Plasma Levels of Hormones and Metabolites as Affected by the Forages Type in Two Different Types of Crossbred Holstein Cattle

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ABSTRACT : An experiment was carried out to study plasma levels of hormones and metabolites of crossbred Holstein cattle during late pregnancy (28 days pre partum), early lactation (30 days post partum), mid-lactation (120 days post partum) and late lactation (210 days post partum). Two breed types of Holstein Friesian × Red Sindhi (50:50 = 50%HF) and Holstein Friesian × Red Sindhi (87.5:12.5 = 87.5%HF) were divided into four groups of four animals each. Two groups of each breed were fed with either rice straw treated with 5% urea or pangola hay (Digitaria decumbens) as the source of roughage throughout the experiments. There were a substantial increases in the mean levels of total triiodothyronine (T_3), insulin and glucagon at the onset of lactation, and maintained in a high levels during lactation advance for all groups of experiments. The mean levels of prolactin and thyroxine (T_4) were not significantly different among groups of animals, but the plasma cortisol concentration was slightly higher in both groups of 50%HF in comparison with those of 87.5%HF animals. The mean levels of plasma growth hormone (GH) of both groups of 87.5%HF animals feeding on either hay or urea treated rice straw markedly rose in the early period of lactation and markedly reduced in mid- and late lactation. These changes were accompanied with changes of milk yield. In contrast to 50%HF animals, plasma GH levels were considerably higher in the late pregnant period than in the early period of lactation and it remained constant as its value at the early lactation throughout the experimental period. The high levels of both plasma progesterone and estradiol concentration significantly declined after parturition and remained low through lactating period. The plasma glucose level in the 50%HF animals feeding on either hay or urea treated rice straw was higher than the 87.5%HF animals in all periods of experiments. Changes in plasma FFA levels of both types of crossbred animals were depended on the endocrine status during late pregnancy and lactation. The levels of plasma FFA of 50%HF animals were significantly higher ($p<0.05$) than those of 87.5%HF animals during late pregnancy. Both plasma β-hydroxybutyrate and lactate concentrations were not affected by feeding on either hay or urea treated rice straw during late pregnancy and lactation. These data demonstrate that there were no differences in the physiological performances in the same crossbred animals fed either hay or urea treated rice straw. The 87.5%HF animal has the genetic potential for a high milk yield and homeorhetic adaptation for mammary function differed from 50%HF animals during periods of lactation. Altering lactation persistency in 87.5%HF is regulated mainly by chronically acting growth hormones through the period of lactation. *(Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 10 : 1359-1366)*

Key Words : Plasma Hormone, Plasma Metabolites, Forages Type, Crossbred Holstein Cattle

INTRODUCTION

It is known that crossbred cattle between *Bos taurus* and *Bos indicus* has been exploited as an efficient tool for blending the adaptability of tropical cattle with the high milking potential of exotic breeds for increased milk production. There is still a need to answer the question of the type of crossbred cattle most suitable for the tropics and the management necessary for efficient dairy production in a hot climate. Not only genetic potential for milk of crossbred cattle has been considered, but another factor which limits milk production of tropical dairy cattle is an inadequate supply for foraging during the dry, summer months. Animals are fed mainly on crop residues such as rice straw which has a low nutritive value. An improvement in rice straw by treatment with urea to help animals survive during periods of scarcity has been reported (Jayasuriya and Perera, 1982; Promma et al., 1994).

During pregnancy, mammary growth has been known to be a prerequisite for satisfactory lactation (Hanwell and Peaker, 1977) and during lactation, the cow partitions dietary energy between the production of milk and body tissues. In crossbred cattle, the balance between the two determines whether the animal is primarily a milk producer or a meat producer and the importance of this balance has been reported (Bauman and Currie, 1980). Differences between animals in partitioning ability are known to be inherited and are thought to be under endocrine control. However, their effects on blood hormone and metabolite levels have not been clarified, although the role of endocrine regulation in initiation and maintenance of lactation have been extensively

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reviewed (Tucker, 1981). It has been realized that during late pregnancy, lactogenesis occurs concurrently with mammary development and many hormones are needed for maximal stimulation of lactogenesis (Tucker, 1981, 1987). Little is known about certain hormones control the functioning in normal lactating crossbred animals and attempts to pinpoint specific role in milk production relation to the bodily nutritional status have been limited. Knowledge and understanding of such roles may permit the early identification of the potentially high-milk yielding animals for selection purpose and may make possible the manipulation of nutritional status to enhance production efficiency of dairy crossbred animals. Therefore, the objective of this study was to evaluate the status of circulating hormones from pituitary gland, thyroid gland, adrenal cortex, pancreas and gonad relating to plasma metabolites during late pregnancy and different stages of lactation in crossbred Holstein cattle fed either hay or urea treated rice straw through the period of experiment.

MATERIALS AND METHODS

Animals and management

Sixteen pregnant heifers crossbred Holstein cattle, 23-25 months old and after approximately 150 days of gestation, were selected for the experiments. These animals consisted of eight animals of two breed types, Holstein Friesian×Red Sindhi (50:50 = 50%HF) and Holstein Friesian×Red Sindhi (87.5:12.5 = 87.5%HF). They were divided into four groups of 4 animals each. Each group of animals consisted of four animals from the same breed. Animals from the same breed type in each group were fed with either rice straw treated with 5% urea or pangola hay (*Digitaria decumbens*) as the source of roughage throughout the experiments. All the animals were housed in sheds. The maximum temperature in the shed at noon was 34±1℃ and the minimum temperature at night was 26±1℃. Before parturition, animals were individually fed a concentrate of an average of 4.0 kg/day (DM basis) and roughage to maintain a moderate the body condition score until calving (2.5, scale = 1 to 5). In the lactation period, animals received an average of 4-5 kg/day of roughage in combination with the same concentrated mixture (7-10 kg/day) (table 1). Each day, half of the food was given at between 0600-0700 h and the other half between 1600-1700 h. Animals were adequately supplied with water and a lick block of minerals throughout the experiment. Animals were fed their respective rations for at least 3 months before the first experimental periods.

The urea treated rice straw was prepared by mixing urea solution (5 kg urea dissolved in 100 litres water per 100 kg dry rice straw) with dry straw. Rice straw sprayed with urea solution was mixed thoroughly and stored under airtight conditions in a cement pit for 21 days. A continuous supply of treated rice straw was made available by using a 2 pit ×21 day system of urea treatment. After 21 days, the treated rice straw with 5% urea was offered to the animals.

Experimental procedures

Four consecutive periods of experiments were carried out in each group. Period 1 was designed to begin 21 days (20-23 days) before parturition (late pregnancy). Period 2 began 30 days postpartum (early lactation). Period 3 began 120 days postpartum (mid-lactation) and period 4 began 210 days postpartum (late lactation). Animals were fed the same ration through the completion of period 4. In lactating periods of experiments, animals were normally milked at around 0600 h and 1700 h. On the day of the experiment at around 1100 h, a blood sample was taken from the jugular vein into the heparinized tube and an arterial blood sample was collected from the coccygeal artery by venipuncture with a #21 needle into heparinized tube. Blood samples in heparinized tube were kept in crushed ice and then centrifuge at 3000 rpm for 30 min at 4℃. Plasma from the venous blood samples were kept in aliquots at -40℃ until hormone concentrations were assayed. An arterial plasma samples were kept at -40℃ for chemical studies. Milk yield was recorded by milking machine in each lactating period of study.

Hormone assay

Plasma samples in aliquots were collected and frozen at -40℃ until time of hormone assays.

Radio immono assay (RIA) of growth hormone (GH). Bovine GH (bGH) were performed on all plasma samples using 100 μl in duplicated as described as following. Highly purified bovine growth

Table 1. Chemical composition of experimental diet and nutrient analysis as a percentage of dry matter

	Pangola hay	Urea treated rice straw	Concentrate
Dry matter	92.1	58.0	89.4
Crude protein	4.3	8.9	17.8
Acid detergent fibre	48.9	61.2	21.5
Neutral detergent fibre	81.0	67.2	28.8
Lignin	6.6	8.8	7.0
Ash	10.2	16.8	5.6

Concentrate formation: ingredients by fresh weight (100 kg $^{-1}$) consisted of soy bean meal (30 kg), cotton seed (25 kg), cassava (25 kg), rice bran (15 kg), dicalcium phosphate (2 kg), sodium bicarbonate (1.7 kg), potassium chloride (0.7 kg) and premix (0.6 kg).

hormone (Batch no. B.980953, Biogenesis Ltd.) was used for iodination and reference standard for GH. Double antibodies RIA standardize in our lab was used for estimation of bGH in plasma samples.

Radio iodination of bGH. Highly purified bGH was labeled with carrier free iodine ($Na^{125}I$, Amersham, UK) at room temperature. Five μg bGH in 0.05 M carbonate buffer pH 9.5 (1 μg/5 μl) was mixed with 50 μl of 0.5 M sodium phosphate buffer (pH 7.5) in separate vial in which iodination was carried out subsequently. To this vial, 1 mCi of carrier free iodine ^{125}I was added and contents were mixed gently. Chloramine T, 15 μg (1 μg/1 μl) in 0.05 M sodium phosphate buffer (pH 7.5) was added to the reaction mixture which was then shaken gently for 60 sec. The reaction was terminated by the addition 50 μg sodium metabisulphite (1 μg/1 μl) in 0.05 M sodium phosphate buffer (pH 7.5) transfer solution.

Radio chromatography of labeled preparation of bGH. The separation of iodinated bGH from free iodine was carried out on two separate Sephadex G75 column (1×15 cm). The whole content of the reaction vial was layered on the Sephadex G75 column. The vial was immediately rinsed with 100 μl of rinse solution containing 0.05 M sodium phosphate buffer (pH 7.5) and 25 ml of 0.05 M sodium phosphate buffer (pH 7.5) and the latter again layered on the column. 1.0 ml fractions were collected in tubes containing 0.5 ml of 2% bovine serum albumin in 0.01 M sodium phosphate buffer (pH 7.5) (2% BSA-PBS). All the iodinated fractions were counted in Auto Gramma Counter. Typical elution pattern on Sephadex G75 showing the separation of bGH ^{125}I from free ^{125}I. The first peak was of bGH ^{125}I whereas the second peak represented free ^{125}I. The fraction of tube no.5 showing the first peak of bGH which was devided in aliquots and store at -20℃.

Assay protocol of bGH. Plasma samples (0.1 ml) were pipetted in dispossible plastic tubes (10×75 mm). Simultaneously, a series of standards ranging from 1.0-40.0 ng/ml were also pipetted. 0.2 ml of 2% BSA-PBS (pH 7.5) was added to each tube. The bGH antiserum (rabbit, Biogenesis Ltd., Batch no. D.980263) was diluted to 1:10,000 with 0.01 M PBS (pH 7.5) and 0.1 ml diluted antiserum was added to all the tubes. The tubes were than vortexed and incubated at refrigerator temperature (4℃) for 48 hr. 0.1 ml of labelled ^{125}I bGH (20,000 cpm) was then added to all the tubes. The tubes were vortexed and incubated for another 72 hr at 4℃. Following this incubation, appropriately diluted 0.5 ml of sheep purified anti-rabbit gramma globulin (Biogenesis Ltd., Batch no. B.981281) was added to all the tubes and incubated 30 min at room temperature. 0.5 ml of 0.01M BSA-PBS buffer was added to each tube. The antibody bound hormone complex was separated from free labelled hormone by centrifuging at 3,000 rpm for 30 min at 4℃. The supernatant was decanted and assay tubes were kept inverted on the absorbent paper. The assay tubes were subsequently counted in Auto Gramma Counter Programmed for hormone quantitation. The assay sensitivity for bGH was 1.0 ng/tube. Intraassay and interassay coefficients of variation were obtained by replicating a single pool containing 12.1 ng GH/ml six times in five consecutive assays were 6.6% and 9.2%, respectively.

Other hormones assays. Plasma insulin concentration was quantified using a radio immuno assay (RIA) kit (Coat-a Count® Insulin, Diagnostic Products Corporation, Los Angeles, CA, USA.). Plasma glucagon concentration was measured using a RIA kits (Glucagon double antibody, Diagnostic Products Corporation, Los Angeles, CA, USA.). Plasma prolactin was measured by RIA kits (Prolactin double antibody, Diagnostic Products Corporation, Los Angeles, CA, USA.). Plasma cortisol was quantified by RIA kits (Coat-a-count® Cortisol, Diagnostic Products Corporation, Los Angeles, CA, USA.). Plasma estradiol was quantified by RIA kits (Coat-a-count® Estradiol, Diagnostic Products Corporation, Los Angeles, CA, USA.). Plasma progesterone was quantified by RIA kits (Coat-a-count® Progesterone, Diagnostic Products Corporation, Los Angeles, CA, USA.). Total plasma thyroxine (T_4) and total plasma triiodothyroxine (T_3) were quantified by RIA kits (Coat-a-count® T_4, T_3, Diagnostic Products Corporation, Los Angeles, CA, USA.).

Metabolites determinations

Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase. Plasma free fatty acids (FFA, C_{16}-C_{18}) concentrations were measured by using gas chromatography (Shimazu GC-7AG Gas Chromatograph) in comparison with the internal standard. The internal standard of heptadecanoic acid was used for estimation of plasma FFA as described by Thomson et al. (Thomson et al., 1979). Plasma β-hydroxybutyrate concentrations were assayed using enzymatic reaction in the presence of β-hydroxybutyrate dehydrogenase (Sigma Chemical Co.). Plasma lactate concentrations were assayed using enzymatic reaction in the presence of lactate dehydrogenase (Sigma Chemical Co.).

Statistics

The experimental results were evaluated by analysis of variance; the significant differences between groups and treatments were compared by Duncan's multiple range test. Values were compared among periods in each group using the paired t-test. Mean values are

presented as mean ± SD.

RESULTS

Concentrations of hormones during late pregnancy and different stages of lactation

Table 2 shows the mean value of plasma hormones of 50%HF and 87.5%HF animals fed either hay or urea treated rice straw. In both groups of crossbred HF animals, feeding on either hay or urea treated rice straw did not significantly affect total plasma thyroxine (T_4) levels during late pregnant periods and lactating periods among groups of animals. There was a substantial increase in the mean level of total triiodothyronine (T_3) at the onset of lactation, it maintained in a high levels during lactation advance for all groups of experiments. Over the entire experiment the mean levels of prolactin were not significantly different among groups of animals either late pregnant periods or during lactating periods. The levels of plasma GH of both groups of 87.5%HF animals markedly rose in the early period of lactation after parturition, thereafter there was a substantial reduction in the mean level of plasma GH in mid- and late lactation ($p<0.05$) in both groups of 87.5%HF animals. In contrast to both groups of 50%HF animals feeding on either hay or urea treated rice straw, the levels of plasma GH were considerably higher in the late pregnant period than in the early period of lactation. During lactation advance to mid- and late lactation, the mean level of GH of both groups of 50%HF animals remained constant as its value at the early lactation. In early lactation, the concentration of plasma GH in the 87.5%HF animals was higher than that in the 50%HF animals feeding either hay or urea treated rice straw. The mean plasma cortisol concentration of both groups of 50%HF was higher by approximately 2 folds than those of 87.5%HF animals fed either hay or urea treated rice straw, although the differences did not attain statistical significance at all periods of experiments.

Table 3 shows that mean plasma insulin concentration of both types of crossbred animals fed either hay or urea treated rice straw increased during the lactating period as compared to the late pregnant and it remained constant in a higher level throughout the lactating period. During late pregnancy and early lactation, glucagon concentrations in plasma were not significantly different among groups of crossbred animals feeding on either hay or urea treated rice

Table 2. Concentrations of triiodothyronine, thyroxine, prolactin, growth hormones and cortisol in plasma of crossbred HF animals feeding on hay or urea treated rice straw during late pregnancy and different stages of lactation

Hormone	Period of experiment	Hay+concentration		Urea treated rice straw+concentration	
		HF:RS (87.5:12.5)	HF:RS (50:50)	HF:RS (87.5:12.5)	HF:RS (50:50)
Triiodothyronine (ng/100 ml)	Late pregnancy	78.8 ±13.6^{a}	81.5 ± 29.9^{a}	96.8 ±18.0^{a}	93.5 ±19.8^{a}
	Early lactation	93.8 ±41.5^{a}	131.5 ± 16.4^{a†}	107.2 ± 8.2^{a}	133.0 ±44.7^{a}
	Mid lactation	111.0 ± 2.8^{a}	120.5 ± 21.2^{a}	118.7 ±17.5^{a}	142.5 ±28.1^{a}
	Late lactation	107.0 ±32.0^{b}	111.5 ± 7.4^{ab}*	122.7 ±19.0^{ab}	141.2 ±19.5^{a}
Thyroxine (T4) (ug/100 ml)	Late pregnancy	3.00± 0.68^{b}	3.93 ± 0.49^{ab}	4.33± 0.95^{a}	3.63± 0.88^{ab}
	Early lactation	3.29± 0.41^{a}	4.15 ± 0.29^{a}	4.05± 0.93^{a}	3.83± 0.90^{a}
	Mid lactation	4.09± 1.16^{a}	3.88 ± 0.68^{a}	3.78± 0.85^{a}	3.50± 0.59^{a}
	Late lactation	3.98± 0.95^{a}	3.78 ± 0.67^{a}	3.41± 0.68^{a}	3.64± 0.98^{a}
Prolactin (ng/ml)	Late pregnancy	3.94± 0.39^{a}	9.36 ± 5.42^{a}	4.83± 1.15^{a}	4.08± 2.47^{a}
	Early lactation	4.80± 1.23^{ab}	9.83 ± 7.48^{a}	5.83± 2.16^{ab}	3.56± 1.52^{b}
	Mid lactation	4.93± 1.52^{a}	9.68 ± 6.27^{a}	7.27± 5.56^{a}	3.80± 0.63^{a}
	Late lactation	7.38± 3.16^{a}	11.48 ± 7.98^{a}	9.65± 6.01^{a}	4.10± 0.88^{a}
Growth hormone (ng/ml)	Late pregnancy	10.72± 4.95^{ab}	15.80 ± 4.56^{a}	6.18± 1.55^{b}	14.53± 4.07^{a}
	Early lactation	15.10± 6.39^{a}	9.45 ± 4.27^{ab}	9.00± 1.50^{ab}	8.62± 1.37^{b}
	Mid lactation	11.30± 0.93^{a}	8.20 ± 3.98^{a}	6.90± 2.94^{a}	9.33± 4.67^{a}
	Late lactation	9.12± 3.81^{a}*	9.27 ± 3.19^{a}	6.70± 0.79^{a}*	10.25± 4.23^{a}
Cortisol (ng/ml)	Late pregnancy	10.3 ±11.0^{ab}	23.5 ± 14.5^{ab}	7.5 ± 2.8^{b}	30.2 ±20.1^{a}
	Early lactation	19.8 ±18.4^{a}	29.9 ± 24.9^{a}	8.6 ± 6.9^{a}	28.6 ±18.6^{a}
	Mid lactation	7.5 ± 3.4^{a}	25.7 ± 25.4^{a}	4.0 ± 1.9^{a}	25.0 ±19.2^{a}
	Late lactation	6.8 ± 6.9^{b}	26.8 ± 22.9^{ab}	5.7 ± 7.0^{b}	45.0 ±19.2^{a}

P-values by paired t-test. $^{\dagger}p<0.05$ with respect to the late pregnant period in each group.
*$p<0.05$, **$p<0.01$ with respect to the early period of lactation in each group.
^{a,b} Mean values within a row indicated with different superscripts are significantly different ($p<0.05$).

Table 3. Concentrations of insulin, glucagon, progesterone and estradiol in plasma of crossbred HF animals feeding on hay or urea treated rice straw during late pregnancy and different stages of lactation

Hormone	Period of experiment	Hay+concentration		Urea treated rice straw+concentration	
		HF:RS (87.5:12.5)	HF:RS (50:50)	HF:RS (87.5:12.5)	HF:RS (50:50)
Insulin (μU/ml)	Late pregnancy	9.40± 3.82[a]	14.93± 9.09[a]	15.30± 4.46[a]	18.17±13.10[a]
	Early lactation	16.94±10.87[a]	18.93± 5.57[a]	20.37±10.36[a]	27.13±15.65[a]
	Mid lactation	21.45± 9.46[a]	25.07±18.43[a]	25.97± 6.96[a]	20.75± 6.56[a]
	Late lactation	17.13± 4.71[a]	17.63± 4.82[a]	25.00± 1.86[a]	28.25±16.35[a]
Glucagon (pg/ml)	Late pregnancy	31.8 ±11.3[a]	48.6 ±19.8[a]	51.8 ±22.7[a]	65.5 ±46.48[a]
	Early lactation	29.5 ± 5.4[a]	59.9 ±17.7[a]	80.0 ±52.5[a]	74.6 ±39.0[a]
	Mid lactation	46.6 ± 6.2[b]	105.3 ±46.7[ab]	118.8 ±45.3[a]*	131.9 ±43.2[a]*
	Late lactation	53.5 ±35.7[c]	97.7 ±12.3[ab]**	77.5 ± 6.1[bc]	124.5 ±29.3[a]*
Progesterone (ng/ml)	Late pregnancy	4.30± 1.60[a]	5.70± 1.10[a]	3.40± 1.10[a]	3.20± 1.50[a]
	Early lactation	0.16± 0.08[a]††	0.15± 0.09[a]††	0.61± 1.02[a]†	0.10± 0.01[a]††
	Mid lactation	0.10± 0.01[b]	2.70± 2.30[a]	1.35± 1.46[ab]	0.15± 0.06[b]
	Late lactation	1.15± 1.25[ab]	3.76± 3.84[a]	3.40± 0.80[ab]	0.56± 0.37[b]
Estradiol (pg/ml)	Late pregnancy	129.8 ±56.2[a]	171.3 ±79.8[a]	115.0 ±56.1[a]	180.0 ±81.69[a]
	Early lactation	12.0 ± 6.2[a]†	17.5 ± 5.7[a]†	16.0 ± 7.9[a]†	19.1 ± 7.8[a]†
	Mid lactation	9.3 ± 6.8[b]	18.7 ±13.1[ab]	14.5 ± 7.5[b]	25.5 ± 9.2[a]
	Late lactation	10.5 ± 2.3[b]	23.6 ±13.5[ab]	18.0 ± 9.9[ab]	28.3 ± 3.6[a]

P-values by paired t-test. † $p<0.05$, †† $p<0.01$ with respect to the late pregnant period in each group,
* $p<0.05$, ** $p<0.01$ with respect to the early period of lactation in each group.
[a,b,c] Mean values within a row indicated with different superscripts are significantly different ($p<0.05$).

straw. A trend toward increased plasma glucagon concentrations during lactation advance to mid- and late lactation. In these periods the increases in the mean glucagon levels, which were substantially higher in both 50%HF and 87.5%HF animals feeding on urea treated rice straw as compared to those animals feeding on hay ($p<0.05$). Levels of plasma progesterone and estradiol concentrations showed considerable individual variation. Both plasma progesterone and estradiol levels increased during late pregnancy. The high levels of both plasma progesterone and estradiol declined markedly after parturition and remained low for a whole lactating period.

Changes in arterial plasma metabolite concentrations during late pregnancy and different stages of lactation (table 4)

Plasma glucose concentrations remained stable throughout periods of study in each group. However, the plasma glucose level in the 50%HF animals feeding on either hay or urea treated rice straw was higher than the 87.5%HF animals in all periods of experiments. The mean plasma FFA levels of both types of crossbred animals differed during periods of experiments, mainly the greatly increased levels at the late pregnant period in comparison to periods of lactation. The levels of plasma FFA of 50%HF animals were significantly higher ($p<0.05$) than those of 87.5%HF animals during late pregnancy. β-hydroxybutarate (BHB) in plasma of all groups of crossbred HF did not show different among groups of animals feeding on either hay or urea treated rice straw or different periods of experiment in the same group. During late pregnancy and lactation, crossbred HF animals feeding on either hay or urea treated rice straw did not affect to the concentrations of plasma lactate. During early lactation, milk yield of both groups of 87.5%HF animals was significantly higher ($p<0.05$) than those of 50%HF animals feeding on either hay or urea treated rice straw. However, in mid- and late lactation, milk yield significantly fell from the early lactating period in both groups of 87.5%HF animals. In contrast to 50%HF animals feeding on either hay or urea treated rice straw, the trend for persistency was observed as for milk yield throughout lactating periods.

DISCUSSION

The present results show that there were no significant differences in the mean plasma thyroxine (T_4) concentration during experiments in all groups of crossbred HF animals. However, plasma triiodothyronine (T_3) concentrations of both types of crossbred HF animals feeding on hay or urea treated rice straw, were lowered in late pregnancy as compared to lactating periods. The difference of the pattern of changes between T_3 and T_4 at the onset of lactation may be suggestive of an active and rapid transformation of T_4 to T_3. An increase in the rate of

Table 4. Arterial concentration of metabolites in plasma of crossbred HF animals feeding on hay or urea treated rice straw during late pregnancy and different stages of lactation

Metabolite	Period of experiment	Hay+concentration		Urea treated rice straw+concentration	
		HF:RS (87.5:12.5)	HF:RS (50:50)	HF:RS (87.5:12.5)	HF:RS (50:50)
Glocose (μmol/ml)	Late pregnancy	3.67± 0.32^{a}	4.06± 0.59^{a}	3.49± 1.04^{a}	3.60± 0.81^{a}
	Early lactation	3.40± 0.53^{b}	4.46± 0.42^{a}	3.54± 0.19^{b}	4.16± 0.13^{a}
	Mid lactation	3.15± 0.46^{b}	3.77± 0.35^{ab}	3.39± 0.56^{ab}	3.99± 0.45^{a}
	Late lactation	3.54± 0.40^{a}	3.86± 0.19^{a}	3.79± 0.33^{a}	3.52± 0.17^{a}
Free fatty acid	Late pregnancy	369.5 ± 83.0^{b}	526.7 ±135.3^{ab}	393.7 ± 90.3^{ab}	573.3 ±165.6^{a}
(C16-18) (μmol/l)	Early lactation	302.0 ±111.3^{a}	314.4 ±115.8^{a}	317.5 ±171.5^{a}	446.5 ±223.5^{a}
	Mid lactation	260.7 ±191.7^{a}	375.1 ±191.3^{a}	200.7 ± 50.3^{a}	298.8 ±146.4^{a}
	Late lactation	182.5 ± 62.4^{b}	350.4 ±129.3^{a}	237.9 ± 76.0^{ab}	288.8 ±117.7^{ab}
β-hydroxybutyrate	Late pregnancy	680.2 ±153.6^{a}	646.0 ±140.3^{a}	539.0 ±160.4^{a}	800.5 ±569.9^{a}
(μmol/l)	Early lactation	648.0 ± 51.8^{a}	508.0 ±125.6^{ab}	397.3 ± 68.4^{b}	536.7 ±195.9^{ab}
	Mid lactation	563.0 ±154.2^{a}	563.7 ± 64.6^{a}	432.5 ±119.9^{a}	517.7 ±102.9^{a}
	Late lactation	531.3 ± 79.6^{a}	523.0 ± 67.0^{ab}	408.0 ± 74.2^{c}	431.3 ± 19.5^{bc}
Lactate (μmol/l)	Late pregnancy	124.2 ± 21.2^{a}	138.0 ± 47.1^{a}	188.0 ± 66.1^{a}	138.3 ± 52.9^{a}
	Early lactation	134.5 ± 54.0^{a}	114.0 ± 20.8^{a}	95.4 ± 24.7^{a}	127.4 ± 46.3^{a}
	Mid lactation	146.4 ± 35.7^{a}	96.6 ± 29.1^{b}	119.4 ± 23.1^{ab}	108.2 ± 30.2^{ab}
	Late lactation	110.3 ± 28.4^{ab}	96.5 ± 21.3^{ab}	91.2 ± 15.5^{b}	124.3 ± 19.3^{a}
Mik yield (kg/d)	Early lactation	19.76± 4.47^{a}	10.98± 1.17^{b}	16.51± 5.92^{ab}	12.91± 1.58^{b}
	Mid lactation	11.00± 1.61^{a}*	10.52± 1.34^{a}	11.72± 0.93^{a}	12.33± 2.46^{a}
	Late lactation	10.11± 0.69^{ab}*	10.47± 0.81^{ab}	9.18± 1.21^{b}*	12.26± 2.51^{a}

P-values by paired t-test. *p<0.05, **p<0.01 with respect to the early period of lactation in each group.
^{a,b,c} Mean values within a row indicated with different superscripts are significantly different (p<0.05).

this transformation is very likely, since plasma T_4 has been shown to transform to T_3 in tissue before it becomes biologically active (Boonnamsiri et al., 1979). In the present results, the plasma T_3 concentrations were maintained in high levels in all lactating periods, indicating the thyroid hormones act as important factor in the regulation of lactation. Since T_3 is the metabolically active form of thyroid hormone (Meites, 1966), an elevation of T_3 in both types of crossbred HF animals feeding on either hay or urea treated rice straw would exert its metabolic effect through increased oxygen consumption, thereby, increased rate of glucose utilization during lactation. An evidence for an increase in total glucose entry rate during the onset of lactation has been previously reported (Chaiyabutr et al., 1998). An elevation of plasma insulin levels during the onset of lactation may also be a factor involved in changes in glucose turnover rate. However, in the present results the mean plasma glucose concentration in all groups was not accompanied by an increase in the plasma insulin concentration throughout periods of experiments. The plasma glucose concentrations of 50%HF animals in both pregnant and lactating periods were higher than those of 87.5%HF animals fed either hay or urea treated rice straw. The differences in plasma glucose levels between 50% and 87.5%HF animals could not be explained as both types of crossbred animals were given identical rations. The higher levels of plasma cortisol may play a role for a rise of the plasma glucose level in 50%HF animals. The hyperactivity of adrenal cortex to produce cortisol in 50%HF was probably higher than that of 875%HF animals in all periods of experiments.

Changes in endocrine status during the transition period from late pregnancy to lactation would influence metabolism and the nutritional status. The pattern of differences in insulin concentrations between late pregnancy and early lactation could not be attributed to diurnal variation (Bines et al., 1983) and to feed effect (Bassett, 1974). Since in the present study, blood was withdrawn from animals after four hours of feeding on same amount of concentrate and roughage and therefore the feed effect during morning was eliminated. A lipogenic role would be expected for an elevation of plasma insulin levels during lactation by the documented fall in plasma FFA concentrations which occurred throughout lactation. During late pregnancy, plasma FFA concentrations were higher than that of lactating periods in all groups of crossbred HF animals which coincided with a low level of the plasma insulin concentration. Several mechanisms could propose to contribute to the changes in lipogenesis. A low plasma insulin level would favor the movement of energy substrates away from the adipose tissues stores and causing an elevation of plasma FFA (Yang and Baldwin, 1973). At the onset

of lactation, the decrease in the sensitivity of adipose tissue to insulin has been reported (Faulkner and Pollock, 1990). Evidence for adipocytes becoming insulin resistant during lactation has also been found with laboratory species with both *in vitro* and *in vivo* studies (Venon, 1996). During late pregnancy, mammary growth and foetus development could account for energy deficity relating to the elevation of plasma FFA concentrations, and it seems reasonable that the depressed plasma insulin represent part of the mechanism permitting mobilization of energy store during late pregnancy. However, the higher level of plasma progesterone during pregnancy in both types of crossbred HF would be the other factor that contribute to an increase in plasma FFA concentration. Since, the correlation between the concentration of plasma progesterone and quantity of FFA in plasma in the pregnant ruminant was also noted (Shevah et al., 1975). Estradiol, primarily estrone of placental origin, markedly increased in plasma during late pregnancy and dramatic decreases after parturition in all groups of crossbred animals. Changes in estradiol may decreased feed intake at the period of prepartum (Grummer et al., 1990). The extent of the decrease of feed intake probably occurred at the prepartum period which may be another factor determining the animals develop high plasma FFA levels.

During early lactation, crossbred HF animals were in a similar state of energy balance, i.e. the metabolic demands of lactation were not being met by dietary intake, causing mobilization of body tissues as indicated by the higher levels of FFA in all groups. The higher levels in plasma GH during late pregnancy in both groups of 50%HF animals could account for an increase in plasma FFA concentrations in comparison to those of 87.5%HF animals. In 87.5%HF animals, the higher levels of circulating GH in early lactation would still possess an intrinsic lipolytic activity (Lee et al., 1974). During lactation advance in 87.5%HF animals feeding on either hay or urea treated rice straw, the decrease in the level of GH coincided with the decrease in the plasma FFA concentration. Therefore, it is possible that GH causes the depletion of adipose tissue reserve in ruminant with an increase in FFA, which being associated with greater requirement for energy.

In the present study, rapidity of the decrease in milk yield during lactation advance in both groups of 87.5%HF animals which was likely effected via the action of GH. Since, the circulating GH level significantly decreased in mid- and late lactating periods of 87.5%HF animals. The higher level of GH in early lactation of both 87.5%HF animals would exert its influence on mammary blood flow in this period. An increase in mammary blood flow would relate to increase milk yield by contributing to a partitioning of nutrients to the mammary gland (Davis and Collier, 1985; Peel and Bauman, 1987). However, changes of the levels of circulating GH in different stages of lactation had no effect on the plasma glucose concentration in all groups. These results conformed with the earlier reports (Smith et al., 1976; Hart et al., 1979). Therefore, an increase in milk yield in early lactating 87.5%HF animals relating to GH levels might not exert an effect directly on glucose available to the mammary gland for milk production, although glucose is known to be the major precursor of lactose synthesis and lactose secretion determines milk secretion as a whole water follows lactose (Linzell and Peaker, 1971).

The control mechanism for the mammary function during transition period from pregnancy to lactation probably differed between 50%HF and 87.5%HF animals. In the present result, the higher level of GH during late pregnancy comparing to early lactation in both groups of 50%HF appeared to have no effect on mammary blood flow. Since, the triggering of mammary blood flow and lactogenesis would involve a complex interaction of hormonal events. In late pregnancy the onset of copious lactation would be overcome by the inhibition action of progesterone. Falling concentrations of progesterone after parturition would release the mammary gland from this inhibition and the rate of milk synthesis and MBF rise to a value that becomes limited by new factors-perhaps the actions of GH. Plasma prolactin concentrations varied within narrow limits and did not differ between 50%HF and 87.5%HF animals either in late pregnancy or during lactation. These results for prolactin were not unexpected as many studies in cows have shown that, once lactation is established, milk secretion can be maintained in the presence of very low circulating levels of the hormone (Hart, 1973; Smith et al., 1974). Furthermore, it has been shown that circulating prolactin in cattle can be raised or lowered by day length (Bourne and Tucker, 1975) or ambient temperature (Wetteman and Tucker, 1974).

The levels of β-hydroxylbutyrate (BHB) in the plasma of crossbred animals did not show differences among groups of animals or different periods of experiment. Since all groups of crossbred animals received the similar ration of concentration, one would expect that production of butyric acid and therefore the plasma BHB concentration would be similar in all groups of crossbred animals. At the late lactation, all crossbred HF animals were suspected to be in positive energy balance, plasma BHB levels were still in similar range, and did not differ from that in the early stage of lactation. Therefore, appearance of the low plasma glucose levels in both groups of 87.5%HF animals compared to those of 50%HF animals in all periods of experiment could not reflect of BHB production. The concentrations of plasma lactate were similar in all groups of crossbred HF animals and

during late pregnancy and lactation. Since lactate is derived from propionate produced in the rumen, which should have been present in roughly equal amounts in all periods of studies in crossbred HF fed either hay or urea treated rice straw. This result suggests that during transition period from late pregnancy to the onset of lactation and during lactation advance, endocrine status does not influence the rate of utilization of this metabolite.

In conclusion, the present study has shown that there were no differences in the physiological performances in the same crossbred animals fed either hay or urea treated rice straw. The 87.5%HF animal has the genetic potential for a high milk yield and homeorhetic adaptation for mammary function differed from 50%HF animals during periods of lactation. Altering lactation persistency in 87.5%HF is regulated mainly by growth hormones chronically acting growth hormones through the period of lactation.

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