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Plasma Hormones and Metabolites in Multiparous and Primparous Lactating Holstein Cows

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PLASMA HORMONES AND METABOLITES IN MULTIPAROUS AND PRIMIPAROUS LACTATING HOLSTEIN COWS

By

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ABSTRACT

PLASMA HORMONES AND METABOLITES IN MULTIPAROUS AND PRIMIPAROUS LACTATING HOLSTEIN COWS

By

Gabriel Garcez Ghirardi

Four multiparous and four primiparous lactating Holstein cows were monitored during the first ten weeks of lactation for milk production, feed intake and body weight. Serial blood samples (every 20 minutes during a 12 hours period) were taken during early lactation. Plasma insulin, growth hormone, glucose and glycerol were measured. Milk production at time of bleeding averaged 33.55 kg/day for multiparous and 22.13 kg/day for primiparous cows. Insulin (uIU/ml), growth hormone (ng/ml), glucose (mg/dl) and glycerol (ug/ml) averaged 9.52, 5.32, 66.80 and 9.14 for multiparous versus 12.09, 5.35, 74.67 and 8.53 for primiparous cows. Insulin ($P<.05$) and glucose ($P<.10$) were significantly higher in primiparous animals. When results were adjusted based on milk production, the differences persisted. The results suggest that differences in milk production do not explain different levels of insulin and glucose in multiparous and primiparous cows.

To my wife Maria Luiza and my daughter
Ana Carolina with endless love.

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INTRODUCTION

Dairying has experienced a 100% increase in milk output per cow in the last 30 years in the USA. This increase resulted from the utilization of new technologies such as artificial insemination, improved feed and nutrition, better reproductive management and other herd management practices.

Predictions are that by 1989, the average herd size will rise to 70 cows from the present 50, production per cow will be 6% higher and the number of dairy farms will decline 22% relative to 1985.

In this scenario, efficiency in production and financial and market management will be essential for success in dairying. Present increases in milk production per cow imply a more selective approach in deciding which animals have to be excluded from the herd and substituted for a first lactation cow.

Milk production records of dairy cows show that the peak is reached in the fifth lactation, when an animal is 7 or 8 years old. However in commercial herds a general culling rate of 25 to 30% per year, keeps the average age of the animals about 6 years with 4 lactations.

Increases in milk production from first to subsequent lactations, are accounted for by an increase in body weight, which includes increase in the mammary gland size and by the effects of recurring pregnancies and lactations.

Hormonal differences between cows producing different ammounts of milk has been the subject of several works (Hart et al., 1978, Kensinger et al. 1984). However as differences in the genetic potential for milk production between the groups are often observed the cause-effect nature of differences observed is usually difficult to determine.

The objective of the present work is to investigate hormonal profile of multiparous and primiparous lactating Holstein cows during early lactation.

REVIEW OF LITERATURE

Dietary energy consumed by ruminants is used productively in the body for synthesis of secretory products and additional body tissues or lost from the body as excreta, heat, and gases. Fast growing animals or high producing dairy cows generally retain a greater proportion of dietary nutrients in body tissues or milk than less productive animals (Trenkle,1981). Energy requirements of dairy cows during early lactation are much higher than the maintenance requirements. Energy intake in this stage of lactation is not sufficient to meet energy demands, and mobilization of body reserves provides the extra energy necessary (Sartin et al., 1985). Partitioning of nutrients is envisioned as resulting from the stimulatory or inhibitory effects of hormones on organs and tissues, which results in establishment of different tissue priorities for available nutrients (Trenkle,1981). Even when the supply of nutrients is adequate, there are marked contrasts in the extent to which lactating cows will partition energy preferentially towards either milk or body tissue, and these contrasts are apparent within breeds as well as between breeds (Bines and Hart, 1978). The term homeorhesis defining

the orchestrated or coordinated changes in metabolism of body tissues necessary to support a physiological state , has been suggested by Bauman and Currie (1980). Although it is likely that many hormones influence nutrient partition either directly or indirectly, three of the most important are insulin, growth hormone (GH) and glucagon, but there are few data from dairy cows on the glucagon (Bines and Hart,1982).

INSULIN

Concentrations of insulin are negatively correlated with milk yield, being low in early and high in late stages of lactation (Koprowski and Tucker, 1973). The role of insulin in the control of metabolism is essentially anabolic. Glucose uptake and utilization by many peripheral tissues are stimulated by insulin, whereas synthesis of glucose and its release from the liver are inhibited (Bines and Hart, 1982). The extensive fermentation of carbohydrates in the rumen leads to a situation where ruminants are dependent upon gluconeogenesis to furnish 80 to 100% of the glucose needed for metabolism (Trenkle,1981). Propionate, amino acids, glycerol and lactic acid are the primary gluconeogenic precursors in ruminants. In dairy cows glucose, the carbon source for synthesis of lactose, is synthesized primarily by the liver. Long chain fatty acids and acetate provide the majority of energy for milk synthesis and mammary gland oxidative needs in the cow. The synthetic pathways for triglyceride storage are de novo

lipogenesis and fatty acids esterification, whereas release of fatty acids and glycerol occurs through triglyceride hydrolysis. These pathways tend to be reciprocal in that lipogenesis predominates during positive energy balance and lipolysis during negative energy balance (Collier et al.,1984). Insulin, glucagon, catecholamines and glucocorticoids are primarily responsible for regulating both the supply of gluconeogenic substrates and regulating gluconeogenic pathways in the liver (Exton,1979). Insulin plays an inhibitory role both in tissue mobilization and gluconeogenesis. By inhibiting the releasing of glucogenic and other amino acids from skeletal muscle and by antagonizing the stimulatory effects of glucagon and catecholamines on lipolysis, insulin reduces both the supply of gluconeogenic substrates and possibly the availability of triglycerides and amino acids for milk fat and protein synthesis, respectively (Hart,1983). Ability of insulin to influence tissue metabolism depends on the circulating levels of the hormone, the rate of delivery of the hormone to the target tissues, the number and affinity of insulin receptors present, the responsiveness of post-receptor events to hormone action and synergistic and antagonistic effects of other hormones (Weekes,1986). Treatment of lactating cows with insulin causes reduction in milk yield which can be reversed by infusing glucose (Kronfeld et al.,1963). Hart et al.(1978) compared circulating insulin in high and low yielding cattle. During lactation the hormone

was significantly higher in the plasma of low yielding cows, which were in energy surplus and gaining body weight, than in the high yielders, which were in energy deficit and losing weight during early lactation. The difference in insulin disappeared when the animals ceased to lactate and throughout lactation a significant positive correlation was noted between changes in insulin and changes in body weight. Based on those observations, it was suggested that when high yielding cows are in energy deficit insulin is suppressed, thus reducing the removal of metabolites by body tissue and increasing the rate of gluconeogenesis, lipolysis and proteolysis. As the uptake of glucose by the mammary gland in ruminants is not insulin-dependent (Hove,1978) it is possible that the mammary gland is at an advantage when competing for glucose with other tissues that require higher levels of insulin for glucose uptake (Hart,1983). In fact 60 to 80% of total glucose turnover is utilized by the mammary gland in lactating cows (Bauman and Collier,1985).

The adaptation to lactation by adipose tissue is exemplary of homeorhetic function (Collier et al.,1984). Even before lactation begins in late gestation, adipose tissue synthetic rates decline (McNamara and Bauman, 1978; Vernon et al.,1981) in many species and rates of lipolysis increase (Sidhu and Emery, 1972). Glucose and insulin are homeostatic regulators of lipid metabolism in adipose tissue. Consistent with the continuous need for lipid mobilization during early lactation in a dairy cow,

adaptation also has occurred in adipose tissue such that lipolytic rates are unaffected by glucose during this period (Mertz and van der Bergh, 1977). The role played by insulin on fat deposition in ruminants is not completely understood. Direct stimulatory effects of insulin on adipose tissue metabolism or inhibitory effects on lipolysis have been difficult to demonstrate in vitro using either tissues slices or isolated adipocytes. Early experiments using ruminant adipose tissue, reviewed by Vernon (1980), indicated a low responsiveness of adipose tissue metabolism to insulin, although half maximal effects on glucose oxidation and fatty acid synthesis were achieved at insulin concentrations within the physiological range. More recently Broad et al., (1983) failed to demonstrate an effect of insulin on lipogenesis from glucose in isolated sheep adipocytes and Smith (1984) could not detect an effect on lipogenesis from acetate in bovine adipose tissue.

GROWTH HORMONE

The growth hormone released into the blood during the episodes of enhanced secretion exerts a growth promoting action on many tissues of the body including cartilage, skeletal muscle, heart, liver, kidney, adipose tissue, and lymphoid organs (Isaksson et al.,1985). The growth promoting effect of growth hormone is primarily manifested as a stimulation of cell multiplication (Kostyo,1985). It has been generally held that growth hormone does not influence cell proliferation directly, but that it does so indirectly

by increasing the circulating concentration of somatomedins or insulin-like growth factors (IGF-1), which are synthesized and released by the liver and probably other tissues in response to growth hormone (Daughaday, 1981). It has been known for many years that growth hormone is also important for the maintenance of ruminant lactation. Growth hormone concentrations during lactation are related to stage of lactation (Koprowski and Tucker, 1973) and growth hormone plasma concentration is higher in high producers than in low producing cows (Hart et al., 1978). Injection of growth hormone in cattle results in increased milk yield (Bauman et al., 1985, Peel et al., 1983). Responses to growth hormone are primarily related to post absorptive use of nutrients. The extensive array of physiological processes altered by growth hormone include metabolism of carbohydrate, protein, lipid and minerals.

There is increasing evidence that nutritional status plays a major role in determining circulating growth hormone concentrations particularly in the ruminant (Gluckman et al., 1987). During periods of nutritional deficit, elevated concentrations of circulating growth hormone have been reported for several species including pigs (Atinmo et al., 1978), man (Merimee and Fineberg, 1974), and sheep (Driver and Forbes, 1981). Plasma concentrations of growth hormone are higher in underfed compared with adequately fed lactating cows (Hart et al., 1978).

The most obvious shift in nutrient partitioning in dairy cows involves glucose metabolism (Bauman and Collier, 1985). The extra glucose required to accommodate the increase in lactose secretion in growth hormone treated cows could be provided by increased gluconeogenesis and/or by reduction of oxidation of glucose. In a study in which milk yield responses to growth hormone caused cows to be in a substantial negative energy balance, feed intake was not affected but growth hormone treatment increased rates of glucose irreversible loss and reduced rates of glucose oxidation. These adaptations were sufficient to provide the glucose needed for the increase in milk lactose secretion (as cited in Bauman and Collier, 1985).

Growth hormone treatment also affects partitioning of amino acids in the lactating cow. If treated cows are in positive protein and energy balance, milk crude protein content is unchanged so that the percent increases in milk protein are identical to those in milk yield (Bauman et al.,1982; Peel et al., 1982, 1983; Fronk et al., 1983; Eppard et al., 1985). Conversely if treated animals are in negative energy and nitrogen balance, protein content of milk decreases so that the increase in yield of milk protein is smaller than the corresponding response in milk yield (Peel et al., 1981, 1983; Tyrrel et al., 1982).

The increase in growth hormone release associated with limited nutrient availability is postulated to mobilize energy from adipose tissue to satisfy the needs for

metabolism and facilitate the transfer of metabolites from adipose to lean tissue when intake of dietary nutrients is not adequate (Bauman and Currie, 1980; Bauman et al., 1982).

Studies concerning the effects of ruminant growth hormone on adipose tissue are limited. Acetate incorporation into lipid was increased by bovine growth hormone in adipose tissue removed from cows in mid to late lactation (Keys and Capuco, 1985). However a direct effect of bovine growth hormone on lipolysis in ruminant adipose tissue, remains to be demonstrated.

When growth hormone treatment causes cows to be in negative energy balance, milk fat content increases so that the response in milk fat yield markedly exceeds the response in milk yield. In this situation plasma concentration of free fatty acids (FFA) are chronically elevated and rates of FFA irreversible loss (lipid mobilization) are increased (Peel et al., 1981, 1982, 1983). This increase in FFA irreversible loss is highly correlated with the degree of negative energy balance (Peel et al.,1982; Tyrrel et al., 1982) and the composition of milk fat shifts to a greater proportion of long chain fatty acids which are characteristic of body fat reserves. When animals are in positive energy balance, milk fat percentage is not altered so that increases in milk fat yield are similar to increases in milk yield (Bauman et al., 1985; Peel et al., 1982, 1983; Fronk et al., 1983; Eppard et al., 1985). Although FFA turnover has not been quantified under these conditions,

blood FFA concentrations and milk compositions are unchanged (Peel et al., 1982, 1983; Eppard et al., 1985).

In a short term study, growth hormone treated cows showed an increased milk yield, but the calcium and phosphorous composition of the milk was not altered, suggesting that growth hormone also alters the partitioning of minerals (Eppard et al., 1985).

The mechanism by which growth hormone responds to homeostatic signals in supportive tissues (e.g. adipose tissue) is not known. It appears that effects on adipose tissue are direct because metabolic changes are observed when growth hormone is added to adipose tissue incubations (Vernon, 1982; Etherton and Walton, 1986). On the other hand simply providing extra nutrients to the mammary gland of cows fed their required nutrients does not alter synthesis of milk (Peel et al.,1982), which suggests that growth hormone effects, whether direct or indirect, may be at the level of the mammary cell. Davis et al.(1984) and Peel et al.(1985), observed an increased level of somatomedin C in growth hormone treated cows, suggesting that a portion of the specific tissue effects of growth hormone may be mediated by this small peptide hormone. The mammary gland has no receptors for growth hormone (Gertler et al.,1983, 1984) and does not respond to close arterial infusion of growth hormone (McDowell and Hart, 1984). However Gregor and Burleigh (1985) have demonstrated the presence of somatomedin receptors in porcine mammary tissue. Although

somatomedin receptors in dairy cow mammary glands have not been detected yet, somatomedin C is therefore one of the most likely mediators of the effects of growth hormone at the level of mammary gland (Tucker and Merkel, 1987).

GROWTH HORMONE AND INSULIN INTERACTIONS

Studies of the effects of growth hormone on adipose tissue are based either on clinical observations in man or on in vitro studies in rats. Thus, extrapolation to the ruminant may be misleading.

The initial response to growth hormone in adipose tissue is insulin-like (Goodman and Coiro, 1981); it consists of increased transport and oxidation of glucose and accelerated glucose metabolism to fatty acids and carbon dioxide. This response also includes increased oxidation of leucine and antagonism of the lipolytic actions of epinephrine.

Because insulin resistance and carbohydrate intolerance have been reported in acromegaly, it was postulated that growth hormone has diabetogenic properties, producing hyperglycemia, glucose intolerance and causing insulin resistance and hyperinsulinemia (Gause et al., 1985). This diabetogenic effect of growth hormone requires chronic exposure of the organism or tissue culture to high doses of the hormone (Kostyo et al., 1984), and presumably represents interference with a post-receptor step in insulin action (Rosenfeld et al., 1982). Cameron et al. (1985) compared the effects of different vertebrate growth hormones in rat

adipose tissue and showed that the diabetogenic activities are intrinsic properties of growth hormone. This remains a confusing area, and there is conflicting evidence with reports relating the differential actions of growth hormo to pituitary contaminants, different fragments of the growth hormone molecule or possibly to different post-translational products of the growth hormone gene.

In non ruminants growth hormone has been shown to exert a diabetogenic effect (Kostyo et al. 1984), and in at least one recent work in ruminants, both highly purified and recombinant growth hormone showed a diabetogenic and lipolytic effect when administered to sheep (Hart et al. 1984).

During the last years, the galactopoieitic effect of growth hormone has been the subject of several studies in dairy cows (Bauman et al. 1985a, Peel et al. 1985, Peel et al. 1983). Early studies reported an increase in glucose, insulin and fatty acids in the blood of treated animals, suggesting that growth hormone could supply extra glucose and fatty acids through a diabetogenic and lipolytic effect (Bourne et al. 1977; Kronfeld, 1965). More recent studies have demonstrated that administration of highly purified preparations of bovine growth hormone to dairy cows (either pituitary or recombinant) has no effect on circulating concentrations of glucose or insulin (Peel et al., 1981, 1982, 1983; Eppard et al., 1985).

One of the possible explanations for this fact could be that the diabetogenic and lipolytic activities of bovine growth hormone are not intrinsic properties of the molecule, but rather contaminations with low molecular weight compounds during the purification technique (Bauman and Collier 1985). With the advent of recombinant bovine growth hormone and improvements in the purification technique of the pituitary growth hormone, these types of responses are not observed (Peel et al. 1982, 1983).

Lipolytic and diabetogenic activities of bovine growth hormone have been studied recently (Hart et al., 1984). In contrast to pituitary-extracted bovine growth hormone, recombinant-derived bovine growth hormone was not lipolytic in studies using rat adipose tissue in vitro. However, both natural and recombinant bovine growth hormone were of similar potency in increasing concentrations of plasma free fatty acids in non lactating sheep (Hart et al., 1984a).

The energy status of the animal seems to be a more important determinant of the hormonal profile than stage of lactation (Peel et al., 1983; Bauman and Collier, 1985).

From the review of literature, we can see that there are only two major studies of the effects of level of milk production on blood levels of insulin, growth hormone and glucose. Hart et al. (1978) found lower levels of insulin in cows producing 40 than in cows producing 15 lbs. of milk per day. Differences in breed of the animals (dairy versus dairy-beef cross bred were used in this experiment), may

have accounted for the different hormonal profiles observed in the two groups. Kensinger et al. (1984) working with superior cows (50 kg of milk per day) and with good producers (34.5 kg of milk per day), did not detect any difference in insulin levels, while plasma growth hormone was higher in the superior animals. In this case, however, serial blood samples were not taken and failure in detecting differences in plasma hormones might be due to an episodic pattern of release.

Consequently, in this work plasma levels of hormones and metabolites will be studied using cows with different levels of milk production but the same genetic potential and at the very same stage of lactation. Serial blood samples will be taken during an interval of time long enough to detect variations caused by episodic releases of hormones and metabolites.

MATERIAL AND METHODS

Four multiparous high milk producers and four primiparous Holstein cows were used in the experiment. All the animals calved during March of 1986 and were housed in the dairy cattle facilities at Michigan State University. Cows were moved to the parlor twice daily for milking at 0400 and 1500 hrs.

Cows were fed ad libitum a total mix ration throughout the experimental period twice daily at 0200 and 1400 hrs. Weigh back was individually recorded daily at 1400, hrs and feed consumption calculated for each animal.

Weekly feed samples were collected , composite monthly and analyzed for crude protein, energy, acid detergent fiber, calcium, phosphorus and minerals, in the Research Extension Analytical Laboratory of The Ohio State University (Wooster, Ohio). The ingredients and composition of the total mix ration are presented in table 1.

Body weight was determined once a week for each animal.

Once weekly, a.m. and p.m. milk samples were obtained for analyses of lactose, fat and protein. Milk components were analyzed in the DHIA laboratory by infra red techniques.

TABLE 1 - Composition of the total mixed ration fed throughout the experiment.

Ingredients and Nutrients	%
Ingredients (as fed)	
Corn Silage	37.50
High moisture corn	33.14
Haylage	19.84
Supplement	9.12
Trace mineral salt	0.11
Sodium bicarbonate	0.28
Composition (dry matter basis)	
Net energy, lactation, Mcal/kg	1.65
Crude protein	17.20
Acid detergent fiber	11.00
Calcium	1.10
Phosphorus	0.56
Magnesium	0.37

The net energy (NE) balance for each animal was calculated on a weekly basis using the energy requirements for maintenance and production from the NRC (1978) based on body weight and fat corrected milk production. For the primiparous animals the requirements for maintenance were based in their actual body weight and the correction to allow for growth was not considered.

A theoretical gross efficiency of milk production, (EFF), was then calculated using the actual fat corrected milk produced during the week when blood samples were collected, divided by the NE calculated from NRC (1978) requirements for maintenance and milk, using the actual fat corrected milk and body weights for the animals at that time.

A ratio between amount of feed ingested and body weight, (FEED/BW), was calculated for each animal dividing the average daily consumption of feed during the week when blood samples were taken by the body weight at that point.

Serial blood samples were collected when cows averaged 72 and heifers 65 days in lactation respectively. One day before sampling catheters were placed in the jugular veins of the animals. The catheters were held in place by elastic tape. In the day of sampling, a 20 ml blood sample was taken via the catheter every 20 minutes from 0900 to 2000 hrs. Plasma was obtained by centrifugation and stored at -20 C before analysis.

Double antibody methods of radioimmunoassay were used to measure serum concentrations of growth hormone (Gorewit R.C.,1981). The procedure to determine insulin plasma concentrations was a solid phase, coated tube RIA system (Micromedics, Inc. 102 Witne Road, Horsham, PA), validated by Gowan, McCann and Ross (1982). Glucose was determined using the glucose oxidase method (Sigma Chemical Company, St. Louis, MO). Glycerol was determined using the coupled enzyme reaction as described by Garland and Randle (1962), with th following modifications: 250 ul of plasma was added to 650 ul of double distilled water and 250 ul of a solution containing glycylglycyne buffer (pH 7.4); 91 ug/ml of NADH; 2 mg/ml of ATP; 1 mg/ml of PEP and magnesium sulfate. 10 ul of a solution containing 600 U/ml of pyruvate kinase and 550 U/ml of lactate dehydrogenase was then added to each

cuvette. All the reagents were then mixed and after 25 minutes the absorbance at 340 nm was read in a spectrophotometer. 10 ul of a solution containing 85 U/ml of glycerokinase was then added to each cuvette. The contents were then mixed and stood for 25 minutes when a new read at 340 nm was performed. Concentrations of glycerol were determined using a standard curve generated by the substitution of plasma for samples with known concentrations of glycerol. All the solutions used in the procedure were obtained from a glycerol kit from Boheringer Mannheim Biochemica (GMBH).

Statistical analyses of the data were performed using the Statistical Analyses System (SAS) for a split plot design with repeated measurements for the model,

$$Yijk = u + Ai + Dj + Bk + (AB)ik + E(ijk)$$ where:

u = overall mean;
A = effect of number of lactation;
D = effect of animal within lactation number;
B = effect of time at the various sampling points;
AB = interaction between lactation number and time of sampling;
E = residual error.

For each animal the average concentration of hormones and metabolites for each particular hour was determined using the average for the three samples taken during that period.

Analyses were performed considering the total period of sampling and for the pre and post-feeding period, using the modifications suggested by Gill (1986) for experiments with few animals per treatment. Two different sets of analyses

were performed: one using the original data and the other with the adjusted means (least square means) of the hormones and metabolites using milk production as covariate.

Comparisons between the two groups of animals as well as analysis of the general profiles of hormones and metabolites for each group were performed.

The averages for milk production, energy balance, body weight, gross efficiency of milk production and the ratio kg of feed ingested per kg of body weight were analyzed using Student's t test.

RESULTS

A summary of the results for milk production, energy balance, body weight, gross efficiency and feed intake per kg of body weight, is presented in table 2.

Average milk production (4.0% fat corrected), the estimated energy balance and body weights for the first ten weeks of lactation for the two groups of animals, are presented in figures 1, 2 and 3 respectively. The peak of production for the multiparous cows occurred at the 4th week of lactation (35.18 kg/day), and at the 5th week (22.89 kg/day) for the primiparous cows. The milk production average when the animals were bled was 33.55 and 22.13 kg/day for multiparous and primiparous cows respectively ($P<.01$).

Both groups started the lactation in negative energy balance and at the end of the 10th week were in slightly positive energy balance (3.83 and 3.72 Mcal/day for multiparous and primiparous cows respectively). The overall mean for the 10 week period was -2.84 and -0.55 Mcal/day for primiparous and multiparous cows.

Table 2. Milk production, energy balance, body weight, estimated milk production efficiency and feed intake per unit of body weight for multiparous and primiparous cows at sampling time.

	Multiparous	Primiparous	SE	P
Milk kg (FCM 4.0%)	33.55	22.13	2.798	<.01
En. Bal. Mcal/day	3.83	3.12	7.19	ns
Body weight kg	584	501.25	43.141	<.05
Efficiency FCM/NEI	.984	.837	.027	<.01
Feed intake/BW	.062	.053	.014	ns

NEI = Net energy intake.
Feed intake/BW = Average feed consumption ("as is"basis) per kg of body weight.
Multiparous n=4
Primiparous n=4

Despite the fact that the younger animals started lactation with a higher energy balance than the multiparous cows it took more time for them to reach a positive energy balance status. At the week when blood samples were collected the average energy balance was 3.83 and 3.12 Mcal/day for multiparous and primiparous cows respectively (P>.20).

When blood samples were taken, multiparous animals averaged 584 kg and primiparous 501.5 kg of body weight (P<.05). Considering the period from the beginning of lactation until the point were blood samples were collected, multiparous animals gained 5.22 kg of body weight, while primiparous cows gained 9.59 kg . Despite the magnitude of this difference, it was not statistically significant (P>.20), probably due to the small number of animals per group and the variation between animals.

Figure 1. Weekly average milk production (kg of fat corrected milk 4.0%) for multiparous and primiparous cows during the first ten weeks of lactation.

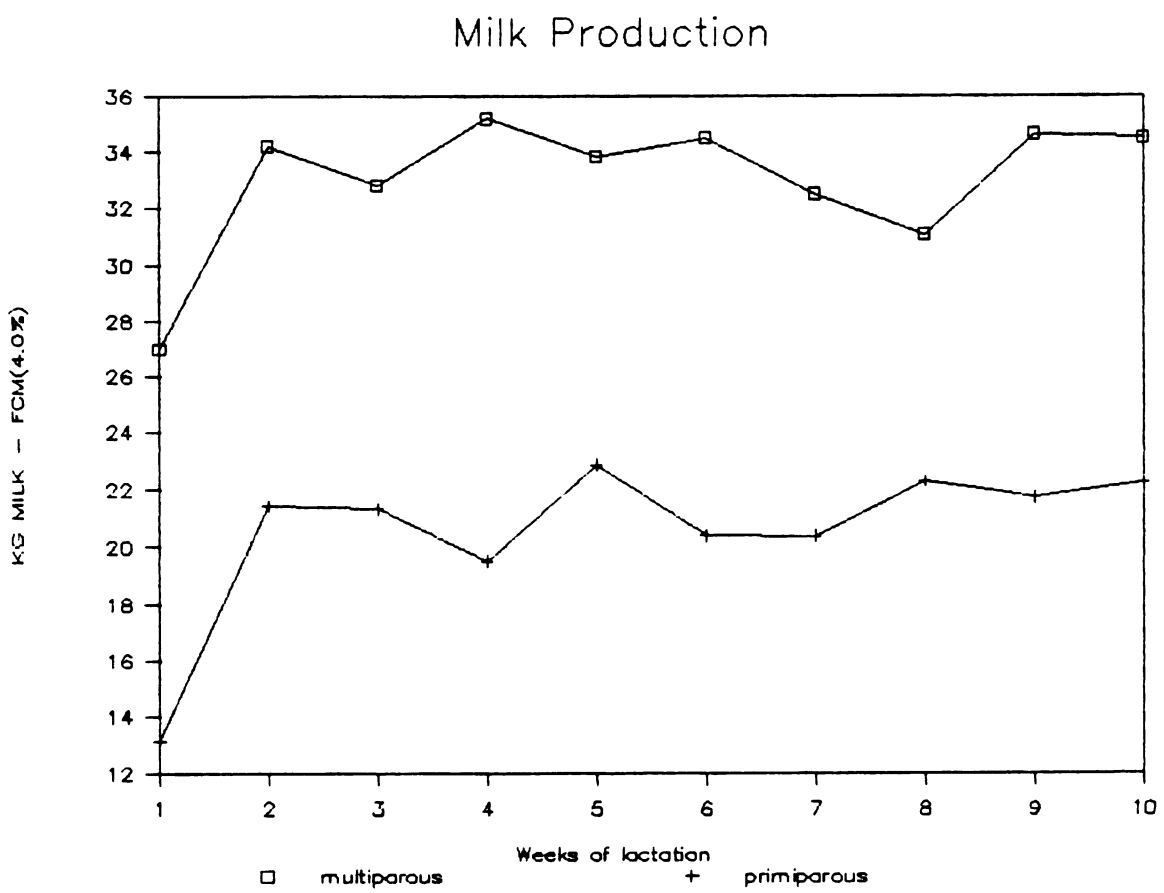


Figure 2. Weekly estimated energy balance (Mcal/day) for multiparous and primiparous cows during the first ten weeks of lactation.

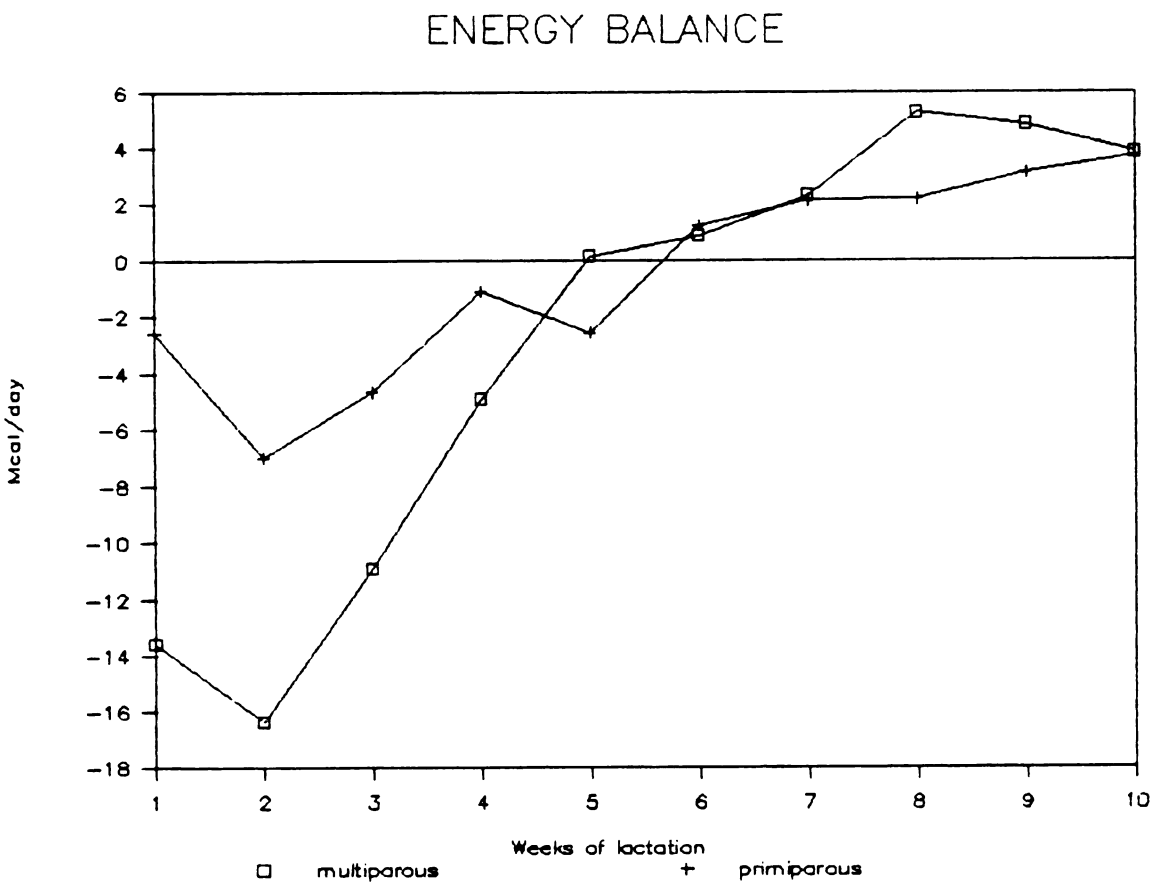
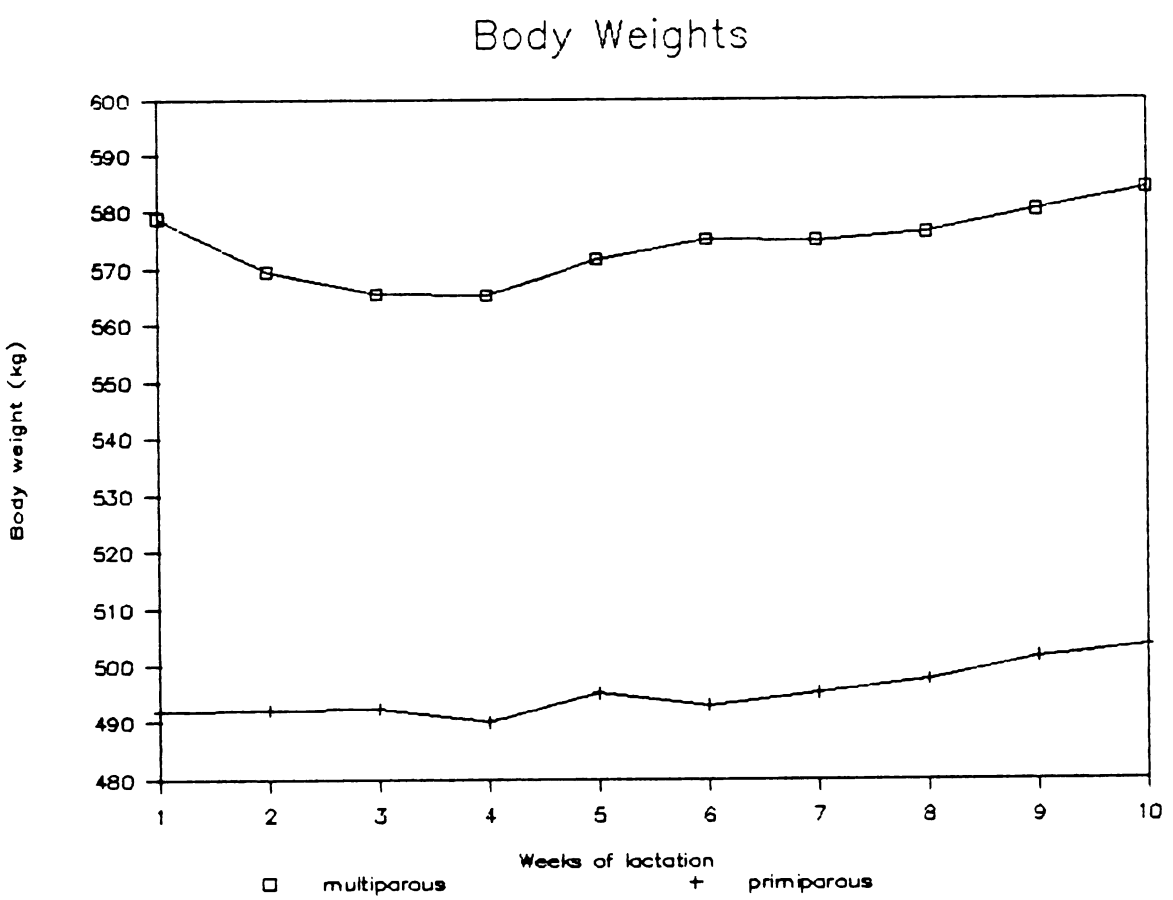


Figure 3. Weekly body weights (kg) for multiparous and primiparous cows during the first ten weeks of lactation.



Multiparous animals had a higher body weight at time of bleeding (P<.05) and also were more efficient converting energy to milk (P<.01). However, no statistical difference was detected in intake per kg of body weight (P>.20).

The averages for plasma hormones and metabolites for groups in the pre and post feeding period are shown in table 3.

Insulin was significantly higher during the post feeding period for multiparous (P<.05) and primiparous animals (P<.01).

There was no significant change in plasma glucose concentration for multiparous and primiparous animals. However, there was a trend toward decreasing glucose during the post feeding period in the primiparous group (P<.10).

Table 3. Average plasma insulin, glucose, growth hormone and glycerol in the pre and post feeding period for multiparous and primiparous cows.

	Pre	Post	SE	P
		Multiparous		
Insulin uIU/ml	7.99	11.06	0.89	<.05
Glucose mg/dl	67.32	66.28	2.41	ns
GH ng/ml	6.21	4.42	0.47	<.01
Glycerol ug/ml	10.38	7.89	1.18	<.05
		Primiparous		
Insulin uIU/ml	10.33	13.85	0.89	<.01
Glucose mg/dl	77.37	71.98	2.41	<.10
GH ng/ml	5.59	5.10	0.47	ns
Glycerol ug/ml	10.08	6.99	1.18	<.05

Plasma growth hormone concentration markedly decreased after feeding the multiparous animals (P<.01).

However, no differences were detected for the primiparous animals.

Both groups had a lower level of plasma glycerol in the post feeding period when compared with the pre feeding period ($P<.05$, table 3).

A summary of the comparisons between the two groups is presented in table 4.

Average insulin concentration was lower ($P<.05$) in multiparous than in primiparous cows (figure 4) when the total period was considered. Primiparous animals also had a higher level of insulin during the pre feeding and post feeding period ($P<.20$ and $P<0.01$ respectively) when compared to the multiparous cows. When the total period was considered, there was a significant effect of time of sampling on insulin concentration ($P<0.01$) and for both groups of animals insulin levels were higher during the post feeding period. However, no effect of time was detected when either pre or post feeding periods were considered alone ($P>0.20$). There was no evidence that the concentration of insulin changed in time in a different pattern between both groups of animals in all the periods analyzed ($P>0.20$).

Treatment mean comparisons for each time showed a significant difference in insulin concentration at 1700 and 1800 hrs ($P<.05$) and at 1400, 1600 and 2000 hrs ($P<.10$).

Plasma glucose concentration for both groups is presented in figure 5. First lactation cows showed an overall higher level of glucose when compared with the multiparous animals

(P<.10). The difference tended to be higher in the pre feeding period (77.36 vs. 67.32 mg/100 ml) than in the post feeding period (71.98 vs. 66.28 mg/100 ml). No effects of time were detected in any of the periods analyzed. No interaction between time and lactation number was detected in the overall or pre feeding period. However in the post feeding period there was some evidence that the concentration of plasma glucose showed a different trend in time for primiparous cows when compared with multiparous animals (P<.15).

Table 4. Average plasma insulin, glucose, growth hormone and glycerol in the total, pre and post feeding period of multiparous and primiparous cows.

	Multiparous	Primiparous	SE	P
	1 - TOTAL PERIOD			
Insulin uIU/ml	9.52	12.09	.299	<.05
Glucose mg/dl	66.80	74.67	3.771	<.10
GH ng/ml	5.32	5.35	.461	ns
Glycerol ug/ml	9.14	8.53	.727	ns
	2 - PRE FEEDING PERIOD			
Insulin uIU/ml	7.99	10.33	1.106	ns
Glucose mg/dl	67.32	77.37	4.275	<.10
GH ng/ml	6.21	5.59	.570	ns
Glycerol ug/ml	10.38	10.08	1.238	ns
	3 - POST FEEDING PERIOD			
Insulin uIU/ml	11.06	13.85	.440	<.01
Glucose mg/dl	66.28	71.98	3.526	ns
GH ng/ml	4.42	5.10	.489	ns
Glycerol ug/ml	7.89	6.99	.545	ns

Figure 4. Plasma insulin concentration for multiparous and primiparous cows.

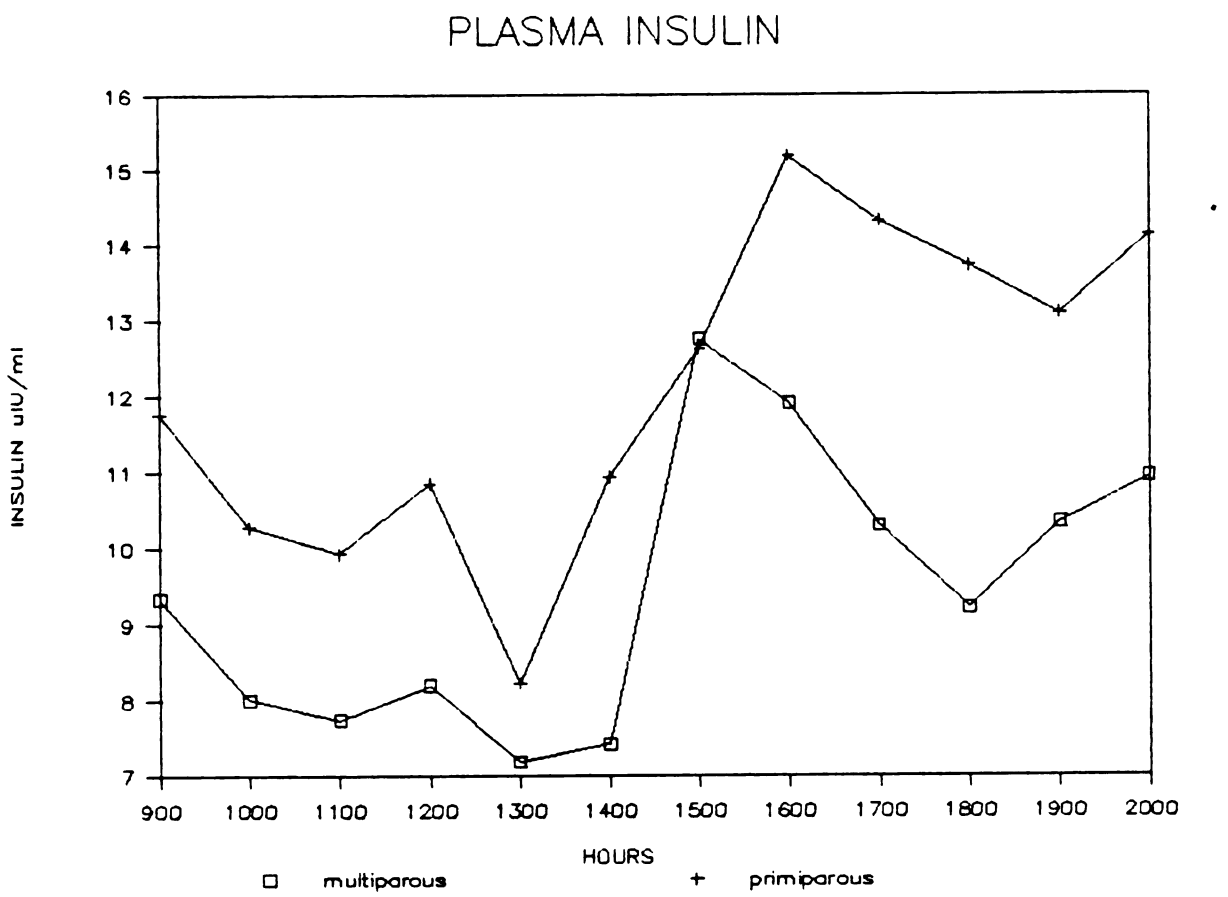
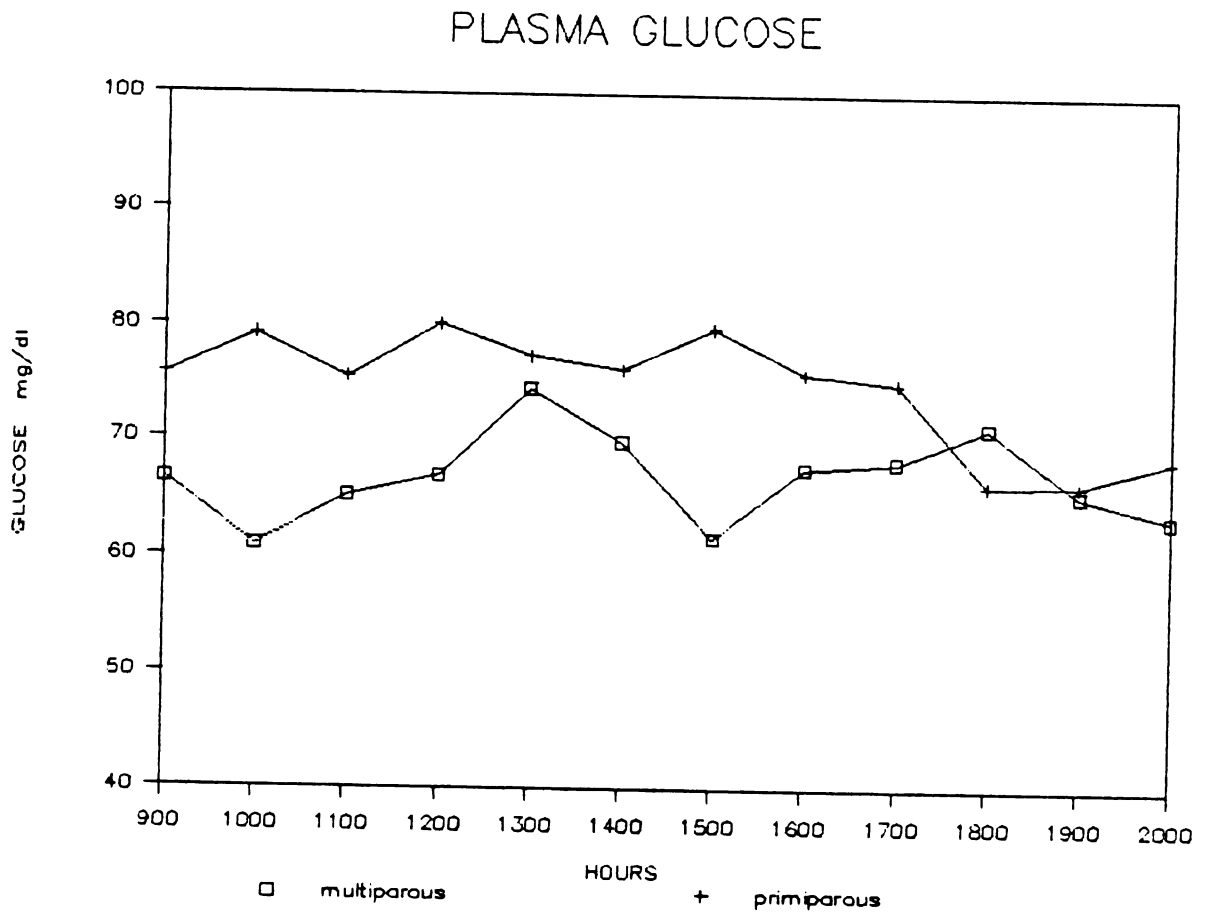


Figure 5. Plasma glucose concentration in multiparous and primiparous cows.



Glucose concentration differed between the two groups at 1000 and 1500 hrs ($P<.05$) and at 1100 and 1200 hrs ($P<.10$, fig.4).

The average growth hormone concentration for primiparous and multiparous cows is shown in figure 6.

Although the multiparous animals presented a wider range of growth hormone concentrations, no significant difference was detected in the total, pre or post feeding period ($P>0.20$). Both groups tended to present a lower level of growth hormone in the post feeding period when compared to pre feeding period and the effect of sampling time was significant during the total ($P<0.01$), pre ($P<.05$) and post feeding period ($P<.10$). No interaction was observed for time of sampling and lactation number for any of the periods analyzed.

In a time by time comparison, growth hormone plasma concentration was significantly higher in multiparous animals at 1300 hrs ($P<.10$) and 1400 hrs ($P<.05$), and lower at 2000 hrs P($<.10$).

Although there was a trend for a higher concentration of glycerol in the multiparous animals (figure 7), no statistical difference between the two groups was detected in any of the periods analyzed ($P>0.20$). Time of sampling presented a significant effect on glycerol concentration when the total, pre feeding or post feeding period was considered ($P<0.01$, $P<.05$ and $P<0.01$ respectively), and the post feeding average tended to be lower for both groups of

Figure 6. Plasma growth hormone concentration in multiparous and primiparous cows.

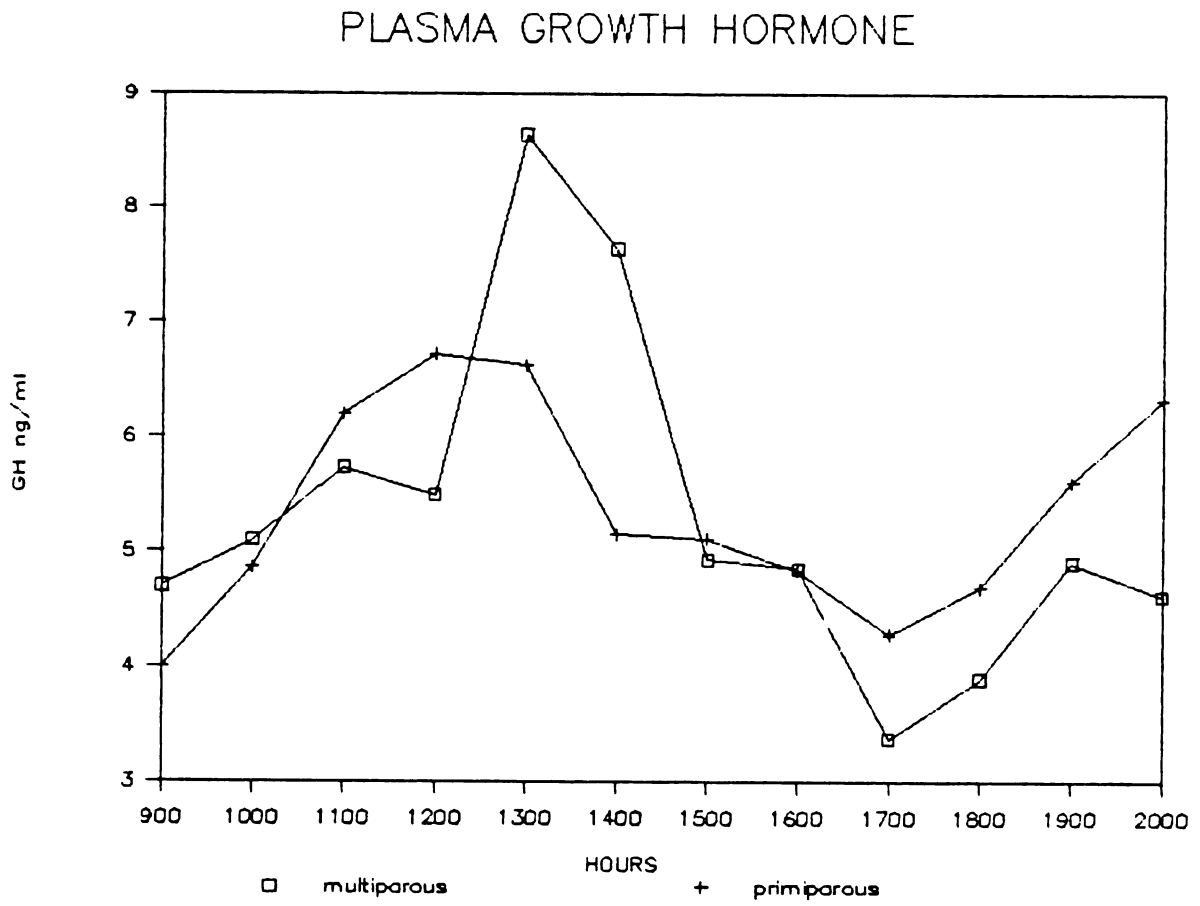
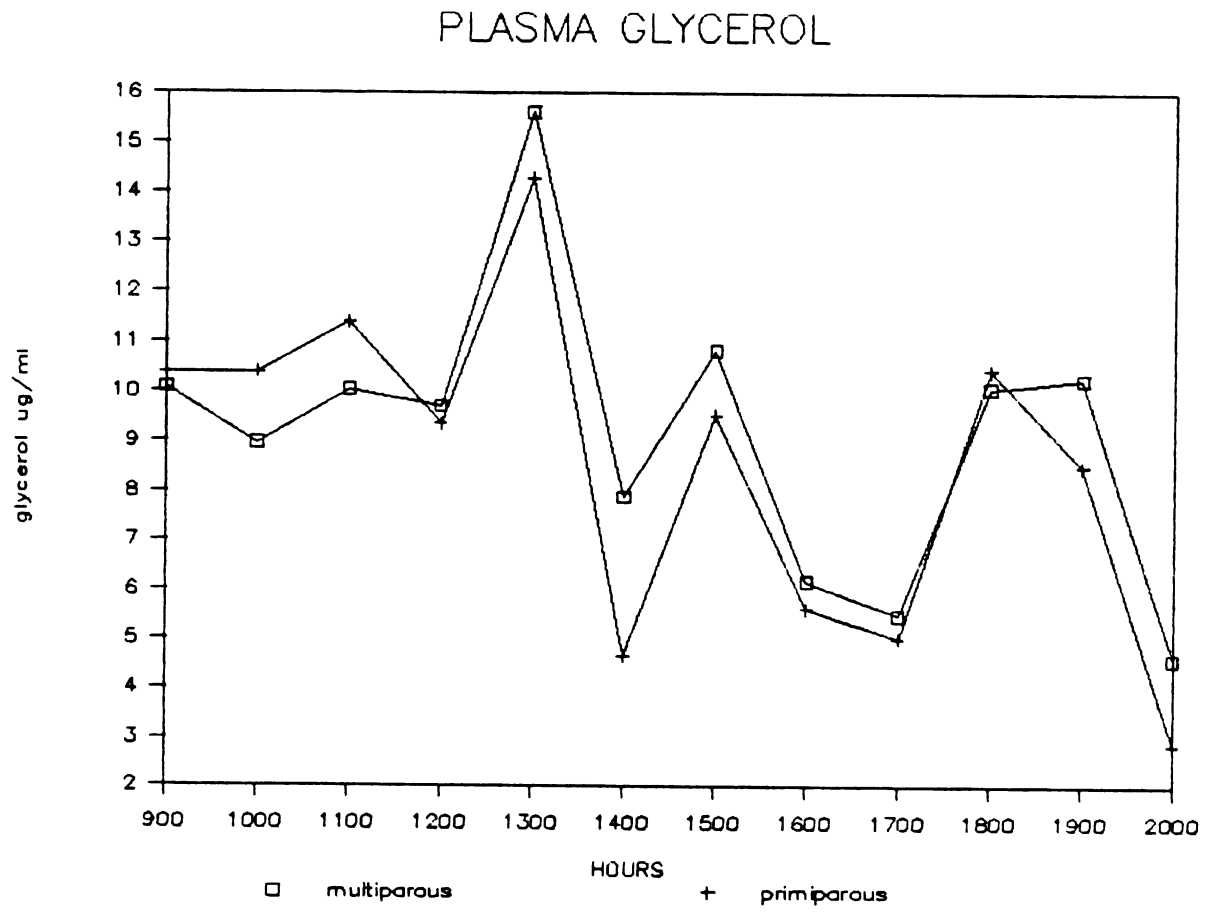


Figure 7. Plasma glycerol concentration in multiparous and primiparous cows.



animals. No effects of interaction between time and lactation number was detected.

In a time by time comparison no significant difference was detected between the two groups.

Least square means for plasma hormones and metabolites are presented in table 5.

The general pattern of a higher level of insulin in the first lactation group was maintained at the same level of significance.

Glucose concentration was significantly higher for the primiparous animals ($P<.05$) during the post feeding period. There was a general tendency to be higher in the other two periods ($P<.10$).

Growth hormone tended to be higher ($P<.10$) in multiparous animals when the data were adjusted based on milk production.

Although no significant difference was detected in glycerol concentration for any of the periods analyzed, the results in this case show an opposite trend when compared to the ones obtained from the unadjusted means. Primiparous animals had an overall tendency for a higher level of glycerol during the total, pre, and post feeding period.

Table 5. Average plasma insulin, glucose, growth hormone and glycerol in the total, pre and post feeding period for multiparous and primiparous cows, using milk production as covariate.

	Multiparous	Primiparous	SE	P
	1 - TOTAL PERIOD			
Insulin uIU/ml	9.73	12.02	.635	<.05
Glucose mg/dl	65.63	75.84	3.761	<.10
GH ng/ml	5.72	4.95	.442	<.10
Glycerol ug/ml	8.12	9.55	.684	ns
	2 - PRE FEEDING PERIOD			
Insulin uIU/ml	8.06	10.26	1.111	ns
Glucose mg/dl	66.15	78.53	4.216	<.05
GH ng/ml	6.61	5.19	.566	<.10
Glycerol ug/ml	9.36	11.09	1.222	ns
	3 - POST FEEDING PERIOD			
Insulin uIU/ml	11.39	13.77	.507	<.01
Glucose mg/dl	65.11	73.15	3.570	<.10
GH ng/ml	4.82	4.70	.450	ns
Glycerol ug/ml	6.88	8.00	.463	ns

DISCUSSION

The general profile observed for insulin in both groups of animals, with increased levels after feeding, is in accordance with previous works (Bassett, J.M., 1974, 1978). Low insulin levels during early lactation has been suggested to be related to energy balance rather than stage of lactation per se (Collier et al., 1984). In the present study a dramatic difference in insulin levels was observed in early lactation when both groups of animals were in positive and equivalent energy balance. Lower levels of insulin in the multiparous group, could lead to a situation were glucose is preferentially shunted towards mammary metabolism. In fact multiparous animals produced 50% more milk and were more efficient in converting energy to milk when compared with first lactation cows.

The fact that insulin levels are negatively correlated with milk production has been shown previously (Vasilatos and Wangsness, 1981, Koprowski and Tucker, 1973). Hart et al. (1978), also showed a lower level of insulin in high producers than in low producers . However in this study, insulin levels differed between the two groups even when the averages were adjusted based on milk production. This means that the difference in insulin levels between primiparous

and multiparous animals can not be explained only by the difference observed in milk production.

Since feed intake was not different per unit of body weight, differences in plasma insulin concentrations are not due to feed intake. Although differences in body weight gain were not significant between the two groups, there was a trend for the primiparous animals to gain more weight than the multiparous. A difference in rate of body weight gain could be one of the explanations for the higher levels of insulin in primiparous cows. The higher level of insulin would increase the uptake of nutrients by peripheral tissues, reducing the availability of nutrients for milk synthesis, decreasing therefore the efficiency of milk production, while furnishing the required nutrients to allow for growth.

Because the total feed intake was smaller for the primiparous animals, even at the same level of weight gain, primiparous cows would have been more efficient than multiparous in increasing body weight. Although carcass composition or body scores were not performed in the present work, one question that could arise at this point is about the composition of the body gain for the two groups. Little information is available on the effects of insulin on energy metabolism in muscle tissue of ruminants. Prior and Christenson, (1978), working with ewes, showed a decrease in plasma levels of amino acids caused by insulin infusion, probably due to an increase in the uptake by muscle tissue.

Goldberg et al.(1980), also suggested that the level of insulin is probably the most important factor regulating protein balance in skeletal muscle. One explanation for the better efficiency in primiparous animals, could be that the increase in body weight for this category in early lactation would be basically due to synthesis of skeletal muscle rather than adipose tissue. However this is an hypothesis that should be tested in further experiments.

For both groups plasma insulin was about 35% higher in the post feeding period than in the pre feeding period. This result is consistent with the idea that differences in insulin response do not relate to lactation at all, but rather are related to the energy balance of the animals (Hedeskov and Capito, 1974)

Plasma glucose levels were higher in primiparous than in multiparous animals. Positive correlation between plasma glucose and insulin, have been reported by Lomax et al. (1979). In addition to that, glucose uptake by the mammary gland is probably contributing for the difference. During the pre feeding period, plasma glucose was higher in primiparous animals, but insulin levels were not different between the two groups. Glucose levels only decreased in first lactation cows after a prolonged exposure to high levels of insulin. These results are in agreement with the ones described by Hove (1978). When the results were adjusted for milk production the difference in glucose was maintained. These results are difficult to explain if 60 to

80% of total glucose turnover is utilized by the mammary gland in lactating ruminants (Bauman and Collier, 1985). An increase in gluconeogenesis in first lactation cows, in order to supply extra nutrients to allow for growth, could be one possible explanation for this fact. However the high level of insulin during the total period does not support this theory. These results could also suggest a difference in insulin sensitivity for mature and first lactation cows.

Plasma growth hormone concentrations were higher in the pre feeding period for multiparous but not for primiparous cows. It is generally accepted that feeding suppresses growth hormone in ruminants, but this type of response was not always consistent. Barnes et al. (1985), working with first lactation Holstein heifers reported an increase in growth hormone but not in insulin after feeding. In the present study, primiparous animals tended to show a higher level of growth hormone when compared with multiparous cows in the post feeding period. Because growth hormone is lipolytic and does increase protein deposition in ruminants (Trenkle A., 1981), this result could also support the theory that the changes in body weight observed in primiparous cows would be predominantly due to an increase in muscle mass rather than fat deposition. Adjusted growth hormone concentrations were higher for multiparous than primiparous cows, which means that at the same level of milk production, heavier (multiparous) animals have higher levels

of growth hormone. These results are in agreement with recent data presented by Verde and Trenkle (1987).

Higher concentrations of glycerol were observed in both groups of animals during the pre feeding period, suggesting that mobilization of body reserves was taking place at that interval of time. These results are consistent with the glycerol patterns described by DiMarco et. al (1981) in fasting and refed steers. The lower level of insulin during the pre feeding period and the consequent reduction of its lipogenic influence was probably one of the reasons contributing to this situation. However when both groups were compared, no difference in glycerol concentration was observed, despite the fact that multiparous cows presented a general tendency for higher levels in all periods. It would be difficult to affirm that multiparous and primiparous cows were mobilizing body reserves at the same rate, because hepatic uptake of glycerol for gluconeogenesis could be occurring at a different level in each group. No statistical difference between the two groups was observed when glycerol levels were adjusted based on milk production.

In conclusion, the hormonal differences between multiparous and primiparous cows during early lactation can not be analyzed considering only a quantitative basis. Insulin, for example, presented the same pattern in time for both groups, although a huge difference in the concentration of this hormone at each particular time was observed when the two groups were compared. The main effect of insulin,

however, could very well be different in multiparous and primiparous cows. In primiparous animals, high levels of insulin in the presence of relatively constant levels of growth hormone would promote an increased uptake of plasma amino acids by peripheral tissue resulting in an increase in body weight due to an increase in skeletal muscle. This idea is supported by the fact that primiparous animals were less efficient in milk production but more efficient in body weight gains when compared to multiparous animals. However due to a paucity of information concerning the role of insulin on energy metabolism in ruminant muscle , further experiments should be performed to clarify this point.

SUMMARY

Plasma glycerol did not show any difference between the two groups during the twelve hour period of observation. However plasma glycerol levels decreased in multiparous and primiparous animals after feeding, suggesting that less fat mobilization was occurring during this period for both groups. Therefore, there was an effect of time of sampling for multiparous and primiparous animals.

Plasma glucose was higher in first lactation cows. But, there was no effect of time of sampling for either groups. However, there was some evidence ($P<.15$) that glucose concentration was changing in time in a different pattern for both groups. While in multiparous cows plasma glucose levels were about constant in the post feeding period, it decreased for primiparous animals during the same period.

Mammary uptake of glucose for lactose synthesis is probably the major explanation for the lower levels of plasma glucose in multiparous cows. Levels of glucose were higher in primiparous animals even after adjusting averages based on milk production. Because primiparous but not multiparous cows presented a decrease in glucose levels when increased levels of insulin were observed during the post feeding period.Therefore there maybe a different insulin sensitivity between groups could.

Despite the fact that multiparous cows presented a wider variation in plasma concentration of growth hormone, when the average concentration for the total period of observation was considered, no differences between the two groups were observed over the total sampling time. Multiparous but not primiparous animals presented a decrease in plasma growth hormone concentration after feeding. After adjusting the results based on milk production, multiparous animals showed a higher level of plasma growth hormone, probably due to differences in body weight.

Insulin was definitely higher in primiparous animals during all the periods considered. Even after adjusting the results based on milk production, insulin was still higher in first lactation cows . Differences in insulin levels in the present work could not be accounted for by differences in energy balance, feed intake per unit of body weight or milk production. Since the primiparous animals are growing this may be the main reason why insulin levels are higher.

However, if both groups were gaining weight at the same rate, differences in body gain composition could be occurring. It is suggested that higher levels of insulin associated with relatively constant levels of growth hormone in primiparous animals would drive nutrients towards skeletal muscle accretion rather than adipose tissue.

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