3-Hydroxybutyrate (Kat. RB 1007) and NEFA (Kat. ¸FA 115) kits (Randox Laboratories Ltd, Ardmore, UK). Total plasmatic proteins, urea, cholesterol were determined using commercial kits (Weiner Lab Kit Bs As, Argentina). The intra-assay coefficient of variation was ≤7.3 % for all the parameters, and the interassay coefficient of variation was ≤9.7 %.

Statistical analyses

Metabolites and hormonal concentrations in plasma were analysed using the mixed procedure of SAS (SAS 2000, SAS Institute Inc., Cary, North Carolina, USA.) with a linear model that included the effect of strain, lactation number, day from calving, and their interactions as fixed effects and length of dry period and body condition score 60 days before calving, as covariates. The covariance structure was autoregressive order 1 and the Kenward-Rogers procedure was used to adjust the denominator degree of freedom when testing the significance of fixed effects. Tukey-Kramer tests were conducted to perform multiple comparisons between means. Reproductive variables were evaluated with a generalized lineal model using the

FIGURE 1: Non-esterified fatty acids (NEFA) (a and b), IGF-1 (c and d), and insulin (e and f) concentrations in blood, of Uruguayan Holstein x New Zealand Holstein Friesian (UH-NZHF) and Uruguayan Holstein (UH) cows in the second (L2) or third (L3) lactation group.



GENMOD procedure with a model that included the fixed effect of strain and lactation number and their interaction.

RESULTS

NEFA concentrations increased around calving; UH L3 and UH-NZHF L2 cows showed the greatest NEFA peripartum increase, P <0.05 (Figure 1a and b). While NEFA concentrations returned to basal levels one month after calving, levels in UH-NZHF L3 decreased more slowly.

BHB concentrations were similar between strains and parities and increased from 20 days before calving and not returning to basal levels during the course of the trial. A marked elevation in BHB concentrations were observed only in UH L3 cows at Day 20 after calving (P <0.02) (I. Pereira, Unpublished data).

Cholesterol concentrations started to decrease before calving, and levels recovered around Day 40 after calving (I. Pereira, Unpublished data). Greater concentrations were observed in UH-NZHF than UH cows (3.55 ± 0.09 vs. 3.37 ± 0.09 g/L; P = 0.01).

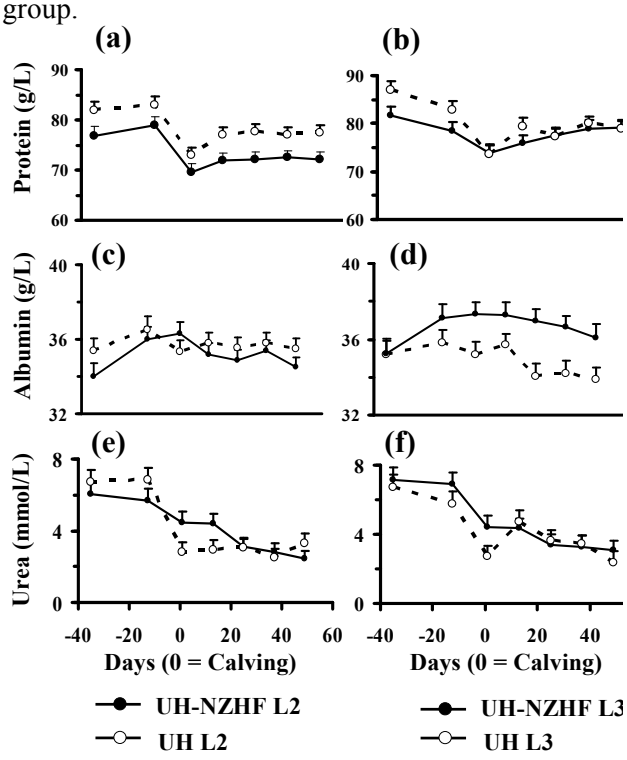
IGF-1 concentrations decreased around calving and did not recover to the initial levels in the postpartum period (Figure 1c and d). IGF-1 concentrations at 30 days before calving were greater in UH-NZHF cows than UH cows (P = 0.01). While IGF-1 levels in UH-NZHF cows were already diminished at 15 days before calving, a decrease in IGF-1 was apparent in UH cows in the first week postpartum. UH-NZHF L3 cows had a greater decrease in IGF-1 than UH-NZHF L2 cows. On the other hand the concentration of IGF-1 was greater in UH-NZHF L3 cows than in UH-NZHF L2 cows in the week before calving (P <0.05).

Insulin concentration in plasma started to decrease 35 days before calving and remained at a low value until 40 days after calving (Figure 1e and f). Insulin concentrations 35 days before calving tended to be higher in UH-NZHF L2 than in UH-NZHF L3 cows (P = 0.06).

Concentrations of total protein in plasma were significantly lower in UH-NZHF than in UH cows (75.7 ± 0.61 vs. 79 ± 0.62 g/L, P <0.0001). Parity affected protein concentrations, as L3 cows had higher total protein levels than L2 cows (P = 0.005). Total protein concentrations started to decrease before calving, reaching a minimum at calving and then recovering immediately after calving. Total protein concentrations in UH-NZHF L2 cows remained consistently low throughout the experiment compared with UH L2 cows (Figure 2a).

Albumin concentrations were greater in UH-NZHF cows than in UH cows (P = 0.005) before calving and increased around calving in UH-NZHF cows, remained constant in UH cows. In UH L3 cows, albumin concentrations had decreased by Day 20 after calving and stayed reduced until the end of the experiment (Figure 2c and d). Plasma urea concentration was similar in both strains; decreased before calving (P <0.01), more drastically in UH cows than in UH-NZFH cows, and remained low until the end of the experiment (Figure 2e and f).

FIGURE 2: Total protein (a and b), albumin (c and d), and urea (e and f) concentrations in plasma, of Uruguayan Holstein x New Zealand Holstein Friesian (UH-NZHF) and Uruguayan Holstein (UH) cows in the second (L2) or third (L3) lactation group.



Days from calving to first oestrus, calving to conception, first service to conception and number of services per conception were similar for both strains. Pregnancy rate at 6 weeks after the start of the mating period was greater in UH-NZFH than in UH cows (61 vs 35%, P = 0.04) with overall pregnancy rate due to artificial insemination followed by natural mating tending to be greater in UH-NZFH cows than in UH cows (87% vs. 71%. P = 0.08).

DISCUSSION

Studies investigating the potential metabolic signals for the reproductive axis have been focused primarily on blood metabolites and metabolic hormones that are known to fluctuate during altered states of energy metabolism (Butler, 2003). The increase in NEFA and BHB concentrations around parturition is associated with the mobilization of body energy reserves observed in the same period as reported previously (Meikle *et al*. 2004; Lucy,

2001). The greater NEFA and BHB concentrations that were observed during early postpartum in UH L3 cows, may be associated with the greater lipid reserves that were mobilised in these cows with a greater loss of body condition score (Pereira, 2010). As previously reported (Chilliard, 1999) changes in body lipids in the lactating cows are affected by three factors: requirements for milk yield, quality and quantity of dietary nutrients and body fat reserves at the time of calving. Greater cholesterol concentrations were observed in UH-NZHF than UH cows. It has been reported that increased cholesterol could be due to lipid mobilisation or to an increase in the synthesis of plasmatic lipoproteins (Margolles, 1983). The latter explanation is more likely in our trial where there was less lipid mobilisation in UH-NZHF cows than in UH cows.

Concentration of total protein decreased at calving as has been reported previously (Cavestany *et al*., 2005). This is likely to be related to the decrease in intake preceding calving, as well as being associated with colostrum production. UH-NZHF L2 cows exhibited their lowest plasma protein levels during the postpartum period, reflecting a decrease in feed intake. The greater concentration of plasma albumin observed in UH-NZHF L3 cows may have been associated with their higher intake at this time. Plasma urea concentrations were not affected by the strain of cow. While levels decreased at calving, probably due to decreased intake, they did not return to their prepartum level during the experiment. This is the opposite of what was reported by Cavestany *et al*. (2005). The great fluctuations in plasma urea concentrations found in this experiment may be due to changes of feeding frequency, and the quantity and quality of feed offered during the prepartum and postpartum periods.

The decrease in insulin concentration at calving observed was consistent with Holtenius *et al*. (2003) and Meikle *et al*. (2004). Few studies have investigated the effect of strain on insulin concentrations. No differences between strains were found in our study, in agreement with Chagas *et al*. (2009). On the other hand, the latter study found that glucose fractional turnover rate was lower in NAH cows, and suggested that the differences in milk production between NAH and NZHF cows in early lactation can, at least in part, could be explained by the greater degree of insulin resistance in the NAH cows.

IGF-1 plasma concentrations decreased around parturition in line with the process of nutrient partitioning for milk production. There was apparently a decreased liver sensitivity to growth hormone and thus reduced IGF-1 production as reported previously (Lucy, 2001). Although circulating IGF-1 levels in UH-NZHF were greater at 30 days before calving, IGF-1 profiles from 10 days before calving to 40 days after calving, did not differ between strains. A recent study (Lucy *et al*., 2009), showed that NAH cows presented a greater somatotropic axis uncoupling than NZHF cows which was consistent with strain differences in milk production and body condition score. In contrast, in this study the decrease in IGF-1 concentrations was more important in UH-NZHF cows than in UH cows. The smaller decrease of IGF-1 concentrations in UH-NZHF L2 vs L3 cows, suggests that the somatotropic axis uncoupling was less effective in L2 cows which could be also associated with lower milk production. This is in agreement with Wathes *et al*. (2007) who reported that IGF-1 concentrations were greater in young animals on account of a role in regulating growth. In our experiment, differences in live weight between L2 and L3 cows of each biotype, 20 kg in UH-NZHF cows and 16 kg in UH cows, may represent approximately 25% to 30% greater growth energy requirements for UH-NZHF L2 than UH L2 cows (National Research Council, 2001). This difference suggests that UH-NZHF L2 cows required more energy for growth, which may be related to the greater IGF-1 levels found in this group. Besides, since UH-NZHF L2 cows were the lightest cows in the herd, it can be also speculated that they may have been restricted in their feed intake because of feed competition.

Several studies have shown that both insulin and IGF-1 are relevant for ovarian follicle growth and are indicators for the reproductive axis regarding the metabolic status of the animal as a whole (Spicer *et al.,* 1995; Butler, 2003). In the present experiment no differences due to strain were found in the anovulatory period (Pereira *et al*., 2010) in agreement with Chagas *et al*. (2009) where a lack of relationship between insulin resistance and the duration of the postpartum anovulatory period was reported. In this experiment, UH-NZHF cows had higher pregnancy rates during the early stages of the insemination period (61 vs 35 % at six weeks after the start of the mating period ($P = 0.04$) as well as greater prepartum IGF-1 concentrations than UH cows. Wathes *et al*. (1998) reported that IGF-1 has an important role in promoting embryo growth and uterine preparation for gestation. On the other hand, De Feu *et al*. (2008) found no differences among IGF-1 between NAH vs NZHF cows, but the proportion of embryo recovered and the rate of development were greater in NZHF cows. This was associated with a different nutrient partitioning from the time of peak lactation through to the end of lactation.

The metabolic profiles reported here suggest that UH cows, especially L3 cows, have a more pronounced negative energy balance than UH-NZHF cows. This may reflect a distinct difference

in nutrient partitioning according to the type of cow, and may be related to the different pregnancy rates found.

ACKNOWLEDGEMENTS

Funding was provided by Sector Productivo-CSIC. We thank Maggie Handel and Laura Nuñez for their technical help.

REFERENCES

Butler, W.R. 2003: Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. *Livestock Production Science* **83:** 211-218.

Cavestany, D.; Blanc, J.E.; Kulcsar, M.; Uriarte, G.; Chilibroste, P.; Meikle, A.; Febel, H.; Ferraris A., Krall, E. 2005: Studies of the transition cow under a pasture-based milk production system: metabolic profiles. *Journal of Veterinary Medicine A* **52**: 1-7.

Chagas, L.M.; Lucy, M.C.; Back, P.J.; Blache, D.; Lee, J.M.; Gore, P.J.; Sheahan, A.J.; Roche, J.R. 2009: Insulin resistance in divergent strains of Holstein-Friesian dairy cows offered fresh pasture and increasing amounts of concentrate in early lactation. *Journal of Dairy Science* **92:** 216-22.

Chilliard, Y. 1999: Metabolic adaptations and nutrient partitioning in the lactating animal. *In*: Biology of Lactation. Martinet, J.; Houdebine, L.M.; Head. H.H., eds. Inserm/INRA, Paris, France. p. 503-552.

De Feu, M.A.; Patton, J.; Evans, A.C.; Lonergan, P.; Butler, S.T. 2008: The effect of strain of Holstein-Friesian cow on size of ovarian structures, periovulatory circulating steroid concentrations, and embryo quality following superovulation. *Theriogenology* **15:** 1101-1110.

Holtenius, K.; Agenäs, S.; Delavaud, C.; Chilliard, Y. 2003: Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. *Journal of Dairy Science* **86:** 883-891.

Kolver, E.S.; Muller, L.D. 1998: Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. *Journal of Dairy Science* **81**: 1403-1411.

Kolver, E.S.; Roche, J.R.; de Veth, M.J.; Thorne, P.L.; Napper, A.R. 2002: Total mixed rations vs pasture diets: evidence of a genotype x diet interaction in dairy cow performance. *Proceedings of the New Zealand Society of Animal Production* **62**: 246-251.

Lucy, M.C. 2001: Reproductive physiology and management of high yielding dairy cattle. *Proceedings of the New Zealand Society of Animal Production* **61**: 120-127.

Lucy, M.C.; Verkerk, G.A.; Whyte, B.E.; Macdonald, K.A.; Burton, L.; Cursons, R.T.; Roche, J.R.; Holmes, C.W. 2009: Somatotropic axis components and nutrient partitioning in genetically diverse dairy cows managed under different feed allowances in pasture system. *Journal of Dairy Science* **92**: 526-539.

Macdonald, K.A,, Verkerk, G.A., Thorrold, B.S., Pryce, J.E., Penno, J.W., McNaughton, L.R., Burton, L.J., Lancaster, J.A., Williamson, J.H., Holmes, C.W. 2008: A comparison of three strains of Holstein-Friesian grazed on pasture and managed under different feed allowances. *Journal of Dairy Science* **91**:1693-707.

Margolles, E. 1983: Metabolitos sanguıneos en vacas altas productoras durante la gestacion-lactancia en las condiciones de Cuba y su relacion con trastornos del metabolismo. *Revista Cubuna de Ciencias Veterinarias* **10**: 228-235.

Meikle, A.; Kulcsar, M.; Chilliard, Y.; Febel, H.; Delavaud, C.; Cavestany, D.; Chilibroste, P. 2004: Effects of parity and body condition score at calving on endocrine and reproductive parameters of the dairy cow under grazing conditions. *Reproduction* **127**: 727-737.

National Research Council. 2001: Nutrient Requirements of Dairy Cattle. 7th Revised Edition. National Academy of Science, Washington, D.C., USA.

Pereira, I.; Laborde, D.; Carriquiry, M.; Lopez-Villalobos, N.; Meikle, A. 2010: Productive and reproductive performance of Uruguayan Holstein and Uruguayan Holstein x New Zealand Holstein Friesian cows in a predominantly pasture-based system. *Proceedings of the New Zealand Society of Animal Production* **70**: 306-310.

Spicer, L.J.; Echternkamp, S.E. 1995: The ovarian insuline and insuline-like growth factor system with an emphasis in domestic animals. *Domestic Animal Endocrinology* **12**: 223-245.

Wathes, D.C.; Bourne, N.; Cheng, Z.; Mann, G.E.; Taylor V.J.; Coffey, M.P. 2007: Multiple correlation analysis of metabolic and endocrine profiles with fertility in primiparous and multiparous cows. *Journal of Dairy Science* **90**: 1310-1325.

Wathes, D.C.; Reynolds, T.S.; Robinson, R.S.; Stevenson, K.R. 1998: Role of the insulin-like growth factor system in uterine function and placental development in ruminants. *Journal of Dairy Science* **81:** 1778-1789.