

รายงานวิจัยฉบับสมบูรณ์
การควบคุมการทำงานของต่อมน้ำนมในโคนมลูกผสม
: ผลของการให้อาหารหยาบต่างชนิดกัน

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Control of mammary function in crossbred dairy cattle : Effects of feeding different types of roughage in combination with concentrate.

Abstract

The present study was to evaluate the effect of prolonged feeding of urea treated rice straw compared with feeding of hay on various physiological changes related to the mechanism responsible for the control of the mammary function in dairy crossbred Holstein Friesians (HF).

Sixteen pregnant crossbred Holstein heifers (23-25 months of age) were selected for the experiments including eight animals of two breed types between Holstein Friesian (HF) and Red Sindhi, 87.5%HF and 50%HF, were selected for the experiment. They were divided into four groups of 4 animals each. Animals from the same breed type in each group were fed either 5% urea treated rice straw or pangola hay (*Digitaria decumbens*) as the source of roughage in combination with a similar concentrate throughout the experiments. Experiments were carried out to measure body fluids, hormonal levels, body glucose metabolism, mammary circulation and mammary utilization of substrates during late pregnancy (21 days prepartum) and three consecutive lactating periods of early lactation (30 days postpartum), mid-lactation (120 days postpartum) and late lactation (210 days postpartum).

It was found that the body weight of 87.5%HF animals fed either hay or urea treated rice straw significant decreased in the early stage of lactation when compared with the late pregnant period while body weight of 50%HF animals did not change significantly. The water turnover rate and blood volume as percentage of body weight were significantly higher in early lactating animals than in pregnant animals while total body water did not change in all groups. The glucose turnover rate as determined by [U-¹⁴C] and 3-[³H] glucose infusion increased significantly during early lactation as compared to late pregnancy in all groups, which coincided with increases of uptake, arteriovenous differences and extraction ratio for glucose across the udder. The glucose turnover rate was not different among breeds during the course of lactation. Recycling of glucose carbon as percentage of glucose turnover increased when lactation advanced in all groups. Udder blood flow was nearly three times higher in early lactating animals than in late pregnant animals in all groups. The udder blood flow and milk secretion of 87.5%HF animals were significantly higher in early lactation in comparison with those of 50%HF animals fed either hay or treated rice straw. Milk yield of both 87.5%HF animals rapid declined after peak from the early stage of lactation. The rate of decline of milk yield was proportional to the decrease in the rate of udder blood flow in 87.5%HF. More persistent lactations were apparent of both groups of 50%HF animals throughout periods of lactation. There were no significant differences in mean arterial plasma concentration and mammary extraction ratio of glucose, acetate, β -hydroxybutyrate, glycerol, triacylglycerol and free fatty acids among periods of lactation in each group. The net uptake by the udder for glucose, acetate and β -hydroxybutyrate markedly decreased when lactation advanced in both groups of

87.5%HF animals. The utilization of glucose by the mammary gland for synthesis of milk lactose and milk citrate decreased while it increased for synthesis of milk triacylglycerol during late lactation in both groups of 87.5%HF animals. Intracellular glucose 6-phosphate metabolized via the pentose phosphate pathway accounted for NADPH (reducing equivalent) of fatty acid synthesis in the udder was higher in 87.5%HF animals during mid-lactation. A large proportion of metabolism of glucose via the Embden Meyerhof pathway in the udder was more apparent in both group of 50%HF animals than those of 87.5%HF animals during early and mid-lactation while it markedly increased for both groups of 87.5%HF animals during late lactation. In the present study, the values of the plasma concentration of protein, urea and electrolytes (Na, K, Cl, Ca, Pi) in all groups were not affected by feeding on either hay or urea treated rice straw. No significant differences were apparent for protein, electrolytes, urea-N and allantoin-N concentrations in milk in all groups throughout all periods of lactation. Mean average values of the plasma hormone concentrations of thyroxine, cortisol and prolactin showed no significant changes during lactating periods in each group. The plasma hormone concentrations of triiodothyronine, insulin and glucagon were significantly higher in lactating periods than in pregnant period while the levels of plasma progesterone and estradiol markedly decreased during lactation in all groups.

It can be concluded from the results that no differences in physiological functions are apparent for prolonged feeding of urea treated rice straw or pangola hay as a sources of roughage in the same crossbred animals. The relatively stable levels of both milk urea and milk allantoin concentrations indicate a constant level of feeding and synthesis of microbial protein in the rumen during feeding on either hay or urea treated rice straw. The difference between breeds is found in the 87.5%HF animals which have a higher milk yield but a lower adjustment for the regulation of body fluid and mammary blood flow during lactation causing poorer lactation persistency in comparison to 50%HF animals. This results encourage further experiments to elucidate the physiological signals responsible for the rapid decline of the mammary blood flow and milk yield during the course of lactation in high yielding animals (87.5%HF). The different mechanisms for homeorhesis in relation to a persistent lactation should be further studied between 87.5%HF animals and 50%HF animals.

Keywords : Crossbred Holstein cattle; Body fluids; Glucose metabolism; Mammary metabolism; Urea treated rice straw

การควบคุมการทำงานของต่อมหน้ามในโคนมลูกผสม : ผลของการให้อาหารหยาดต่างชนิดร่วมกับอาหารข้น

บทคัดย่อ

การวิจัยในครั้งนี้เพื่อศึกษาผลของการเลี้ยงโคนมจากการให้ฟางปรุ้งแต่งด้วยยูเรีย 5% เปรียบเทียบกับหญ้าแห้งติดต่อกันเป็นเวลานานกับการเปลี่ยนแปลงทางสรีรวิทยาและกลไกควบคุมการทำงานของต่อมหน้ามในโคนมลูกผสม

โคนมลูกผสม 2 สายพันธุ์ ระหว่าง Holstein Friesian และ Red Sindhi อายุระหว่าง 23-25 เดือน จำนวน 16 ตัว ใช้ในการวิจัยโดยแบ่งออกเป็น 2 กลุ่ม ได้แก่ กลุ่มโคนมลูกผสมสายพันธุ์ 87.5% Holstein Friesian (87.5%HF) จำนวน 8 ตัว และกลุ่มโคนมลูกผสมสายพันธุ์ 50% Holstein Friesian (50%HF) จำนวน 8 ตัว โดยในแต่ละกลุ่มแบ่งสัตว์ทดลองในจำนวนเท่ากัน 4 ตัว โดยให้กินฟางปรุ้งแต่งด้วยยูเรีย 5% หรือหญ้าแห้งพันธุ์ Pangola (*Digitaria decumbens*) ร่วมกับอาหารข้นชนิดเดียวกันตลอดการทดลอง ทำการวัดปริมาณน้ำในร่างกาย ระดับฮอร์โมนต่าง ๆ กลูโคสเมแทบอลิซึม ระบบไหลเวียนเลือดภายในต่อมหน้ามและการใช้สารอาหารโดยต่อมหน้ามในขณะที่สัตว์ตั้งท้องระยะท้าย (21 วันก่อนคลอด) และในระยะให้นมซึ่งแบ่งเป็น 3 ช่วง ได้แก่ ช่วงให้นมระยะแรก (30 วันภายหลังคลอด) ช่วงให้นมระยะกลาง (120 วันภายหลังคลอด) และช่วงให้นมระยะท้าย (210 วันภายหลังคลอด)

ผลจากการศึกษาพบว่าโคนมลูกผสม 87.5%HF ที่เลี้ยงด้วยหญ้าแห้งและฟางปรุ้งแต่งพบน้ำหนักตัวลดลงอย่างมีนัยสำคัญในช่วงให้นมระยะแรกเมื่อเปรียบเทียบกับขณะตั้งท้องระยะท้าย โดยที่กลุ่มโคนมลูกผสม 50%HF ไม่พบการเปลี่ยนแปลง อัตราการหมุนเวียนของน้ำในร่างกาย และปริมาตรของเลือดภายในร่างกายเทียบกับน้ำหนักตัวพบว่าเพิ่มขึ้นในช่วงให้นมระยะแรกมากกว่าขณะตั้งท้องระยะท้ายในทุกกลุ่ม อัตราการหมุนเวียนของกลูโคสภายในร่างกายศึกษาโดยการฉีด [$U-^{14}C$] และ $3-[^3H]$ กลูโคส พบว่าจะเพิ่มขึ้นอย่างมีนัยสำคัญในช่วงให้นมระยะแรกเมื่อเปรียบเทียบกับขณะท้องในระยะท้าย การเปลี่ยนแปลงนี้พบร่วมไปกับการเพิ่มขึ้นของการใช้สารกลูโคสและสัดส่วนการใช้กลูโคสของต่อมหน้ามในสัตว์ทดลองทุกกลุ่ม อัตราการหมุนเวียนของกลูโคสภายในร่างกายในขณะที่ให้นมไม่พบความแตกต่างกันระหว่างโคนมลูกผสมต่างสายพันธุ์ แต่เปอร์เซ็นต์การหมุนเวียนกลับการใช้ธาตุคาร์บอนของกลูโคสจะเพิ่มขึ้นตามระยะเวลาการให้นม ปริมาณของเลือดที่ไหลสู่ต่อมหน้ามเพิ่มขึ้นประมาณ 3 เท่าในช่วงให้นมระยะแรกเมื่อเทียบกับขณะท้องระยะท้ายในโคนมทุกกลุ่ม อย่างไรก็ตามปริมาณเลือดที่ไหลสู่ต่อมหน้ามและการหลั่งน้ำนมในโคนมลูกผสม 87.5%HF จะมีปริมาณมากกว่ากลุ่มโคนมลูกผสม 50%HF ในช่วงให้นมระยะแรก การหลั่งน้ำนมและปริมาณเลือดสู่ต่อมหน้ามของโคนมลูกผสม 87.5%HF จะลดลงอย่างรวดเร็วหลังจากช่วงให้นมระยะแรก การหลั่งน้ำนมในกลุ่มโคนมลูกผสม 50%HF ค่อนข้างคงที่ตลอดระยะเวลาการให้นม การศึกษาค่าเฉลี่ยความเข้มข้นของสารต่าง ๆ ที่อยู่ภายในเลือด ได้แก่ กลูโคส อะซิเตรด เบต้า-ไฮดรอกซีบิวทาเรท กลีเซอรอล ไตรกลีเซอไรด์ และกรดไขมันอิสระ ไม่แตกต่างในช่วงต่าง ๆ ของระยะการให้นมของโคนมแต่ละกลุ่ม การใช้สารอาหารโดยต่อมหน้ามของ กลูโคส อะซิเตรด และ เบต้า-ไฮดรอกซีบิวทาเรท จะลดลงอย่างมีนัยสำคัญในช่วงระยะท้ายการให้นมในกลุ่มโคนมลูกผสม 87.5%HF การใช้กลูโคสภายในต่อมหน้ามเพื่อการสังเคราะห์แลคโตส และ ซิเตรทในน้ำนมจะลดลงอย่างชัดเจน แต่การสังเคราะห์ไตรกลีเซอไรด์ในน้ำนมจะเพิ่มขึ้นในช่วงระยะท้ายของการให้นมในกลุ่มโคนมลูกผสม 87.5%HF ที่กินทั้งหญ้าแห้งและฟางปรุ้งแต่ง การใช้กลูโคสภายในเซลล์ต่อมหน้ามในรูปกลูโคส 6-ฟอสเฟตผ่านวิถีเพนโตสให้ NADPH เพิ่มขึ้นเพื่อใช้ในการสังเคราะห์กรดไขมันในช่วงระยะกลางของการให้นมของกลุ่มโคนมลูกผสม 87.5%HF ขบวนการเมแทบอลิซึมของกลูโคสผ่านวิถี Embden Meyerhof ภายใน

ต่อมน้ำนม ในช่วงระยะต้นและระยะกลางของการให้นมจะพบอย่างเด่นชัดในกลุ่มโคนมลูกผสม 50%HF มากกว่ากลุ่มโคนมลูกผสม 87.5%HF แต่ขบวนการเมแทบอลิซึมดังกล่าวจะเพิ่มขึ้นอย่างชัดเจนในกลุ่มโคนมลูกผสม 87.5%HF เมื่อเข้าสู่ช่วงระยะท้ายของการให้นม ในการศึกษาครั้งนี้ไม่พบการเปลี่ยนแปลงความเข้มข้นของสารในพลาสมา ได้แก่ โปรตีน ยูเรีย อิเล็กโทรไลต์ (Na, K, Cl, Ca, และ Pi) ในโคนมลูกผสมทุกกลุ่มที่เลี้ยงด้วยฟางปรุงแต่งและหญ้าแห้ง ความเข้มข้นของสารอาหารในน้ำนม ได้แก่ โปรตีน ยูเรีย อิเล็กโทรไลต์ (Na, K, Cl, Ca, Pi) และ allantoin ไม่พบการเปลี่ยนแปลงในโคนมทุกกลุ่มตลอดช่วงระยะการให้นม ระดับความเข้มข้นของฮอร์โมนคอร์ติซอล โปรแลคติน ไม่พบการเปลี่ยนแปลงในช่วงระยะการให้นมในโคนมลูกผสมทุกกลุ่ม ระดับฮอร์โมนไทรไธโรซีน อินซูลิน กลูคากอน จะเพิ่มขึ้นในช่วงระยะการให้นม แต่ระดับฮอร์โมนโปรเจสเตอโรนและเอสตราไดออลจะลดลงอย่างชัดเจนในโคนมลูกผสมทุกกลุ่ม

จากผลการศึกษาสรุปได้ว่าผลของการให้กินอาหารหยาบต่างชนิด ฟางปรุงแต่ง และหญ้าแห้ง ไม่พบข้อแตกต่างของหน้าที่ทางสรีรวิทยาของร่างกายเมื่อเปรียบเทียบกับโคนมลูกผสมในสายพันธุ์เดียวกัน แต่ความแตกต่างระหว่างโคนมลูกผสมต่างสายพันธุ์พบว่าโคนมลูกผสม 87.5%HF ที่ให้น้ำนมสูงจะมีการปรับตัวในการควบคุมปริมาณของน้ำในร่างกายและอัตราการไหลของเลือดสู่ต่อมน้ำนมในช่วงระยะการให้นมต่ำกว่าโคนมลูกผสม 50%HF จากผลการศึกษาที่น่าจะมีการศึกษาเพิ่มเติมเพื่อหาสัญญาณทางสรีรวิทยาที่บ่งบอกถึงการลดลงของปริมาณเลือดที่ไหลสู่ต่อมน้ำนมและการหลั่งน้ำนมในกลุ่มโคนมลูกผสม 87.5%HF ซึ่งเป็นสายพันธุ์ให้น้ำนมสูง กลไกการปรับตัวแบบ homeorhesis ที่แตกต่างกันในส่วนที่เกี่ยวกับการคงไว้ของระดับของการหลั่งน้ำนมจำเป็นที่จะต้องศึกษา

วิจัยต่อไปเพื่อนำมาประยุกต์ใช้ในการแก้ปัญหาการให้น้ำนมน้อยในโคนมลูกผสมในประเทศไทย

คำหลัก : โคนมสายพันธุ์ลูกผสม; ปริมาณของเหลว; กลูโคสเมแทบอลิซึม; เมแทบอลิซึมในต่อมน้ำนม; ฟางปรุงแต่ง

Chapter I

General Introduction

The major problem for the Thai dairy practices are low milk yield and short lactation period of either pure exotic or crossbred dairy cattle. Many factors can affect milk production in dairy cattle in the tropics. For examples high environmental temperature, less genetic potential for milk production of indigenous cattle and inadequate supply for foraging during summer months. Selecting the types of suitable crossbreeding of indigenous and exotic cattle for the tropics is practiced. Milk secretion is a continuous process and requires a continuous supply of substrate for milk productions. Low levels of nutrition of the animal are still a major cause of such a low productivity. Several approaches have been utilized in attempting to improve dairy productivity by using different types of feeds, particularly different types of crop residues have been used to feed animals as a roughage. For example rice straw which has a low nutritive value, was treated with urea to help animals survive during period of scarcity (Jayasuriya and Perera, 1982, Promma et al., 1994). Until now there have been very few studies on the mechanism acting within the bodies of crossbred cattle concerning the profitability of efficient utilization of the treated rice straw for dairy production.

The aim of the work described in this report was to obtain quantitative data for improvement of milk production in crossbred dairy cattle feeding on different types of roughage. A study was performed in crossbred dairy cattle during prolonged feeding of urea treated rice straw compared with normal feeding of hay on various physiological responses in relation to the mechanism response for the control of milk secretion in both conscious pregnant and lactating crossbred Holstein cattle.

It is well known that mammary growth during pregnancy has been known to be a prerequisite for satisfactory lactation in all mammals. Thus in this study of late pregnant

crossbred dairy cattle were chosen as experimental animals those which would give some clue as to the nature of the developed mechanism of the mammary gland. During pregnancy maternal bodily functions are altered e.g. cardiac output and heart rate (Hanwell and Peaker, 1977). Mammary blood flow increases during pregnancy which may be related to growth of the gland during this period. However, little is known of the changes in the mammary circulation. Therefore, the initial work of the study was concerned with determining, the effects of feeding on different types of roughage in combination with the concentrate on cardiovascular function, water balance and mammary circulation in the late pregnant and early period of lactation in crossbred Holstein cattle. (Chapter III)

During pregnancy the mammary gland is competing with many other organs for nutrition to sustain growth. Although mammary secretory activity in dairy cattle is known to be initiated from pregnancy albeit it is at a low level. Little is known about the utilization of substrates in the mammary gland in pregnant crossbred cattle. By using a technique for measuring mammary blood flow and combining this with measurement of mammary arteriovenous difference of the concentration of substrates, the mammary uptake of substrates was measured. Glucose utilization by the mammary gland and related glucose turnover in the whole animals are also determined in pregnant cattle. The effects of prolonged feeding different types of roughage in combination with the concentrate on the glucose metabolism in late pregnancy and early lactation in crossbred dairy cattle were investigated (Chapter IV).

During lactation, many bodily functions are altered e.g. there is an increase in general circulation and body fluid. However, little is known about the regulation of water balance, general circulation and mammary circulation during lactation in different types of crossbred dairy cattle, although there is well known in the relation between mammary blood flow and milk yield. To obtain more evidence on this matter. The effects of feeding different types of roughage in combination with the concentrate on cardiovascular function, water balance and

mammary blood flow in different stages of lactation in crossbred dairy cattle were performed (Chapter V).

Glucose is essential for milk secretion which has been shown in the isolated perfused udder (Hardwick et al., 1961). Glucose is known to be important in lactose synthesis and providing the reducing equivalent required for the synthesis of fatty acid de novo in the mammary gland (Chaiyabutr et al., 1980). Very few data are available regarding the dynamics and the regulation of glucose metabolism in the mammary gland of different types of crossbred cattle. Therefore, an experiment to study effects of feeding on different types of roughage in combination with the concentrate on glucose metabolism during different stages of lactation in crossbred cattle, was carried out (Chapter VI). The study also adopts a more direct approach to study the utilization of glucose metabolism for synthesis of milk components in different metabolic pathways and NADPH requirement in the mammary gland of crossbred dairy cattle (Chapter X).

During lactation, milk production will depend on the intake of different quality and quantity of roughage available. The low intake and low contents of digestible energy may occur in crossbred dairy cattle during the feeding of different types of roughage. To ensure that the feeding system will allow maximum possible intake to meet requirements for rumen degradable nitrogen during the experimental period. The secretion of urea and allantoin in milk was performed to indicate nutritional status of animals during feeding different types of roughage in combination with the concentrate throughout the course of lactation (Chapter VII).

The role of endocrine regulation in initiation and maintenance of lactation has been known to occurs in many species. However, hormonal requirement among mammalian species differ considerably, for example, in rabbit prolactin alone can maintain lactation while in cows prolactin is not a rate limiting hormone in established lactation in place growth

hormone become relatively more important (Hart, 1973; Knight, 1993). Very few data are available in the study of circulating hormones in crossbred dairy cattle. The circulating concentrations of some hormones were investigated which would be expected to change and relate to the mechanism responsible for the control of milk secretion during prolonged feeding of urea treated rice straw in different types of crossbred Holstein cattle. Investigation of the plasma levels of thyroid hormone, prolactin, cortisol, growth hormone, insulin, progesterone and estradiol were determined in late pregnancy and different stages of lactation of crossbred animals (Chapter VIII).

It is known that the lactating mammary gland is dependent upon its blood supply to provide substrates at appropriate rates to sustain milk synthesis. The rate of substrates supplying to the mammary gland is determined by substrate concentration in the plasma and mammary blood flow. There is evidence that substrate supply to the mammary gland is often inadequate to maintain the maximum rate of milk synthesis. Little is known about the utilization of substrate in the mammary gland in crossbred cattle. By using techniques for measuring mammary blood flow and combining these with measurement of arteriovenous difference of the concentration of substrates, the mammary uptake of substrates was measured (Chapter IX).

The overall responses of prolonged feeding of urea treated rice straw as a roughage in both types of crossbred animals to the physiological changes in both extra-mammary tissue and intra-mammary tissue are discussed in Chapter XI.

Chapter II

Materials and Methods

Methodological details relevant for this report are presented or referred to in the separate publications (Chapter III to X). This chapter is limited to the experimental protocol.

Animals and management

Sixteen pregnant heifers, 23-25 months old and after approximately 150 days of gestation, were selected for the experiments. These animals comprised eight animals of two breed types, Holstein Friesian x Red Sindhi (50:50 = 50%HF) and Holstein Friesian x 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 tie stall type shed, solid floor with open sides. An ambient temperature in the shed were recorded daily and averaged weekly during experiment. The maximum temperature at noon was $34\pm1^{\circ}\text{C}$ and the minimum temperature at night was $26\pm1^{\circ}\text{C}$. The relative humidity was $68\pm12\%$. Pregnant animals were individually fed a concentrate and roughage at maintenance level for the body condition score at three until calving. Animals were fed their respective rations for at least 3 months before the first experimental periods.

In the lactating period, animals individually received an average of 3-5 kg/day of roughage in combination with the same concentrated mixture (7-10 kg/day) to maintain a moderate body condition score (2.5, scale = 1 to 5). The chemical composition of feeds is presented in Table 1. Each day, the food was given in equal portions at about 06.00 h and 17.00 h when animals were milked. Animals had free access to water and a lick block of

minerals throughout the experiment (the composition of minerals a in 1 kg lick block consisted of Na 136 gm, Ca 140 gm, P 60 gm, Mg 20 gm, K 25 gm, S 12 gm, Fe 1,000 mg, Zn 800 mg, Mn 350 mg, Cu 300 mg, Co 80 mg, I 245 mg and Se 20 mg). Animals were fed their respective rations throughout the experimental period.

Preparation of the urea treated rice straw

The urea treated rice straw was prepared by mixing the urea solution with dry straw (5 kg urea dissolved in 100 litres water per 100 kg dry rice 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 x 21 day system of urea treatment. After 21 days, the rice straw treated 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 before expected parturition but actually averaged 20-23 days prepartum (late pregnancy). Period 2 was designed to begin 30 days postpartum (early stage of lactating period). Period 3 began 120 days postpartum (middle stage of lactating period). Period 4 began 210 days postpartum (later stage of lactating period). Animals were fed the same ration through the completion of period 4. The dry matter intake of each animal was determined by measuring both the concentrate and roughage offered and refused each day. Animals were normally milked at around 0600 h and 1700 h by a milking machine and milk production was recorded daily. On the day of the experiment in each period, measurements of the total body water, water turnover, udder blood flow, arterial blood pressure, heart rate, plasma volume, blood volume and glucose turnover rate were carried out. The rate of milk

secretion was recorded by hand milking in the afternoon and the measurement of udder blood flow was carried out. Animals were weighed after collecting the milk sample.

On the day before the experiment began in all periods of experiments, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm) were inserted into either the left or right milk vein by using a intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, U.K.) under local anesthesia. This was done in standing animals for the measurement of mammary blood flow and for collection of venous blood. The tip of the catheter was positioned near the sigmoid flexure anterior to the point at which the vein leaves the udder. The other catheter was positioned downstream about 20 cm from the first one. The catheter for isotope infusion and dye injection was inserted into an ear vein under local anesthesia. All catheters were flushed with sterile heparinized normal saline and were left in place during the experiment.

Water intake, total body water and water turnover measurements

The estimated water intake values of animals in each period was predicted from the measured daily dry matter intake, daily sodium intake, milk production and minimum ambient temperature. An estimation was based on the following equation developed by Murphy et al. (1983) :

$$\text{Water intake (kg/day)} = 15.99 + 1.58 \times \text{DM intake (kg/day)} + 0.9 \times \text{milk production (kg/day)} + 0.05 \times \text{sodium intake (gm/day)} + 1.2 \times \text{minimum temp. (}^{\circ}\text{C)}$$

The water turnover rate and total body water were determined by tritiated water dilution techniques using a single dose injection of 3,000 μCi per animal of carrier free tritiated water (TOH) in normal saline and the equilibrium time was determined by taking blood samples for 3 days after the injection. Blood samples for measurements of water turnover rate, biological half-life of tritium, TOH space and total body water were performed.

Determination of plasma volume and blood volume

In the studies of plasma volume and blood volume, plasma volume was measured by dilution of Evan's blue (T-1824) dye (E. Merck Darmstadt, Germany). The injection of 20 ml of the dye (0.5 g/100 ml normal saline) into the ear vein catheter was followed by venous samples from the jugular vein taken at 30, 40 and 50 min. Dilution of dye at zero time was determined by extrapolation. Blood volume was calculated from the plasma volume and packed cell volume (PCV). Plasma osmolality was measured using the freezing point depression method (Advance Osmometer model 3, U.S.A.).

Determination of udder blood flow, arterial blood pressure and heart rate

Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion which was adapted from the method of measuring blood flow in the milk veins of lactating goats. A dye (T-1824) was dissolved in sterile normal saline and diluted to a concentration of 100mg/L. The solution was infused by a peristaltic pump (Gilson Medical Electronics, France), at a constant rate of 80 ml/min into the milk vein for 1-2 min. Before infusion, blood was drawn from downstream in the milk vein as a pre-infusion sample. About 10 seconds after starting infusion, 10 ml of blood was drawn from downstream in the milk vein at a constant rate into a heparinized tube. Two consecutive plasma samples were taken during dye infusion. Blood flow of half of the udder was calculated from plasma samples. In lactating cows, quarter milking showed that the yields of the two halves of the udder were similar. Udder blood flow was therefore calculated by doubling the flow measured in one milk vein. Packed cell volume was measured after centrifugation of the blood in a microcapillary tube. In the lactating period, milk yield was recorded by weight. Heart rate and blood pressure were measured directly from the intermediate auricular artery under local anesthesia by venipuncture with a #21 needle

connected with a pressure transducer and chart recorder (Universal Oscillograph, Harvard Apparatus Ltd. U.K.). Mammary resistance was calculated from udder blood flow and mean arterial blood pressure using the standard formula.

Glucose turnover measurements

Glucose kinetic studies of each animal in each experimental period were carried out. Briefly, at about 1100h a priming dose of radioactive glucose in 20 ml of sterile NSS containing 60 $\mu\text{Ci}(3\text{-}^3\text{H})$ glucose and 40 $\mu\text{Ci}(\text{U-}^{14}\text{C})$ glucose was administered intravenously via the ear vein catheter and followed by a constant infusion of 1 ml/min of sterile saline (0.9%) containing 2 $\mu\text{Ci}(\text{U-}^{14}\text{C})$ glucose and 3 $\mu\text{Ci}(3\text{-}^3\text{H})$ glucose for 4h (Peristaltic pump; EYLA Model 3). During the final 1 hour (1400-1500h) of infusion, three sets of blood samples were collected at 20 min. intervals. A venous blood sample was collected from the milk vein via a catheter while an arterial blood sample was collected from the coccygeal artery by venipuncture with a #21 needle. Blood samples in heparinized tubes were kept in crushed ice for chemical studies.

Determinations of other parameters

Plasma and milk samples from each experimental period were kept at -70°C and -20°C respectively for chemical, biochemical enzymes assay, plasma hormones and milk components measurements.

Statistical analysis

All the results were statistically analyzed by analysis of variance (ANOVA); the significant differences between groups and treatments were compared by Duncan's multiple range test. Values were compared among periods of lactation in each group using the paired

t-test. Mean values are presented as mean $\pm$ S.D. In some cases a further comparison of consistent changes was made using Wilcoxon's signed-rank test.

**The regulation of body fluids and mammary circulation during
late pregnancy and early lactation of crossbred Holstein cattle
feeding on different types of roughage**

By N. CHAIYABUTR, S. KOMOLVANICH, S. SAWANGKON, S. PREUKSAGORN and
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Introduction

The problems of dairying in the tropics are multifaceted including nutrition, the hot climate, genetics, disease and management. The genetic potential for milk of most indigenous cattle in the tropics is less than that of dairy cattle in temperate countries, while indigenous cattle have resistance to many tropical diseases and a high level of heat tolerance (NAKAMURA et al. 1987). Therefore, crossbreeding has been exploited as an efficient tool for blending the adaptability of tropical cattle with the high milking potentials of exotic breeds for increase milk production. One of the factors which limits milk production of tropical dairy cattle is an inadequate supply for foraging during the dry, summer months. They are fed mainly on crop residues such as rice straw which has a low nutritive value. An improvement in rice straw by treating with urea to help animals survive during period of scarcity has been reported (JAYASURIYA and PERERA 1982; PROMMA et al. 1994). Several approaches have been utilized in attempting to improve dairy productivity by using different types of crop residues and selecting the types of suitable crossbred cattle for the tropics and their management. However, there is less information concerning the profitability of efficient utilization of the crop residue for dairy production in crossbred cattle, although the crossbred animal has been found to differ from the pure breeds both in body composition (GHARAYBEH et al. 1968) and water turnover rate (MACFARLANE 1964). It is known that values of body water content have been used as an index of nutritional status of the animal (MACFARLANE and HOWARD 1970). Water turnover values have been shown to be related to the food and water intake and metabolism of the animal (MURPHY 1992). During pregnancy, many maternal bodily functions are altered, including body water and the cardiovascular system. Mammary growth during pregnancy has been known to be a

prerequisite for satisfactory lactation (HANWELL and PEAKER 1977). Little is known about these physiological performances in relation to these variables in different types of crossbred dairy cattle. The possibility of comparative examination on the efficient utilization of different types of roughage for milk production of different types of crossbred cattle needs to be explored. The study in animals of both pregnancy and lactation might throw some light on a useful index of adaptability for the efficient utilization of different types of roughage. Thus, the purpose of the present study was to evaluate the effect of prolonged feeding of urea treated rice straw compared with feeding of hay on water turnover rate, total body water, mammary circulation and other physiological parameters in relation to these variables during pregnancy and early stages of lactation in crossbred Holstein Friesians.

Materials and methods

Animals and management

Sixteen pregnant heifers, 23-25 months old and after approximately 150 days of gestation, were selected for the experiments. These animals comprised eight animals of two breed types, Holstein Friesian x Red Sindhi (50:50 = 50%HF) and Holstein Friesian x 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 tie stall type shed, solid floor with open sides. An ambient temperature in the shed were recorded daily and averaged weekly during experiment. The maximum temperature at noon was $34\pm 1^{\circ}\text{C}$ and the minimum temperature at night was $26\pm 1^{\circ}\text{C}$. The relative humidity was $68\pm 12\%$. Pregnant animals were individually fed a concentrate and roughage to maintain the body condition score at three until calving. In the lactation period, animals received roughage in combination with the same concentrated mixture (Table 1). Each day, half of the food was given at between 0700-0800 h and the other half between 1600-1700 h. Animals were adequately supplied with water and a lick block of minerals throughout the experiment (the composition of minerals in 1 kg. lick block consisted of Na 136 gm, Ca 140 gm, P 60 gm, Mg 20 gm, K 25 gm, S 12 gm, Fe 1,000 mg, Zn 800 mg, Mn 350 mg, Cu 300 mg, Co 80 mg, I 245 mg and Se 20 mg). 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 2 pits x 21 days of urea treatment. After 21 days the treated rice straw with 5% urea was offered to the animals.

Experimental procedures

Two consecutive periods of experiments were carried out in each group. Period 1 was designed to begin 21 days before expected parturition but actually averaged 20-23 days prepartum (late pregnancy). The calf birth weight was recorded immediately after parturition. Period 2 began 30 days postpartum (early stage of lactating period). Animals were fed the same ration before parturition through the completion of period 2. In both periods of experiments, dry matter intake of each animal was recorded daily and averaged weekly. In the lactating period, animals were normally milked at around 0600 h and 1700 h and milk productions were recorded daily. On the day of the experiment, measurements of the total body water, water turnover, udder blood flow, arterial blood pressure, heart rate, plasma volume and blood volume were carried out. The rate of milk secretion was recorded by hand milking in the afternoon and the measurement of udder blood flow was carried out. Animals were weighed after collecting the milk sample.

On the day before the experiment began in both periods of experiments, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm) were inserted into either the left or right milk vein by using a intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, U.K.) under local anesthesia. This was done in standing animals for the measurement of mammary blood flow and for collection of venous blood. The tip of the catheter was positioned near the sigmoid flexure anterior to the point at which the vein leaves the udder. The other catheter was positioned downstream about 20 cm from the first one. The catheter for isotope infusion and dye injection was inserted into an ear vein under local anesthesia. All catheters were flushed with sterile heparinized normal saline and were left in place during the experiment.

Water intake, total body water and water turnover measurements

The estimated water intake values of animals in both periods of experiments were predicted from measured daily dry matter intake, daily sodium intake, milk production and minimum ambient temperature. An estimation was based on the following equation developed by MURPHY et al. (1983).

$$\text{Water intake (kg/day)} = 15.99 + 1.58 \times \text{DM intake (kg/day)} + 0.9 \times \text{milk production (kg/day)} \\ + 0.05 \times \text{sodium intake (gm/day)} + 1.2 \times \text{minimum temp. (}^{\circ}\text{C)}$$

The water turnover rate and total body water were determined by tritiated water dilution techniques. For this, the animals were injected intravenously via the ear vein with carrier free tritiated water (TOH) in normal saline at a single dose of 3000 μCi per animal and the equilibration time was determined by taking blood samples for 3 days after injection. Blood samples for water turnover measurements were collected 8, 20, 26, 32, 44, 50, 56 and 68h subsequent to the injection. Preliminary experiments showed that tritiated water was uniformly distributed in the body water 5h after dosing in large animals (CHAIYABUTR et al. 1987). The preparation for sample counting was achieved by the internal standardization technique as described by VAUGHAN and BOLING (1961). The corrected activity of the samples, in d.p.m., were plotted on semilogarithmic paper against time, in hours after dosing, and the extrapolated activity at theoretical zero time of complete mixing of radioisotope was used in determining the TOH space by the following equation:

$$\text{TOH space (L)} = \frac{\text{standard counts (dis/min)} \times \text{dose (ml)}}{\text{sample counts at zero time (dis/min)}}$$

The biological half-life of tritium ($T_{1/2}$) was determined from the slope of the linear regression line obtained from a plot on semilogarithmic paper of the activity of the samples taken over the period of 3 days against time. Then the water turnover rate was calculated as the product of the TOH space and k , where $k = 0.693 / (\text{biological half-life})$ (HOLLEMAN et al., 1982).

The total body water was calculated by using the corrected factor $(1 - \text{fraction of plasma solids}) \times \text{TOH space}$.

Determination of plasma volume and plasma solids concentration

Plasma volume was measured by dilution of Evan's blue (T-1824) dye. The injection of 20 ml of the dye (0.5 g/100 ml normal saline) into the ear vein catheter was followed by venous samples from the jugular vein taken at 30, 40 and 50 min. Dilution of dye at zero time was determined by extrapolation. Blood volume was calculated from the plasma volume and packed cell volume (PCV) (CHAIYABUTR et al. 1980). Plasma osmolality was measured using the freezing point depression method (Advance Osmometer model 3, U.S.A.). The plasma solids concentration was determined by a refractometer.

Determination of udder blood flow, arterial blood pressure and heart rate

Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion which was adapted from the method of measuring blood flow in the milk veins of lactating goats (CHAIYABUTR et al. 1980). A dye (T-1824) was dissolved in sterile normal saline and diluted to a concentration of 100mg/L. The solution was infused by a peristaltic pump (Gilson Medical Electronics, France), at a constant rate of 80 ml/min into the milk vein for 1-2 min. Results of pilot experiments showed that these infusion rates could produce adequate mixing of dye with blood within 4 min. Although the concentration of dye recirculating in arterial blood might increase during the infusion, it was always substantially less than that in the milk vein because of large blood volume in cattle. Before infusion, blood was drawn from downstream in the milk vein as a pre-infusion sample. About 10 seconds after starting infusion, 10 ml of blood was drawn from downstream in the milk vein at a constant rate into a heparinized tube. Two consecutive plasma samples were taken during dye infusion. Blood flow of half of the udder was calculated from plasma samples using the equation derived by THOMPSON and THOMSON (1977). In lactating cows, quarter milking showed that the yields of the two halves of the udder were similar. Udder blood flow was therefore calculated by doubling the flow measured in one milk vein (BICKERSTAFFE et al. 1974). Packed cell volume was measured after centrifugation of the blood in a microcapillary tube. In the lactating period, milk yield was recorded by weight. Heart rate and blood pressure were measured directly from the intermediate auricular artery under local anesthesia by venipuncture with a #21 needle connected with a pressure transducer and chart recorder (Universal Oscillograph, Harvard Apparatus Ltd. U.K.). Mammary resistance was calculated from udder blood flow and mean arterial blood pressure using the standard formula (BURTON 1965).

Statistical analysis

The paired t-test was used to estimate the statistical significance of differences between value obtained from the same animals during pregnant and lactating periods. In some cases a further comparison of consistent changes was made using Wilcoxon's signed-rank test (CAMPBELL 1967). The unpaired t-test was used for statistical analysis in different groups of animals.

Results

Dietary dry matter intake, water intake, milk production and body weight (Table 2)

The data in Table 2 show that the body weight of 87.5%HF animals fed either hay or urea treated rice straw significantly decreased in the early stage of lactation when compared with the late pregnant period ($P<0.05$), while the body weight of 50%HF animals did not significantly change. Live weights of both groups of 87.5%HF animals were significantly higher than those of 50%HF animals. The total daily dry matter intake was significantly higher in lactating period than in pregnant period in all groups which coincided with a marked increase in water intake. Average calf birth weight for both groups of 87.5%HF was slightly higher than those for 50%HF animals. Milk production in early stage of lactation was higher for 87.5%HF than those of 50%HF fed either hay or urea treated rice straw. The ratio of dry matter intake to milk production for 87.5%HF animals fed either hay or urea treated rice straw was significantly lower than that of 50%HF animals.

Water turnover rate and total body water (Table 3)

An average water turnover rate and the water turnover rate per body fat free wet weight ($\text{kg}^{0.82}$) was significantly higher in lactating animals than in pregnant animals in all groups. There were no significant differences in water turnover rate among groups of animals in regard to either the pregnant period or lactating period. Pregnant animals were found to have values of the half-life of tritium in a similar range for all groups of animals. During the lactating period, the half-life of tritium was significantly lower than in pregnant periods for all groups of animals. The TOH space and total body water vary with the size of animals. Since each pregnant animal in the experiment acted as its own control, in the lactating period significant reductions of TOH space and total body water were noted ($P<0.05$) in both groups of 87.5%HF fed either hay or treated rice straw. No significant differences in TOH space and total body water were apparent between the pregnant period and lactating period of 50%HF fed either hay or treated rice straw. However, in each group, values of TOH space or total body water as a percent of body weight showed no significant differences between pregnant and lactating periods.

Plasma volume, blood volume, plasma osmolality and packed cell volume (Table 4)

For both 87.5%HF and 50%HF animals fed either hay or treated rice straw, the values of plasma volume and blood volume as absolute values or as percentages of body weight increased significantly in the lactating period when compared with the pregnant period. The plasma osmolality was not apparently affected in animals fed with different types of

roughage when compared among groups. The packed cell volume significantly decreased during lactating period for both groups of 87.5%HF animals while it did not significantly change in 50%HF animals. Packed cell volume of 50%HF animals was significantly higher than that of 87.5%HF animals fed either hay or treated rice straw.

Mammary and general circulation (Table 5)

There were no significant differences in both heart rate and mean arterial blood pressure between the periods of late pregnancy and early stage of lactation in all groups of animals fed either hay or treated rice straw. Udder plasma flow and udder blood flow were nearly three times higher in lactating animals than those in late pregnant animals while mammary resistance was significantly lower in the early stage of lactation for all groups. Comparing 50%HF and 87.5%HF fed either hay or treated rice straw, udder plasma flow and udder blood flow were significantly higher while mammary resistance was significantly lower in 87.5%HF animals. Mean average values of udder blood flow, plasma flow and mammary resistance for lactating periods showed no significant differences among groups of animals. Milk secretion of 87.5%HF animals fed either hay or treated rice straw was significantly higher than that of 50%HF animals. The ratio of udder blood flow to milk secretion ratio was in a similar range for all groups of experimental animals.

Discussion

The chemical compositions of feeds used in this experiment are shown in Table 1. It is clear from the data that crude protein content increased nearly 2 folds and N.D.F. decreased in urea (ammonia) treated rice straw as compared to hay. This value was not very different from the value reported by BADURDEEN et al. (1994) for urea-treated rice straw. It indicates that the treatment of rice straw with 5% urea increased the crude protein level in it when compared with 4.9% crude protein by dry rice straw (HUQUE and CHOWDHURY 1997). In the present results both 50%HF and 87.5%HF animals feeding on urea treated rice straw did not show any under nutritional effects in comparison to those fed with hay either pregnancy or lactation. It is probably that urea treatment of rice straw can increase nitrogen availability and optimizing the rumen environment while this availability in the rumen is limited in dry rice straw (HUQUE and CHOWDHURY 1997).

It is evident from the present results that the distribution of body fluids and mammary circulation of both 50%HF and 87.5%HF animals vary during pregnancy and lactation. The

present results indicate that the live weight of 50%HF were significantly lower than those of 87.5%HF animal both the pregnant and lactating period. Such differences could be attributable to disparities in breed. However, the body weight of both crossbred animals fed either hay or treated rice straw decreased in the early lactating period in comparison to the prepartum period. Most of the weight loss might not only be due to expulsion of the calf and fluids since calf birth weights among groups were not significantly different. It is noteworthy that the degree of reduction of body weight during early lactating period of 87.5%HF was greater than that of 50%HF. This reduction coincided with decreases in both TOH space and total body water in 87.5%HF, which it were not apparent in 50%HF animals fed either hay or treated rice straw. The differences in these results between 87.5%HF and 50%HF without reduction in feed intake may be explained, in part, on the basis of the difference in breed of animals used. An increase in DM intake in lactating period for all groups was mainly because of an increase in consumption of concentrate to compensate the energy requirements for milk production. The ratio of dry matter intake to milk production was lower in both groups of 87.5%HF animals as compared to 50%HF animals. It indicates that the energy output in milk and for maintenance for 87.5%HF animals was greater than the energy consumed in the food and body tissue was being mobilized to make up this deficit. The 50%HF animals were approximately in energy equilibrium, there being no loss or gain of weight. The high blood level closing to exotic *bos taurus* breed of 87.5%HF cattle may lead to poor adjustment to the tropical environment, while yielding high milk production. These would, in turn, lead to lower TOH space and total body water, not only because of water loss through milk but also water loss in the process of evaporative cooling to the surrounding environment. The decrease in total body water of 87.5%HF during early stages of lactating period occurred rather rapidly which may be attributed to a relatively lower efficiency in the water retention mechanism although the estimated water intake was slightly higher in 87.5%HF animals. A low water content may be related to the poor adaptation of 87.5%HF to this tropical environment (SPRINGELL 1968). In comparison to 87.5%HF, no significant differences of total body water of both groups of 50%HF were noted between pregnant and lactating periods. These results show an important physiological significance in 50%HF animals. A greater water reserve would not only provide a greater reservoir of soluble metabolites for biosynthesis of milk but it is useful in slowing down the elevation in body temperature of this breed during lactation in hot conditions (NAKAMURA et al. 1987). The pregnant 87.5%HF showed a lower water turnover rate while having a higher total body

water in comparison with the lactating 87.5%HF animal. It indicates that 87.5%HF probably do not require greater amounts of water, while still retaining more water in their bodies in the late pregnant period. In the present study, the signs of udder edema were observed to a high degree in the late pregnant 87.5%HF more than that of 50%HF animals. It is consistent with the report by VESTWEBER and AL-ANI (1984) that more udder edema was apparent in late pregnant HF animals.

The present results showed that average values of $T_{1/2}$ for body water of both crossbred cattle were similar to the values reported for *bos taurus* cattle (BLACK et al. 1964). However, the biological half-life of tritium was significantly shorter while the water turnover rate was clearly higher in the lactating period than in the prepartum period in the same breed of both crossbred animals. This elevation was due to the fact that the process of lactation requires more water. More loss of water secretion in milk which is generally known about 87%, would account for these phenomena. In the present study, animals were housed in the same shed under the same environment. Thus, a rise in the water turnover rate of both types of crossbred cattle was not enhanced by environmental conditions, although marked differences of water turnover rate and half-life of body water in animals occurred during winter and summer (RANJHAN et al. 1982).

The values of plasma volume and blood volume, either as basis values or as a percent of body weight were higher in the lactating period than in the late pregnant period in both types of crossbred cattle. The plasma volume and blood volume as estimated by the dye dilution technique, were not proportional to a higher body weight in pregnant cattle. Because Evan's blue dye does not cross the placenta, measurements of the plasma volume in the pregnant period are not inflated by fetal circulation (REYNOLDS 1953). Therefore, the difference in blood volume between the late pregnant and lactating periods might be a reflection of the different state of water balance. In 87.5%HF lactating animals feeding on either hay or treated rice straw, increased blood flow to the udder may allow the plasma volume to remain nearly constant despite the loss of body weight and total body water. It might indicate that different control mechanisms are at play in the maintenance of body fluids in the lactating period for 87.5%HF and 50%HF animals.

The studies on mammary circulation in relation to the general circulation of crossbred cattle feeding on different types of roughage are the first reported in which variables have been measured in the same animals at stages of late pregnancy and early lactation. The data in the present studies indicate that the pattern of mammary growth varies during pregnancy

and lactation for both 87.5%HF and 50%HF. The mean changes in mammary resistance relative to the changes in mammary blood flow in different periods of studies are shown. Both of these are indices of the extent of local changes, since blood pressure did not change significantly. It is clear that the degree of local vasoconstriction was greater during pregnancy and less in lactation for both types of crossbred cattle. In late pregnancy, the mammary circulation of 50%HF appeared to be less than that of 87.5%HF animals. The difference in mammary circulation could be due to variations in the development of mammary blood vessels and mammary cells. The secretory activity of the mammary cells has increased, albeit at a slow rate in the late pregnant 50%HF cattle. If the hypothesis that mammary blood flow is largely controlled by local vasodilators produced by the active cells (HANWELL and PEAKER 1977; LACASSE et al. 1996) is accepted, then clearly a slow rate in cellular activity would arise in the resistance of the vascular bed and a lower in mammary blood flow in 50%HF in comparison to 87.5%HF animals. Mammary blood flow is a major determinant of the rate of substrate supply for milk synthesis. Whether the marked differences in the mammary circulation between 50%HF and 87.5%HF crossbred animals at the stage of late pregnancy have consequent effects on the lactating length relating to the mammary metabolism and milk secretion should be further investigated.

Summary

In this study, sixteen pregnant crossbred Holstein Friesian (HF) heifers, consisting of eight animals of two breed types, 87.5%HF and 50%HF were selected and each breed was randomly allocated into two groups. Each group, consisting four animals from the same breed, was fed either 5% urea treated rice straw or pangola (*Digitaria decumbens*) hay as the source of roughage. Animals from each breed type were maintained on the ration in combination with a similar concentrate throughout the experiments. Two consecutive periods of experiments were carried out in each groups. Period 1 was designed to begin 21 days before parturition (late pregnancy) and period 2 began 30 days postpartum (early stage of lactating period). The body weight of 87.5%HF animals fed either hay or urea treated rice straw significantly decreased in the early stage of lactation when compared with the late pregnant period. The body weight of 50%HF animals did not significantly change. The water turnover rate was significantly higher in lactating animals than in pregnant animals in all groups. During the lactating period the half-life of tritium was significantly lower than

during pregnant period. There were no significant differences in the water turnover rate among groups of animals in regard to either the pregnant period or lactating period. In the lactating period the significant reductions of total body water were noted in both groups of 87.5%HF fed either hay or treated rice straw, while this was not apparent for 50%HF animals. Plasma volume and blood volume increased significantly in the lactating period when compared with the pregnant period in all groups. The packed cell volume significantly decreased in the lactating period of both groups of 87.5%HF animals while it did not significantly change in 50%HF animals. The packed cell volume of 50%HF animals was significantly higher than that of 87.5%HF animals fed either hay or treated rice straw. There were no significant differences in heart rate, arterial blood pressure and plasma osmolality between the periods of late pregnancy and early stage of lactation in all groups of animals. Udder blood flow was nearly three times higher in lactating animals than in late pregnant animals in all groups. The udder blood flow of 87.5%HF was significantly higher while mammary resistance was significantly lower than in 50%HF animals. Milk secretion of 87.5%HF animals fed either hay or treated rice straw was significantly higher than that of 50%HF animals. The ratio of udder blood flow to milk secretion ratio was in the similar range for all groups of experiment animals. The ratio of DM intake to milk production for 87.5%HF animals fed either hay or urea treated rice straw was significantly lower than that of 50%HF animals. From these results it can be concluded that no differences in physiological functions are apparent for prolonged feeding of urea treated rice straw or pangola hay in the same crossbred pregnant and lactating animals. The difference between breeds is found in 87.5%HF animals which has a higher milk yield but a lower adjustment for the regulation of body fluids during pregnancy and lactation in comparison to 50%HF animals.

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Table 1 Chemical composition of feed components (% on dry matter basis).

Particulars	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 formulation : ingredient fresh weight (kg/100 kg) 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.

Table 2 Dietary dry matter intake (DM), water intake, milk production and body weight in late pregnancy and early stage of lactation of crossbred Holstein fed with hay and urea treated rice straw (N = 4 in each group)

	Hay + Concentrate				Urea treated rice straw + Concentrate			
	HF:RS (87.5:12.5)		HF:RS (50:50)		HF:RS (87.5:12.5)		HF:RS (50:50)	
	Pregnancy	Lactation	Pregnancy	Lactation	Pregnancy	Lactation	Pregnancy	Lactation
DM intake (kg/d):								
Concentrate	3.74±0.82 ^a	7.05±1.74 [*]	4.54±0.75 ^{ab}	8.41±0.30 ^{**}	4.62±0.29 ^{ab}	7.58±1.55 ^{**}	5.07±0.39 ^b	8.25±0.45 [*]
Hay	4.79±0.74	4.00±1.28	4.75±0.52	3.47±0.49	-	-	-	-
Urea treated rice straw	-	-	-	-	2.53±0.38	2.47±0.24	2.75±0.13	2.75±0.22
Total	8.53±0.12 ^a	11.05±0.47 ^{**}	9.29±0.37 ^b	11.87±0.61 ^{**}	7.15±0.45 ^c	10.29±1.07 ^{**}	7.83±0.32 ^d	11.00±0.56 ^{**}
Water intake (kg/d)	62.38±1.44 ^a	86.15±4.44 ^{***}	64.92±0.84 ^b	80.39±1.96 ^{***}	61.39±0.79 ^a	82.62±6.44 ^{***}	62.67±0.67 ^a	80.68±1.65 ^{***}
Body weight (kg.)	388±33 ^a	360±34 ^{*α}	329±35 ^b	321±37 ^{αβ}	379±14 ^a	341±21 ^{*α}	324±6 ^b	309±14 ^β
Calf birth weight (kg)	30.5±3.4	-	26.4±2.9	-	28.4±1.3	-	27.4±3.0	-
Milk yield (kg./d)	-	19.76±4.47 ^α	-	10.98±1.17 ^β	-	16.51±5.92 ^{αβ}	-	12.91±1.58 ^β
DM intake/milk yield	-	0.55±0.11 ^α	-	1.08±0.08 ^γ	-	0.66±0.18 ^{αβ}	-	0.86±0.12 ^{βγ}

P-values by paired t-test: * P<0.05, ** P<0.01, *** P<0.001 with respect to the period of pregnancy in each group.

^{a-d} Mean values with different superscripts within the same row in regard to the pregnancy effects among groups are significantly different at P<0.05.

^{α-γ} Mean values with different superscripts within the same row in regard to the lactation effects among groups are significantly different at P<0.05.

Table 3 Changes in water turnover rate and total body water in late pregnancy and early stage of lactation of crossbred Holstein fed with hay and urea treated rice straw (N = 4 in each group)

	Hay + Concentrate				Urea treated rice straw + Concentrate			
	HF:RS (87.5:12.5)		HF:RS (50:50)		HF:RS (87.5:12.5)		HF:RS (50:50)	
	Pregnancy	Lactation	Pregnancy	Lactation	Pregnancy	Lactation	Pregnancy	Lactation
Water turnover rate:								
(l/d)	44.7±10.5	64.8±16.5	40.4±9.6	69.1±5.0 ^{**}	42.0±9.1	48.4±7.7 ^(†)	34.4±8.9	56.8±6.9 [*]
(l/100 kg.b.w./d)	11.6±3.0	18.2±4.9 ^{*(†)αβ}	12.2±2.0	21.7±1.9 ^{**α}	11.1±2.5	14.1±1.5 ^{*β}	10.6±2.9	18.4±1.8 ^{*α}
(ml/kg ^{0.82} /d)	338.5±86.0	522.6±140.3 ^{*(†)α}	347.6±60.2	611.2±45.3 ^{**α}	324.3±72.7	404.3±46.2 ^{*β}	301.6±80.1	514.0±52.3 ^{*α}
T _W (d) :	4.6±1.0	2.9±0.7 ^{*(†)}	4.5±0.8	2.5±0.2 [*]	4.7±7.0	3.4±0.3 [*]	4.8±1.2	2.8±0.3 [*]
TOH space :								
(l)	289.9±23.2 ^a	259.3±17.3 ^{*(†)}	257.0±20.8 ^a	253.1±17.6	278.2±24.4 ^a	234.9±18.9 ^{**}	227.1±13.5 ^b	229.9±12.5
(l/100 kg.b.w.)	74.8±4.8	72.2±3.7 ^{αβ}	78.4±4.5	79.2±4.8 ^α	73.5±6.9	68.8±2.1 ^β	70.1±3.8	74.5±1.9 ^α
Total body water :								
(l)	267.0±21.6 ^a	227.8±15.9 [*]	236.5±19.5 ^{ab}	232.6±15.9	256.4±21.3 ^a	214.4±17.0 ^{**}	210.6±12.2 ^b	211.2±10.8
(l/100 kg.b.w.)	68.9±4.3 ^{ab}	65.3±2.7 ^α	72.6±4.3 ^a	72.8±4.4 ^β	67.8±6.1 ^{ab}	62.8±2.0 ^α	65.0±3.4 ^b	68.4±1.4 ^{αβ}

P-values by paired t-test except where marked by ^(†) in which case by Wilcoxon signed-rank test: * P<0.05, ** P<0.01, with respect to the period of pregnancy in each group.

^{a-b} Mean values with different superscripts within the same row in regard to the pregnancy effects among groups are significantly different at P<0.05.

^{α-β} Mean values with different superscripts within the same row in regard to the lactation effects among groups are significantly different at P<0.05.

Table 4 Changes in plasma volume, blood volume, plasma osmolality and packed cell volume in late pregnancy and early stage of lactation of crossbred Holstein fed with hay and urea treated rice straw (N = 4 in each group)

	Hay + Concentrate				Urea treated rice straw + Concentrate			
	HF:RS (87.5:12.5)		HF:RS (50:50)		HF:RS (87.5:12.5)		HF:RS (50:50)	
	Pregnancy	Lactation	Pregnancy	Lactation	Pregnancy	Lactation	Pregnancy	Lactation
Plasma volume (l)	19.87±2.92 ^a	21.20±3.28	15.84±1.32 ^b	19.26±1.92 ^{**}	19.45±0.94 ^a	19.69±1.33	16.19±0.22 ^b	18.58±1.37 [*]
(l/100 kg.b.w.)	5.12±0.62	5.87±0.41 ^{*(†)}	4.83±0.20	6.02±0.36 ^{**}	5.14±0.38	5.80±0.65 ^{*(†)}	4.99±0.69	6.03±0.62 [*]
Blood volume (l)	27.54±3.78 ^{ab}	28.71±4.29	22.98±2.50 ^b	26.45±2.62 [*]	27.10±1.89 ^a	26.72±1.78	24.23±0.97 ^b	26.33±2.69
(l/100 kg.b.w.)	7.10±0.76	7.94±0.46	7.00±0.13	8.28±0.84 [*]	7.17±0.71	7.87±0.94	7.48±0.35	8.56±1.15
Plasma Osmolality (mOsm/kgH ₂ O)	279±3	279±6	280±7	287±4	285±6	282±2	293±7	288±1
Packed cell volume (%)	28±1 ^a	26±2 ^{*α}	31±2 ^b	30±2 ^β	28±3 ^a	25±2 ^{*α}	33±2 ^b	29±3 ^β

P-values by paired t-test except where marked by ^(†) in which case by Wilcoxon signed-rank test: * P<0.05, ** P<0.01, with respect to the period of pregnancy in each group.

^{a-b} Mean values with different superscripts within the same row in regard to the pregnancy effects among groups are significantly different at P<0.05.

^{α-β} Mean values with different superscripts within the same row in regard to the lactation effects among groups are significantly different at P<0.05.

Table 5 Changes in the mammary circulation and general circulation in late pregnancy and early stage of lactation of crossbred Holstein fed with hay and urea treated rice straw (N = 4 in each group)

	Hay + Concentrate				Urea treated rice straw + Concentrate			
	HF:RS (87.5:12.5)		HF:RS (50:50)		HF:RS (87.5:12.5)		HF:RS (50:50)	
	Pregnancy	Lactation	Pregnancy	Lactation	Pregnancy	Lactation	Pregnancy	Lactation
Heart rate (beats/min)	91±11	90±13	91±3	82±14	99±8	87±6	84±15	85±17
Mean arterial blood pressure (mmHg)	79.0±20.0	91.0±17.4	61.7±8.5	71.7±11.5	79.6±19.3	72.0±12.3	62.5±5.9	75.8±16.7
Udder plasma flow (ml/min)	1938±204 ^a	5014±1623 [*]	718±246 ^b	2825±476 ^{**}	1368±275 ^c	3428±875 ^{**}	968±289 ^{cb}	3114±401 ^{***}
Udder blood flow (ml/min)	2696±265 ^a	6845±2266 [*]	1034±355 ^b	3887±543 ^{**}	1876±363 ^c	4482±1152 ^{**}	1396±406 ^{cb}	4314±575 ^{***}
Mammary resistance (dyne.sec/cm ⁵)	2397±796 ^a	1121±285 [*]	7772±3522 ^b	1494±267 [*]	3381±371 ^a	1250±319 ^{**}	3873±1494 ^{ab}	1455±520 ^{**}
Milk yield (ml/min)	-	13.73±3.11 ^α	-	7.62±0.81 ^β	-	11.46±4.11 ^{αβ}	-	8.96±1.09 ^β
Udder blood flow/milk yield ratio	-	489±61 ^{αβ}	-	508±19 ^α	-	401±51 ^β	-	480±23 ^α

P-values by paired t-test: * P<0.05, ** P<0.01, ***P<0.001 with respect to the period of pregnancy in each group.

^{a-c} Mean values with different superscripts within the same row in regard to the pregnancy effects among groups are significantly different at P<0.05.

^{α-β} Mean values with different superscripts within the same row in regard to the lactation effects among groups are significantly different at P<0.05.

Glucose metabolism in vivo in crossbred Holstein cattle feeding on different types of roughage during late pregnancy and early lactation

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ABSTRACT. An experiment was carried out to study the glucose kinetics of crossbred Holstein cattle feeding on either hay or 5% urea treated rice straw during late pregnancy (21 days prepartum) and early lactation (30 days postpartum). Sixteen pregnant heifers (23-25 months of age) were selected for the experiments, including eight animals of two breed types, Holstein Friesian x Red Sindhi (50:50 = 50%HF) and Holstein Friesian x Red Sindhi (87.5:12.5 = 87.5%HF). They were divided into four groups of 4 animals each. 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. The glucose turnover rate in both types of crossbred Holstein cattle was determined using a continuous infusion of (U-¹⁴C) and (3-³H) glucose during late pregnancy and early lactation. Total glucose entry and utilization rates increased significantly during lactation for all groups. Recycling of glucose-C was approximately 20% in both crossbred cattle fed either hay or urea treated rice straw and was unaffected by the stage of late pregnancy or early lactation. Comparing 50%HF and 87.5%HF animals, arterial plasma glucose concentrations were slightly higher during pregnant periods but significantly higher in lactating periods in 50%HF animals. The ratio of specific radioactivity of arterial blood bicarbonate relative to that of arterial blood glucose-¹⁴C in the lactating period significantly decreased in 50%HF animals fed either urea treated rice straw or hay. An increase in udder blood flow during early lactation was significantly higher in 87.5%HF animals than in 50%HF animals. The uptake, arteriovenous differences and extraction ratio for glucose across the udder significantly increased in the lactating period for all crossbred animals. Glucose uptake by the udder of 87.5%HF animals accounted for 65% of the total glucose turnover rate compared to a value of 46% in the lactating 50%HF animals. It can be concluded that both crossbred cattle fed either urea treated rice straw or hay exhibit the same body glucose turnover rate. The 87.5%HF animal has the genetic potential for a high milk yield and has high body and udder glucose metabolisms compared with 50%HF animals.

KEY WORDS. Crossbred Holstein cattle, early lactation, glucose turnover rate, late pregnancy, udder glucose uptake, urea treated rice straw

INTRODUCTION

Most indigenous cattle (*Bos indicus*) in tropical countries have a low genetic potential for milk production but are well adapted to the high ambient temperature (21). Exotic *Bos taurus* breeds have higher milk production, but they have inherent disadvantageous traits (19). Crossbreeding has therefore been exploited as an efficient tool for blending the adaptability of tropical cattle with the high milking potential of exotic breeds (*Bos taurus*) for increased milk production. Even for crossbred dairy cattle (*Bos taurus* x *Bos indicus*) used for selective purposes in the tropics, 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, although some information on the physiological performance of different types of crossbred dairy cattle has been reported (7,21).

One of the problems which may limit milk production of dairy cattle in the tropics 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. To overcome the livestock feed problem, several chemicals such as urea have been used to improve the feeding value of low quality roughage (14). An improvement in rice straw by treating with urea to help animals survive during periods of scarcity has been reported (12,22). However, there is less information on the responses of bodily functions in animals fed with urea treated rice straw as roughage. Glucose is an important intermediary of metabolism in general and is particularly important for foetal growth and lactation. The provision of glucose in a ruminant is entirely by endogenous synthesis from non-carbohydrate sources by gluconeogenesis (4). During pregnancy and lactation the requirement for glucose increases considerably; in the pregnant animal the foetus and uterus utilize glucose as a major energy source (17) and in lactation large quantities of glucose are removed by the mammary glands for lactose synthesis (1). The mechanisms that facilitate the entry of glucose into the acinar cells have been directly investigated in the rat mammary tissue (6,23). However, few data are available on the glucose metabolism in crossbred dairy cattle during feeding with different types of roughage. Knowledge and understanding of glucose metabolism in whole animals and in the mammary glands of crossbred animals may help to identify animals for potential high milk yields and may assist in improving crossbred dairy cattle management. Therefore, the present experiment was conducted to obtain information on whether the responses in glucose metabolism are the same in both breeds of cattle feeding on different types of roughage. In the present glucose kinetic study, both (U-¹⁴C)-glucose and (3-³H)-glucose infusions in crossbred pregnant heifers

were studied at two consecutive periods : in late pregnancy and early lactation, as they were fed either hay or urea treated rice straw through the period of the experiment.

MATERIALS AND METHODS

Animals and management. Sixteen pregnant heifers, 23-25 months old and after approximately 150 days of gestation, were selected for the experiments. These consisted of eight animals of two breed types each, Holstein Friesian x Red Sindhi (50:50 = 50%HF) and Holstein Friesian x Red Sindhi (87.5:12.5 = 87.5%HF). They were divided into four groups of 4 animals each. Animals from the same breed 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 $34\pm 1^{\circ}\text{C}$ and the minimum temperature at night was $26\pm 1^{\circ}\text{C}$. Pregnant animals were individually fed a concentrate of an average of 4.0 kg/day (DM basis) and roughage to maintain the body condition score at three until calving. 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 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 x 21 day system of urea treatment. After 21 days, the treated rice straw with 5% urea was offered to the animals.

Experimental procedures. Two consecutive periods of experiments were carried out in each group. Period 1 was designed to begin 21 days before expected parturition but actually averaged 20-23 days prepartum (late pregnancy). Period 2 began 30 days postpartum (early stage of lactating period). Animals were fed the same ration through the completion of period 2. In both periods of experiments, the glucose turnover rate, mammary udder blood flow, and udder glucose uptake were measured. In the lactating period, animals were normally milked at around 0600 h and 1700 h. On the day of the experiment, milk secretion was recorded by hand milking in the afternoon and the measurement of udder blood flow was carried out. Animals were weighed after collecting the milk sample.

On the day before the experiment began in both periods of experiments, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm) were inserted into either the left or right subcutaneous abdominal vein (milk vein) by using a intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, U.K.) under local anesthesia. This was done in standing animals for the measurement of mammary udder blood flow and for collection of venous blood. The tip of the catheter was positioned near the sigmoid flexure anterior to the point at which the vein leaves the udder. The other catheter was positioned about 20 cm downstream from the first one. The catheter for isotope infusion was inserted into an ear vein under local anesthesia. All catheters were flushed with sterile heparinized normal saline and were left in place during the experiment.

Glucose turnover measurements. For glucose kinetic studies of each animal in both pregnant and lactating periods, at about 1100h a priming dose of radioactive glucose in 20 ml of sterile normal saline solution containing 60 $\mu\text{Ci}(3\text{-}^3\text{H})$ glucose and 40 $\mu\text{Ci}(\text{U-}^{14}\text{C})$ glucose was administered intravenously via the ear vein catheter and followed by a constant infusion of 1 ml/min of sterile saline (0.9%) containing 2 $\mu\text{Ci}(\text{U-}^{14}\text{C})$ glucose and 3 $\mu\text{Ci}(3\text{-}^3\text{H})$ glucose for 4h (Peristaltic pump; EYLA Model 3). This procedure produced a constant specific radioactivity of plasma glucose throughout the final hour of infusion. During the final hour (1400-1500h) of infusion, three sets of blood samples were collected at 20 min. intervals. A venous blood sample was collected from the milk vein via catheter while an arterial blood sample was collected from the coccygeal artery by venipuncture with a #21 needle. Blood samples in heparinized tubes were kept in crushed ice for chemical studies.

Chemical methods. All chemical and biochemical enzymes were obtained from Sigma Chemical Co. Radiochemicals, except for $(\text{U-}^{14}\text{C})$ -glucose and $(3\text{-}^3\text{H})$ -glucose which were obtained from the Radiochemical Center, Amersham Bucks., U.K. The isotopes were dissolved in sterile pyrogen free saline (0.9% NaCl). Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase. The specific activity of labeled plasma glucose was determined by the method described by Chaiyabutr and Buranakarl (8). The radioactivity in blood bicarbonate was measured by acidifying 2 ml of blood with an equal volume of 6% perchloric acid. The $^{14}\text{CO}_2$ was liberated and trapped as K^{14}CO_3 in a plastic cup which contained 0.1 ml 40% KOH.

Udder blood flow measurements. Udder blood flow measurements were performed in duplicate. Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion which was adapted from the

method of measuring blood flow in the milk veins of lactating goats (9). A dye (T-1824) was dissolved in sterile normal saline and diluted to a concentration of 100 mg/l. The solution was infused by a peristaltic pump (Gilson Medical electronics) at a constant rate of 85 ml/min into the milk vein for 1-2 min. Results of pilot experiments showed that these infusion rates could produce adequate mixing of dye with blood. Although the concentration of dye re-circulating in arterial blood might increase during the infusion, it was always substantially less than that in the milk vein because of the large blood volume in cattle. Before infusion, blood was drawn from downstream in the milk vein as a pre-infusion sample. About 10 seconds after starting the infusion, 10 ml of blood was drawn from downstream in the milk vein at a constant rate into a heparinized tube. Two consecutive plasma samples were taken during each dye infusion at about 5 min intervals. Blood flow of half of the udder was calculated from plasma samples using the equation derived by Thompson and Thomson (25). Quarter milking showed that the yields of the two halves of the udder were similar. Udder blood flow was therefore calculated by doubling the flow measured in one milk vein (5). Packed cell volume was measured after centrifugation of the blood in a microcapillary tube.

Calculations. The glucose turnover rate in the whole animal (T), expressed as $\mu\text{mol/min}$, was calculated from the equation.

$$T = I/G_A$$

where I = rate of infusion of (U- ^{14}C) glucose or (3- ^3H) glucose ($\mu\text{Ci/min}$) and G_A = specific activity of ^{14}C - or ^3H -glucose in arterial plasma at equilibrium ($\mu\text{Ci}/\mu\text{mol}$).

Recycling of glucose carbon in the whole animal, expressed as % glucose turnover, was calculated from the equation:

$$\text{Recycling} = (T_3 - T_{14}) \times 100 / T_3$$

where T_3 = total turnover rate of glucose calculated from (3- ^3H) glucose and T_{14} = turnover rate of glucose calculated from (U- ^{14}C) glucose.

Glucose clearance in the whole animal (C_G) was expressed as ml of plasma cleared of body glucose per minute and was calculated from the equation:

$$C_G = T_3/P_A$$

where T_3 = turnover rate of glucose calculated from (3- ^3H) glucose ($\mu\text{mol/min}$) and P_A = arterial plasma glucose concentration ($\mu\text{mol/ml}$).

Uptake of glucose by the udder (U_M), expressed as $\mu\text{mol/min}$, was calculated from the equation:

$$U_M = Q_P \times (P_A - P_V)$$

where Q_P = udder plasma flow (ml/min), P_A = concentration of glucose in coccygeal arterial plasma ($\mu\text{mol/ml}$) and P_V = concentration of glucose in mammary venous plasma ($\mu\text{mol/ml}$).

The extraction ratio of glucose by the udder was calculated by dividing the arteriovenous difference ($P_A - P_V$) by the arterial plasma glucose concentration (P_A).

Glucose oxidation for non-mammary tissue was estimated from the CO_2 produced by the animal. The ratio of specific radioactivity of arterial blood bicarbonate relative to that of arterial blood glucose- C^{14} was measured. The arterial blood CO_2 for calculated specific radioactivity was measured by a blood gas analyzer (238PH/blood gas analyzer, Ciba Corning).

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 between pregnant and lactating periods in each group using the paired t-test. Mean values are presented as mean $\pm$ SD.

RESULTS

Changes in glucose metabolism during late pregnancy and early lactation (Table 2)

The present studies were designed to investigate the glucose turnover rate in crossbred Holsteins by making simultaneous estimates of the total glucose entry rate using ($3\text{-}^3\text{H}$) glucose infusion and utilization rate of glucose using ($\text{U-}^{14}\text{C}$) glucose infusion. Estimates of the ($3\text{-}^3\text{H}$) glucose entry rate invariably exceeded those of the ($\text{U-}^{14}\text{C}$) glucose utilization rate for all groups of crossbred animals. Values of glucose turnover rate expressed as absolute values or as a function of metabolic body size increased markedly in the early lactating period in comparison with the late pregnant period for all groups. In pregnant 50%HF fed either hay or urea treated rice straw, the glucose turnover rate expressed as a function of metabolic body size exhibited a higher rate when compared with that of pregnant 87.5%HF animals. Recycling of glucose-C did not change with advancing pregnancy into early lactation in each group of experimental animals. However, among groups of the same crossbred animals, there were no significant differences in the glucose turnover rate and recycling of glucose-C from the pregnant period to the lactating period. The plasma glucose clearance increased by approximately 20-50% in the lactating period of 87.5%HF fed either hay or urea treated rice straw, while there was not increase for both groups of 50%HF animals. Comparing between the pregnant period and the lactating period, the ratios of the specific radioactivity of arterial

blood bicarbonate relative to that of arterial blood glucose- C^{14} in the lactating period slightly decreased in 87.5%HF but significantly decreased ($P<0.05$) in 50%HF animals fed either hay or urea treated rice straw. This result may indicate that the rate of glucose oxidation of 87.5%HF was more than that of 50%HF animals in the lactating period, whereas there were no apparent differences among groups of pregnant animals. The data in Table 2 show that the body weight of 87.5%HF animals fed either hay or urea treated rice straw significantly decreased in the early stage of lactation when compared with the late pregnant period ($P<0.05$), while the body weight of both groups of 50%HF animals did not significantly change between the two periods of study.

Changes in udder blood flow, udder glucose uptake and arterial plasma glucose concentration (Table 3)

For all groups, the rates of udder blood flow were nearly three times higher in lactating animals than in late pregnant animals. In comparison between 50%HF and 87.5%HF fed either hay or treated rice straw, the udder blood flow was significantly higher in 87.5%HF animals. The packed cell volumes significantly decreased during the lactating period for both groups of 87.5%HF animals while it did not significantly change in 50%HF animals. The packed cell volume of both pregnant and lactating 50%HF animals were significantly higher than those of 87.5%HF animals fed either hay or treated rice straw. The values of plasma glucose concentration were not apparently affected in lactating periods when compared with pregnant periods for all groups. The plasma glucose concentration was slightly higher in the pregnant 50%HF in comparison with the pregnant 87.5%HF fed either hay or treated rice straw. During lactation, the plasma glucose concentrations of 50%HF were significantly higher ($P<0.05$) than those of 87.5%HF fed either hay or urea treated rice straw. The arteriovenous differences and extraction ratio for glucose across the mammary gland markedly increased in lactating periods for all groups of experiments. There were no significant differences in glucose uptake, glucose arteriovenous differences and glucose extraction ratio by the udder of crossbred animals during pregnant periods or lactating periods among groups of animals fed different types of roughage. Mean average values of glucose uptake by the udder relative to the total glucose turnover rate were nearly 8 times ($P<0.01$) higher in the lactating period in comparison with the pregnant period for all groups of experimental animals. In the lactating 87.5%HF, glucose taken up by the udder was 64-65% of the total glucose turnover rate compared to a value of 43-46% in the lactating 50%HF animals.

DISCUSSION

It is evident from the present results that body responses of 50%HF and 87.5%HF animals vary during pregnancy and lactation. During the experiment the body weight of both crossbred animals fed either hay or treated rice straw decreased in the early lactating period in comparison to the prepartum period. Most of the weight loss might be due to expulsion of the calf and fluids. However, the degree of reduction of the body weight during the early lactating period of 87.5%HF was greater than that of 50%HF. The differences in these results between 87.5%HF and 50%HF without reduction in feed intake may be explained, in part, on the basis of the difference in the breed of animals used. During the early lactating period, the 87.5%HF animals gave a high milk yield (by average 18 kg/day), and the energy output in milk and for maintenance might be greater than the energy consumed in the food, and body tissue was being mobilized to make up this deficit. The 50%HF animals (average milk yield 12 kg/day) were approximately in energy equilibrium, there being no loss or gain of weight. The high genetic blood level of 50%HF, close to that of the *bos indicus* breed, leading to good adjustment to a tropical environment, could be another reason (21).

Studies measuring body glucose kinetics in dual isotope experiments have enabled one to determine both the utilization and total entry rates of glucose turnover. Values for the rate of glucose turnover of all groups have been expressed on the basis of metabolic body size, i.e., relative to body weight^(0.75); in this case the turnover rate of glucose is within the range reported in either sheep or goats of comparable body size using the same technique (3,10). In ruminants, endogenous synthesis of glucose from non-carbohydrate precursors (gluconeogenesis) is known to be the main process of glucose production (17). Little glucose is absorbed from the digestive tract when animals are fed roughage diets (13). In the present studies, both 87.5%HF and 50%HF animals were fed either hay or urea treated rice straw while they were maintained on a similar concentrate intake throughout the period of the experiment. An increase in the intake of both concentrate and roughage diets during the lactating period would increase glucogenic precursors which are the source for gluconeogenesis mainly in the liver. This would be attributed to an increase in the glucose turnover rate in lactating animals (24). However, in both the pregnant and lactating periods the proportion of newly synthesized glucose which was derived from glucose-C (i.e., glucose-C recycling) was approximately 20% of the total glucose turnover rate in both types of crossbred HF fed either hay or urea treated rice straw. These results presumably reflect adequate feeding in both crossbred HF animals, although analyses of different foraging

species fed hay or urea treated rice straw indicate differences in percentages of crude protein contents (Table 1). During the lactating period of 50%HF animals fed either hay or urea treated rice straw, the plasma glucose clearance was maintained at values similar to those of the pregnant period. In contrast to lactating 50%HF, the plasma glucose clearance increased while the plasma glucose concentration had a tendency to decrease for both groups of lactating 87.5%HF animals. These different results for 50%HF and 87.5%HF suggest that 87.5%HF animals had a higher body glucose metabolism than 50%HF animals. This reflects the high body utilization of glucose in 87.5%HF which probably resulted from the high rate of mammary glucose drain in 87.5%HF animals. For 50%HF, animals feeding on either hay or urea treated rice straw was sufficient to meet the animals' requirements for glucose metabolism. A slightly higher level in the plasma glucose concentration in lactating 50%HF would be an index for this adjustment. These results also confirmed the finding for different types of cows that the plasma glucose levels were higher in low yielding cattle (20).

It is clear that in the lactating period of both crossbred animals, the increase in the glucose turnover rate was due mainly to an increase in the glucose uptake by the mammary gland. Estimates of the glucose turnover rate in lactating periods increased by approximately 24% compared with the glucose uptake by the udder which increased almost 10 times. This may indicate that in the pregnant period, animals try to maintain homeostasis during the high demand for glucose by the foetus (15). This demand may be met by minimizing glucose utilization by the udder during late pregnancy. However, the rate of glucose uptake by the udder during pregnancy and lactation in both breeds seems to be dependent on both mammary blood flow and activity of the mammary epithelial cells. There was a correlation between milk yield and udder blood flow suggesting that blood flow is the main determinant of quantitative differences in udder metabolism between 87.5%HF and 50%HF animals. This is confirmed by the fact that arteriovenous differences in glucose uptake by the udder in the lactating periods was not significantly different between the breeds nor related to the milk yield. In the present results, marked increases in the extraction ratio and arteriovenous differences of glucose by the mammary gland in the lactating periods of both breeds also indicates an increase in the activity of mammary cells. The existence of such high rates of glucose uptake by the udder relative to the whole body glucose turnover rate in the lactating period means that other non-mammary tissues of the lactating animal exist at a reduced rate of glucose utilization even when fed either hay or urea treated rice straw. The decrease in the rate of glucose utilization by non-mammary tissues of both lactating crossbred HF animals could be calculated when the glucose uptake was subtracted from the total glucose turnover

rate which would be comparable with that during the pregnant period. Another indication of a decreased rate of glucose utilization by the non-mammary tissue was indicated by the decrease in the specific radioactivity of blood bicarbonate relative to that of blood glucose, suggesting a decreased rate of glucose oxidation in the lactating period as compared with the late pregnant period for both types of crossbred HF. Similar phenomena for decreased rates of body glucose oxidation in lactating periods were also noted between lactating and non-lactating cows (2) and between pregnant and lactating goats (10). Hormonal changes have been suggested to involve these metabolic changes without changes in the levels of plasma insulin or cortisol (11). However, the differences of glucose oxidation by the non-mammary tissue were also observed between 87.5%HF and 50%HF animals, in which the values of the ratio of the specific radioactivity of blood bicarbonate to arterial blood glucose- C^{14} was higher in the lactating 87.5%HF than in 50%HF animals which also indicates the higher body glucose oxidation in 87.5%HF animals.

In conclusion, this study has shown that 87.5%HF animal has the genetic potential for a high milk yield and has high body and udder glucose metabolisms compared with 50%HF animals. There were no differences in the physiological performances in the same crossbred cattle fed either hay or urea treated rice straw, which indicates that urea treated rice straw could be used as preserved roughage for the dairy crossbred Holstein cattle during the dry season.

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TABLE 1. Chemical composition (%DM) of feeds used.

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

Concentrate formulation : ingredient fresh weight (kg/100 kg) 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).

TABLE 2. Aspects of glucose metabolism in late pregnancy and early stage of lactation of crossbred Holsteins fed with hay and urea treated rice straw (N = 4 in each group).

	Hay + Concentrate				Urea treated rice straw + Concentrate			
	HF:RS (87.5:12.5)		HF:RS (50:50)		HF:RS (87.5:12.5)		HF:RS (50:50)	
	Pregnancy	Lactation	Pregnancy	Lactation	Pregnancy	Lactation	Pregnancy	Lactation
Body weight (kg) :	388±33 ^a	360±34 ^{*α}	329±35 ^b	321±37 ^{αβ}	379±14 ^a	341±21 ^{*α}	324±6 ^b	309±14 ^β
Glucose turnover using:								
(3- ³ H)glucose infusion	4084±276	5662±566 [*]	4427±1131	4966±564	3606±351	4637±699 [*]	4493±978	5415±444 [*]
(μmol/min)								
(U- ¹⁴ C)glucose infusion	3267±287	4712±747 [*]	3597±807	3980±399	2945±400	3805±451	3572±877	3895±734
(μmol/min)								
(3- ³ H)glucose infusion	46.8±3.0 ^{ab}	68.6±7.5 [*]	56.8±10.6 ^a	65.6±5.8	42.1±5.1 ^b	58.8±10.8 [*]	58.7±12.5 ^a	70.0±10.4 [*]
(μmol/min per kg ^{0.75})								
(U- ¹⁴ C)glucose infusion	37.4±2.9 ^{ab}	56.5±7.4 [*]	46.3±7.1 ^a	52.6±3.4	34.4±5.5 ^b	48.2±7.4 [*]	46.7±11.4 ^{ab}	52.8±8.5
(μmol/min per kg ^{0.75})								
Recycling of glucose-C	20±4	17±7	18±4	20±2	19±5	16±7	21±5	24±7
(%)								
Plasma glucose clearance	1120±161	1702±409	1116±360	1127±197	1087±291	1313±196	1258±193	1288±70
(ml/min)								
Arterial Blood ¹⁴ CO ₂ /	1.29±0.39	1.00±0.13 ^α	1.21±0.25	0.64±0.04 ^{*β}	0.98±0.16	0.74±0.13 ^β	1.02±0.24	0.69±0.14 ^{*β}
Arterial Blood								
(¹⁴ C) glucose (%)								

P-values by paired t-test: * P<0.05, with respect to the period of pregnancy in each group.

^{a-b} Mean values with different superscripts within the same row indicate that effects during pregnancy are significantly different at P<0.05.

^{α-β} Mean values with different superscripts within the same row indicate that effects during lactation are significantly different at P<0.05.

TABLE 3. Udder blood flow, arterial plasma glucose concentration and udder glucose uptake in late pregnancy and early stage of lactation of crossbred Holsteins fed with hay and urea treated rice straw (N = 4 in each group).

	Hay + Concentrate				Urea treated rice straw + Concentrate			
	HF:RS (87.5:12.5)		HF:RS (50:50)		HF:RS (87.5:12.5)		HF:RS (50:50)	
	Pregnancy	Lactation	Pregnancy	Lactation	Pregnancy	Lactation	Pregnancy	Lactation
Udder blood flow (ml/min)	2696±265 ^a	6845±2266 [*]	1034±354 ^b	3887±543 ^{**}	1876±363 ^c	4482±1151 ^{**}	1396±406 ^{cb}	4314±575 ^{***}
Packed cell volume (%)	28.3±1.3 ^a	26.3±2.1 ^{*α}	30.9±2.3 ^b	30.0±1.6 ^β	28.1±2.6 ^a	25.3±1.7 ^{*α}	33.1±2.3 ^b	29.3±2.9 ^β
Glucose :								
Arterial concentration (μmol/ml)	3.68±0.32	3.40±0.53 ^α	4.06±0.59	4.45±0.43 ^β	3.49±1.04	3.53±0.19 ^α	3.60±0.81	4.20±0.17 ^β
A-V difference (μmol/ml)	0.16±0.07	0.74±0.04 ^{***}	0.32±0.20	0.81±0.16 [*]	0.22±0.07	0.87±0.18 ^{***}	0.20±0.07	0.76±0.23 ^{**}
Extraction ratio (%)	4.2 ±1.5	22.1±3.1 ^{**}	8.2±6.6	18.1±2.7 [*]	6.4±2.2	24.7±4.5 ^{**}	5.5±1.1	18.1±5.0 ^{**}
Udder uptake (μmol/min)	301±108	3704±1240 ^{**}	254±226	2270±537 ^{**}	291±91	2974±902 ^{**}	188±65	2339±604 ^{**}
Udder glucose uptake/ Glucose turnover (%)	7.5±3.1	64.9±19.7 ^{**}	5.8±4.7	45.7±9.8 ^{**}	7.9±1.8	64.5±18.1 ^{**}	7.9±7.8	42.9±8.6 ^{**}

P-values by paired t-test: *P<0.05, **P<0.01, ***P<0.001, with respect to the period of pregnancy in each group.

^{a-b} Mean values with different superscripts within the same row indicate that effects during pregnancy are significantly different at P<0.05.

^{α-β} Mean values with different superscripts within the same row indicate that effects during lactation are significantly different at P<0.05.

**Comparative study on the regulation of body fluids and mammary circulation
at different stages of lactation in crossbred Holstein cattle feeding
on different types of roughage**

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SUMMARY. The present study was to evaluate the effect of prolonged feeding of urea treated rice straw compared with feeding of hay on the regulation of body fluids, milk yield and mammary circulation at different stages of lactation in crossbred Holstein Friesians. Sixteen first lactating crossbred Holstein Friesians (HF), consisting of eight animals of two breed types, 87.5%HF and 50%HF, were selected and each breed was randomly allocated into two groups. Each group, consisting of four animals from the same breed, was fed either 5% urea treated rice straw or pangola hay (*Digitaria decumbens*) as the source of roughage in combination with a similar concentrate throughout the experiments. Three consecutive periods of experiments were carried out for early lactation (30 days postpartum), mid-lactation (120 days postpartum) and late lactation (210 days postpartum). During the course of lactation there were no significant differences in body weight, heart rate, mean arterial blood pressure, plasma osmolality, plasma volume and blood volume among groups of 87.5%HF animals and 50%HF animals fed either hay or urea treated rice straw. Water turnover rate, total body water space and total body water as a percentage of body weight of 50%HF animals were significantly higher than those of 87.5%HF animals fed either hay or urea treated rice straw, while there was no significant differences in the biological half-life of body water for all groups of crossbred animals during the course of lactation. The packed cell volume was significantly higher in all lactating periods of both groups of 50%HF animals in comparison to 87.5%HF animals. The ratio of DM intake to milk production for 87.5%HF animals fed either hay or urea treated rice straw was significantly lower than that of 50%HF animals in early lactation. The udder blood flow and milk secretion of 87.5%HF were significantly higher in early lactation and markedly declined when lactation advanced in comparison with those of 50%HF animals fed either hay or treated rice straw. The ratio of mammary blood flow to milk yield for all groups was in a similar range during early lactation while it significantly increased in mid- and late lactation for both groups of 87.5%HF animals. From these results it can be concluded that both 50%HF and 87.5%HF animals feeding on urea treated rice straw as a roughage source do not show any undernutritional effects in comparison with those fed with hay during the course of lactation. The physiological response differences between

breeds are that 87.5%HF animals, which have a genetic makeup closer to the exotic *bos taurus* breed and a high milk yield, show a poor adjustment to the tropical environment and poorer lactation persistency in comparison with 50%HF animals.

Many factors can affect milk production in dairy cattle in the tropics, e.g., a high environmental temperature, less genetic potential for milk of indigenous cattle and an inadequate supply for foraging during the dry, summer months. Several approaches have been utilized in attempting to improve dairy productivity. Types of suitable crossbreeding of indigenous and exotic cattle for the tropics have been selected. Different types of crop residues have been used as roughage to feed animals. For example, rice straw, which has a low nutritive value, was treated with urea to help animals survive during periods of scarcity (Jayasuriya & Perera 1982; Promma et al. 1994). Until now there have been very few studies on the mechanism acting within the bodies of crossbred cattle concerning the profitability of efficient utilization of the treated rice straw for dairy production.

During a study on the regulation of body fluids and mammary circulation in crossbred Holstein cattle (HF) feeding on different types of roughage (Chaiyabutr et al., 1997) it was noted that 50%HF animals showed some differences in the distribution of body fluids and mammary circulation from 87.5%HF animals during late pregnancy and early lactation. The differences between crossbred and purebred animals in body composition and water turnover rate have also been reported (Macfarlane and Howard 1970; Gharaybeh et al. 1968). During lactation, many bodily functions are altered. For example, blood volume and cardiac output are increased (Hanwell & Peaker 1977) and lactating animals metabolize large amounts of water and are affected rapidly by water deprivation (Murphy 1992). No research in crossbred dairy cattle has been conducted to study consequent effects from the utilization of different types of roughage on the changes of body fluids, the mammary circulation and milk secretion in different stages of lactation. The study at different stages of lactation in crossbred animals might throw some light on a useful index for studying adaptability in crossbred cattle which will provide information on choosing suitable crossbred dairy cattle in the tropics for increased milk production. Therefore, the objective of the present study was to evaluate the effect of prolonged feeding of urea treated rice straw compared with feeding of hay on the water turnover rate, total body water, mammary circulation, milk yield and other physiological parameters in relation to these variables at different stages of lactation in crossbred Holstein Friesians.

MATERIALS AND METHODS

Animals and management

Sixteen first lactating crossbred dairy cattle were selected for the experiments. These animals consisted of eight animals of two breed types, Holstein Friesian x Red Sindhi (50:50 = 50%HF) and Holstein Friesian x 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 animals were housed in sheds and tethered in individual stalls and fed twice daily. The maximum temperature in the shed at noon was $34\pm1^{\circ}\text{C}$ and the minimum temperature at night was $26\pm1^{\circ}\text{C}$. The relative humidity was $68\pm12\%$. Animals individually received an average of 3-5 kg/day of roughage in combination with the same concentrated mixture (7-10 kg/day) to maintain a moderate body condition score (2.5, scale = 1 to 5). The chemical composition of feeds is presented in Table 1. Each day, the food was given in equal portions at about 06.00 h and 17.00 h when animals were milked. Animals had free access to water and a lick block of minerals throughout the experiment (the composition of minerals in 1 kg lick block consisted of Na 136 gm, Ca 140 gm, P 60 gm, Mg 20 gm, K 25 gm, S 12 gm, Fe 1,000 mg, Zn 800 mg, Mn 350 mg, Cu 300 mg, Co 80 mg, I 245 mg and Se 20 mg). Animals were fed their respective rations throughout the experimental period.

The urea treated rice straw was prepared by mixing the urea solution with dry straw (5 kg urea dissolved in 100 litres water per 100 kg dry rice 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 x 21 day system of urea treatment. After 21 days, the rice straw treated with 5% urea was offered to the animals.

Experimental procedure

Three consecutive periods of experiments were carried out in each group. Period 1 was designed to begin 30 days postpartum (early stage of lactating period). Period 2 began 120 days postpartum (middle stage of lactating period). Period 3 began 210 days postpartum (later stage of lactating period). Animals were fed the same ration from before parturition through the completion of period 3. The dry matter intake of each animal was determined by measuring both the concentrate and roughage offered and refused each day. Animals were normally milked at around 0600 h and 1700 h by a milking machine and milk production was recorded daily. On the day of the experiment, measurements of the total body water, water turnover, udder blood flow, arterial blood pressure, heart rate, plasma volume and blood volume were carried out. The rate of milk secretion was recorded by hand milking in the afternoon and the

measurement of udder blood flow was carried out. Animals were weighed after collecting the milk sample.

On the day before the experiment began in each period of lactation, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm) were inserted into either the left or right milk vein using a intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, U.K.) under local anesthesia. This was done in standing animals for the measurement of mammary blood flow and for collection of venous blood. The tip of the catheter was positioned near the sigmoid flexure anterior to the point at which the vein leaves the udder. The other catheter was positioned downstream about 20 cm from the first one. The catheter for isotope infusion and dye injection was inserted into an ear vein under local anesthesia. All catheters were flushed with sterile heparinized normal saline and were left in place during the experiment.

Water intake, total body water and water turnover measurements

The estimated water intake values of animals in each period of lactation was predicted from the measured daily dry matter intake, daily sodium intake, milk production and minimum ambient temperature. An estimation was based on the following equation developed by Murphy et al. (1983) :

$$\text{Water intake (kg/day)} = 15.99 + 1.58 \times \text{DM intake (kg/day)} + 0.9 \times \text{milk production (kg/day)} + 0.05 \times \text{sodium intake (gm/day)} + 1.2 \times \text{minimum temp. (}^{\circ}\text{C)}$$

The water turnover rate and total body water were determined by tritiated water dilution techniques using a single dose injection of 3,000 μCi per animal of carrier free tritiated water (TOH) in normal saline and the equilibrium time was determined by taking blood samples for 3 days after the injection. Blood samples for measurements of water turnover rate, biological half-life of tritium, TOH space and total body water were performed as previously described (Chaiyabutr et al., 1997).

Determination of plasma volume and plasma solids concentration

Plasma volume was measured by dilution of Evan's blue (T-1824) dye (E. Merck Darmstadt, Germany). The injection of 20 ml of the dye (0.5 g/100 ml normal saline) into the ear vein catheter was followed by venous samples from the jugular vein taken at 30, 40 and 50 min. Dilution of dye at zero time was determined by extrapolation. Blood volume was calculated from the plasma volume and packed cell volume (PCV) (Chaiyabutr et al., 1980). Plasma osmolality was measured using the freezing point depression method (Advance Osmometer model 3, U.S.A.). The plasma solids concentration was determined by a refractometer.

Determination of udder blood flow, arterial blood pressure and heart rate

Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion which was adapted from the method of measuring blood flow in the milk veins of cattle as previously described (Chaiyabutr et al., 1997). Heart rate and blood pressure were measured directly from the intermediate auricular artery under local anaesthesia by venipuncture with a No.21

needle connected with a pressure transducer and chart recorder (Universal Oscillograph, Harvard Apparatus Ltd., Kent, UK). Mammary resistance was calculated from udder blood flow and mean arterial blood pressure using the standard formula (Burton, 1965).

Statistical analysis

All the results were statistically analyzed by analysis of variance (ANOVA); the significant differences between groups and treatments were compared by Duncan's multiple range test. Values were compared among periods of lactation in each group using the paired t-test. Mean values are presented as mean $\pm$ S.D.

RESULTS

Dietary dry matter intake, water intake and milk yield (Table 2)

The total daily dry matter intake was not significantly different among groups of crossbred HF animals during the course of lactation. Estimated mean values of daily water intake during course of lactation for both groups of 87.5%HF were slightly higher than those for 50%HF animals excepted for the period of late lactation. Daily water intake significantly decreased in mid- and late lactation when compared with early lactation in 87.5%HF animals fed with hay as roughage. In all groups of crossbred animals, milk yield rose for several weeks after parturition. In both groups of 87.5%HF animals feeding on either hay or urea treated rice straw, milk yield peak occurred at week 10, thereafter yield declined in both groups throughout lactating period. In contrast to 50%HF animals, milk yield peak occurred at week 5 after parturition and persistent lactation seemed to be apparent throughout periods of lactation (Fig.1). Milk production in the early stage of lactation was significantly higher for the 87.5%HF animals than for the 50%HF animals fed either hay or urea treated rice straw. In mid-lactation, the milk yield of both groups of 87.5%HF animals significantly declined by approximately 42% from early lactation. In contrast, there were no significant changes of milk yield during lactation advances in 50%HF animals fed either hay or urea treated rice straw. An evaluation of the dry matter intake and milk yield revealed that during early lactation the mean ratios of total dry matter intake to milk yield of both groups of 87.5%HF animals were significantly lower than those of 50%HF animals fed either hay or urea treated rice straw. In mid- and late lactation of both 87.5%HF animals the mean ratios of dry matter intake to milk yield increased significantly when compared to those values in early lactation. There were no significant differences in the ratio of dry matter intake to milk yield during course of lactation in 50%HF animals.

Plasma volume, blood volume, plasma osmolality and packed cell volume (Table 3)

There were no significant differences of the values of plasma volume and blood volume as absolute values or as percentages of body weight for either 87.5%HF or 50%HF animals fed either hay or urea treated rice straw throughout the course of lactation. The packed cell volume of both groups of 50%HF animals were significantly higher than those of both groups of 87.5%HF animal fed either hay or urea treated rice straw. During the course of lactation there were no significant differences of body weight and plasma osmolality among groups of 87.5%HF and 50%HF animals.

Water turnover rate and total body water (Table 4)

There were no significant differences in the average water turnover rate for all groups of crossbred animals during the course of lactation. However, the water turnover rate as a percentage of body weight had a tendency to be higher, particularly at late lactation, in both groups of 50%HF animals when compared with 87.5%HF animals fed either hay or urea treated rice straw. During the course of lactation, the values of half-life of body water were found to be in a similar range for all groups of animals. The TOH space and total body water as percentage of body weight of 50%HF animals were significantly higher than those of 87.5%HF animals fed either hay or urea treated rice straw. In each group, the values of TOH space or total body water showed no significant differences among periods of lactation.

Mammary and general circulation (Table 5)

There were no significant differences in either the heart rate or mean arterial blood pressure among the periods of lactation in all groups of animals fed either hay or urea treated rice straw. Udder plasma flow and udder blood flow of 87.5%HF animals was significantly higher during early lactation than those of 50%HF animals fed either hay or urea treated rice straw. The marked decrease in mammary blood flow from the period of early lactation to mid-lactation of 87.5%HF animals coincided with the decrease in milk yield. The ratio of mammary blood flow to the rate of milk yield did not significantly change during the course of lactation in 50%HF animals. However, the marked increase in the ratio of mammary blood flow to the rate of milk yield during lactation advance was apparent in both groups of 87.5%HF animals.

Table 1 Chemical composition of feed components (% on dry matter basis).

Particulars	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 formulation: fresh weight (kg/100 kg) 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.

Table 2 Dietary dry matter intake, water intake and milk yield in different stages of lactation of crossbred Holsteins fed with hay and urea treated rice straw (n = 4 in each group).

	Period of	Hay+concentration		Urea treated rice straw+concentration	
	lactation	HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Dry matter intake (kg/d)					
Concentrate	Early	7.05±1.74 ^a	8.41±0.30 ^a	7.85±1.55 ^a	8.25±0.45 ^a
	Mid	8.51±1.51 ^{ab}	6.56±1.67 ^b	10.05±0.86 ^a	9.05±0.07 ^a
	Late	6.90±2.76 ^b	7.04±1.35 ^b	10.18±0.82 ^a	8.90±0.10 ^{ab}
Roughage	Early	4.00±1.28 ^a	3.47±0.49 ^{ab}	2.47±0.24 ^b	2.75±0.22 ^b
	Mid	3.43±0.72 ^a	3.64±0.42 ^a	2.60±0.27 ^b	2.29±0.32 ^b
	Late	3.79±0.44 ^b	4.47±0.26 ^a	2.64±0.18 ^c	2.95±0.17 ^c
Total DM intake	Early	11.05±0.47 ^{ab}	11.87±0.61 ^a	10.29±1.07 ^b	11.00±0.56 ^{ab}
	Mid	11.95±1.50 ^{ab}	10.19±1.53 ^b	12.65±0.75 ^a	11.39±0.31 ^{ab}
	Late	10.70±2.41 ^a	11.51±1.60 ^a	12.81±0.67 ^a	11.85±0.19 ^a
Water intake (kg/d)	Early	86.15±4.44 ^a	80.39±1.96 ^a	82.62±6.44 ^a	80.68±1.65 ^a
	Mid	80.92±4.10 ^{ab*}	76.62±3.34 ^b	82.98±2.29 ^a	81.12±2.63 ^{ab}
	Late	77.22±5.12 ^{a**}	79.18±3.78 ^a	81.01±2.06 ^a	81.72±2.17 ^a
Milk yield (kg/d)	Early	19.76±4.47 ^a	10.98±1.17 ^b	16.51±5.92 ^{ab}	12.91±1.58 ^b
	Mid	11.00±1.61 ^{a*}	10.52±1.34 ^a	11.72±0.93 ^a	12.33±2.46 ^a
	Late	10.11±0.69 ^{ab*}	10.47±0.81 ^{ab}	9.18±1.21 ^{b*}	12.26±2.51 ^a
DM intake/Milk yield	Early	0.55±0.11 ^c	1.08±0.08 ^a	0.66±0.18 ^{bc}	0.86±0.12 ^b
	Mid	1.09±0.05 ^{a**}	0.98±0.18 ^a	1.08±0.05 ^{a**}	0.95±0.18 ^a
	Late	1.03±0.18 ^{b*}	1.10±0.16 ^b	1.31±0.24 ^{a***}	1.00±0.21 ^b

P-values by paired t-test: * P<0.05, ** P<0.01, *** P<0.001 with respect to the early stage of lactation in each group.

^{a-c} Mean values with different superscripts within the same row are significantly different at P<0.05.

Table 3 Plasma volume, blood volume and packed cell volume in different stages of lactation of crossbred Holsteins fed with hay and urea treated rice straw (n = 4 in each group).

	Period of	Hay+concentration		Urea treated rice straw+concentration	
	lactation	HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Plasma volume					
(l)	Early	21.24±3.28 ^a	19.26±1.92 ^a	19.69±1.33 ^a	18.58±1.37 ^a
	Mid	19.53±1.26 ^a	16.47±0.35 ^b	19.77±2.57 ^a	17.48±1.07 ^{ab}
	Late	20.38±1.26 ^a	17.11±1.11 ^b	18.69±1.11 ^b	18.04±0.77 ^b
(l/100 kg)	Early	5.88±0.42 ^a	6.02±0.36 ^a	5.80±0.65 ^a	6.03±0.62 ^a
	Mid	5.69±0.51 ^a	4.85±0.51 ^a	5.31±0.68 ^a	5.09±0.56 ^a
	Late	5.85±0.37 ^a	4.62±0.39 ^b	5.02±0.54 ^b	4.92±0.52 ^b
Blood volume					
(l)	Early	28.71±4.29 ^a	26.45±2.62 ^a	26.72±1.78 ^a	26.33±2.69 ^a
	Mid	27.06±2.08 ^a	24.08±0.26 ^a	27.17±2.97 ^a	25.57±1.68 ^a
	Late	28.34±2.16 ^a	25.13±1.97 ^b	26.14±1.78 ^{ab}	26.21±1.36 ^{ab}
(l/100 kg)	Early	7.94±0.46 ^a	8.28±0.84 ^a	7.87±0.94 ^a	8.56±1.15 ^a
	Mid	7.87±0.46 ^a	7.09±0.76 ^a	7.30±0.81 ^a	7.46±0.86 ^a
	Late	8.12±0.57 ^a	6.79±0.66 ^b	7.01±0.83 ^{ab}	7.16±0.87 ^{ab}
Hct (%)	Early	26.3±2.1 ^{bc}	30.0±1.6 ^a	25.3±1.7 ^c	29.3±2.9 ^{ab}
	Mid	27.7±2.6 ^b	31.6±1.1 ^a	27.4±1.8 ^b	31.6±0.5 ^a
	Late	27.7±1.9 ^b	31.8±1.0 ^a	28.7±0.6 ^{b*}	31.1±1.0 ^a
Plasma osmolality (mOsm/kg)	Early	279.0±6.1 ^b	287.0±4.3 ^a	282.0±2.4 ^{ab}	288.5±1.3 ^a
	Mid	281.5±4.0 ^b	285.3±2.8 ^b	284.5±3.3 ^b	290.7±2.2 ^a
	Late	278.5±3.3 ^b	280.2±2.2 ^b	283.0±4.6 ^{ab}	288.0±3.5 ^a
Body weight (kg)	Early	360±33 ^a	321±36 ^{ab}	341±21 ^{ab}	309±14 ^b
	Mid	346±47 ^a	342±35 ^a	372±14 ^a	344±18 ^a
	Late	350±38 ^a	371±23 ^a	375±29 ^a	368±28 ^a

P-values by paired t-test: * P<0.05, with respect to the early stage of lactation in each group.

^{a-c} Mean values with different superscripts within the same row are significantly different at P<0.05.

Table 4 Changes in water turnover rate and total body water in different stages of lactation of crossbred Holsteins fed with hay and urea treated rice straw (n = 4 in each group).

	Period of	Hay+concentration		Urea treated rice straw+concentration	
	lactation	HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Water turnover rate					
(l/d)	Early	64.8±16.5 ^a	69.1±4.9 ^a	53.4±12.7 ^a	56.8±6.9 ^a
	Mid	64.1±5.6 ^a	65.0±12.4 ^a	70.9±15.9 ^a	69.1±8.5 ^a
	Late	50.4±6.9 ^b	72.6±12.9 ^a	58.9±7.6 ^{ab}	73.2±10.6 ^a
(l/100 kg/d)	Early	18.2±4.9 ^{ab}	21.7±1.9 ^a	15.5±2.6 ^b	18.4±1.8 ^{ab}
	Mid	18.7±2.7 ^a	19.0±2.7 ^a	18.9±5.7 ^a	20.1±2.2 ^a
	Late	14.3±2.8 ^c	19.5±2.7 ^{ab}	15.7±1.6 ^{bc}	20.0±3.5 ^a
(ml/kg ^{0.82} /d)	Early	522.6±140.3 ^{ab}	611.2±45.3 ^a	444.7±81.0 ^b	514.0±52.3 ^{ab}
	Mid	535.2±69.2 ^a	525.2±75.4 ^a	550.0±107.8 ^a	572.8±60.4 ^a
	Late	410.1±82.7 ^b	566.4±82.1 ^a	456.0±46.9 ^b	577.6±95.5 ^a
Biological half-life					
(d)	Early	2.9±0.7 ^a	2.5±0.2 ^a	3.1±0.4 ^a	2.8±0.3 ^a
	Mid	2.5±0.3 ^a	2.8±0.3 ^a	2.5±0.3 ^a	2.7±0.3 ^a
	Late	3.4±0.9 ^a	2.8±0.3 ^a	3.1±0.4 ^a	2.6±0.4 ^a
TOH space					
(l)	Early	259.3±17.3 ^a	253.1±17.6 ^{ab}	236.4±17.2 ^{ab}	229.9±12.5 ^b
	Mid	230.4±23.9 ^a	255.8±27.1 ^a	254.8±36.2 ^a	262.8±6.9 ^a
	Late	246.4±38.5 ^a	287.1±22.1 ^a	265.5±43.8 ^a	273.6±9.7 ^a
(l/100 kg)	Early	72.2±3.7 ^b	79.2±4.8 ^a	69.3±1.2 ^b	74.5±1.9 ^{ab}
	Mid	66.8±3.3 ^b	74.9±5.4 ^{ab}	68.2±7.3 ^b	76.5±3.6 ^a
	Late	70.1±3.5 ^b	77.4±4.4 ^a	70.5±6.2 ^b	74.4±3.2 ^{ab}
Total body water					
(l)	Early	227.7±15.9 ^a	232.6±15.9 ^a	214.4±17.0 ^a	211.2±10.8 ^a
	Mid	209.9±22.1 ^a	234.1±24.8 ^a	232.7±32.9 ^a	240.7±7.2 ^a
	Late	223.7±35.5 ^a	262.8±20.7 ^a	241.8±40.0 ^a	250.6±9.1 ^a
(l/100 kg)	Early	65.3±2.7 ^{bc}	72.7±4.4 ^a	62.8±1.9 ^c	68.4±1.4 ^{ab}
	Mid	61.7±1.8 ^b	68.5±5.1 ^{ab}	62.3±6.7 ^b	70.0±3.2 ^a
	Late	63.7±3.2 ^b	70.8±4.2 ^a	64.2±5.7 ^b	68.2±2.9 ^{ab}

P-values by paired t-test: * P<0.05, with respect to the early stage of lactation in each group.

^{a-c} Mean values with different superscripts within the same row are significantly different at P<0.05.

Table 5 Changes in general circulation and mammary circulation in different stages of lactation of crossbred Holsteins fed with hay and urea treated rice straw (n = 4 in each group).

	Period of lactation	Hay+concentration		Urea treated rice straw+concentration	
		HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Heart rate (beats/min)	Early	90±13 ^a	82±14 ^a	87±6 ^a	85±17 ^a
	Mid	81±14 ^a	65±10 ^b	72±7 ^{ab}	80±9 ^{ab}
	Late	70±15 ^a	67±7 ^a	66±5 ^a	66±7 ^a
Mean BP (mmHg)	Early	91.0±17.4 ^a	71.8±11.5 ^a	72.0±12.3 ^a	75.8±16.4 ^a
	Mid	73.5±12.1 ^a	74.6±20.9 ^a	84.0±19.6 ^a	67.9±5.3 ^a
	Late	76.5±14.7 ^a	69.2±7.5 ^a	72.5±4.4 ^a	68.7±4.9 ^a
Udder plasma flow (ml/min)	Early	5243±1275 ^a	2825±476 ^b	4101±1521 ^{ab}	3114±401 ^b
	Mid	3528±744 ^a	2795±256 ^a	2791±682 ^a	3465±712 ^a
	Late	3667±514 ^{ab}	2684±329 ^c	2845±658 ^{bc*}	3695±608 ^a
Udder blood flow (ml/min)	Early	7160±1807 ^a	3887±543 ^b	4619±1149 ^b	4314±575 ^b
	Mid	4745±836 ^a	4090±398 ^a	3843±872 ^a	5068±1054 ^a
	Late	5026±724 ^{ab}	3942±500 ^b	3995±883 ^b	5371±932 ^a
Mammary resistance (dynes/sec.cm ⁵)	Early	1122±285 ^a	1494±267 ^a	1251±319 ^a	1455±520 ^a
	Mid	1254±233 ^{ab}	1467±445 ^{ab}	1876±749 ^a	1105±226 ^b
	Late	1211±75 ^{ab}	1410±139 ^{ab}	1630±628 ^a	1052±234 ^b
Udder blood flow/ Milk yield	Early	519±24 ^a	508±19 ^a	413±51 ^a	480±23 ^a
	Mid	620±37 ^{a*}	563±51 ^{ab}	476±129 ^b	594±66 ^{ab}
	Late	713±63 ^{a*}	540±38 ^b	624±88 ^{ab*}	634±25 ^{ab}

P-values by paired t-test: * P<0.05, with respect to the early stage of lactation in each group.

^{a-c} Mean values with different superscripts within the same row are significantly different at P<0.05.

DISCUSSION

It is evident from the present results and those of Chaiyabutr et al. (1997) that both 50%HF and 87.5%HF animals feeding on urea treated rice straw as roughage did not show any undernutritional effects in comparison to those fed with hay in different periods of lactation. It is probable that urea treatment of rice straw can increase nitrogen availability and optimize the rumen environment while this availability in the rumen is limited in dry rice straw (Huque & Chowdhury 1997). In the present study, total DM intake was not different among groups of animals fed either hay or urea treated rice straw throughout lactating periods. However, the milk yield of 87.5%HF animals fed either hay or urea treated rice straw as roughage was significantly higher in early lactation than those of 50%HF animals while the ratio of total DM intake to milk yield was lower in both groups of 87.5%HF animals as compared to 50%HF animals. This indicates that the energy output in milk and for maintenance for 87.5%HF animals was greater than the energy consumed in the food in the first month of lactation. In mid-lactation, the milk yield of both groups of 87.5%HF animals significantly declined from early lactation. In contrast to 50%HF animals, persistent lactation seemed to be apparent throughout periods of lactation during feeding on either hay or urea treated rice straw. Therefore, this might indicate that different control mechanisms are at play in the regulation of milk production as lactation advances in 50%HF and 87.5%HF animals. The 50%HF animals were approximately in energy equilibrium, there being no change in the ratio of total DM intake to milk yield among periods of lactation. The high genetic similarity of 87.5%HF cattle to the exotic *bos taurus* breed may lead to poor adjustment to the tropical environment, while yielding high milk production in early lactation. Water secreted in milk would increase which would account for most of the increased water intake during early lactation in 87.5%HF animals. However, the lower total body water as a percentage of body weight of 87.5%HF compared to 50%HF animals in all periods of lactation may be attributed to a relatively lower efficiency in the water retention mechanism, although the estimated water intake was slightly higher in 87.5%HF animals. A low water content may be related to the poor adaptation of 87.5%HF to this tropical environment (Springell, 1968). Poorer lactation persistency in higher yielding cows was also noted (Chase, 1993; Coulon et al., 1995). A higher water reserve in 50%HF animals would not only provide a higher reservoir of soluble metabolites for biosynthesis of milk but is also useful in slowing down the

elevation in body temperature of this breed during lactation in hot conditions (Nakamura et al., 1987). In the present study, animals were housed in the same shed under the same environment. Thus, the water turnover rate of both types of crossbred cattle was not influenced by environmental conditions, although marked differences of water turnover rate and half-life of body water in animals occurred during the winter and summer (Ranjhan et al., 1982). The lactating 87.5%HF animals showed a lower water turnover rate especially in late lactation in comparison with 50%HF animals. This indicates that 87.5%HF probably do not require greater amounts of water, while animals could restore their body fluids to equilibrium in all lactating periods.

The present results provide further evidence that the mammary circulation of animals between 87.5%HF and 50%HF varied during different periods of lactation. A higher mammary blood flow during early lactation compared to mid-lactation in 87.5%HF animals feeding on either hay or treated rice straw cannot be attributed to a change in blood volume and plasma volume, which remained nearly constant. Plasma osmolality remained unchanged during the course of lactation in all groups indicating that homeostatis was being maintained throughout all periods of lactation. Such differences of the mammary circulation between 87.5%HF and 50%HF animals could be attributable to disparities in breed. The ratio of mammary blood flow to the rate of milk yield did not significantly change during the course of lactation in 50%HF animals. The marked decrease in the mammary blood flow of 87.5%HF at mid-lactation correlated with the decrease of milk yield. Thus, the rate at which milk yield declined after the peak could have been due primarily to a decreased availability of substrates to the mammary gland. The marked increase in the ratio of mammary blood flow to the rate of milk yield during lactation advance to mid- and late lactation in both groups of 87.5%HF animals indicated a more decreased secretory activity of mammary tissue. The question then arises as to whether mammary metabolism influences mammary blood flow or mammary blood flow influences mammary metabolism, which should be further investigated.

Many studies on mechanisms concerned with regulation of mammary blood flow, local and extramammary production of vasoactive agents and activity of mammary sympathetic nerves have been reviewed comprehensively (Linzell, 1974). The studies on mammary circulation in relation to the general circulation of crossbred cattle feeding on different types of roughage have been measured in the same animals at different stages of lactation. The mean values of mammary resistance relative to mammary blood flow in different periods of lactation in each groups did not significantly change.

Therefore, it is clear that the local changes for vasoconstriction in the udder were not apparent in different periods of lactation for both types of crossbred cattle. It seems that humoral factors are responsible. More research needs to be conducted on the hormone level, especially that of growth hormones, in the regulation of mammary circulation and milk production between 87.5%HF and 50%HF animals, since a number of studies have demonstrated that similar proportion increases in milk secretion and mammary blood flow occurred during growth hormone treatment of goats and cows (Hart et al., 1980; Davis et al., 1983). A marked change in blood flow to the mammary gland during the transitional period from early lactation to mid-lactation was indicated by the constancy of the plasma volume and blood volume. Regulation of mammary blood flow from the early period to mid-period of lactation in 87.5%HF animals could be regarded a major homeorhetic principle (Bauman & Currie, 1980).

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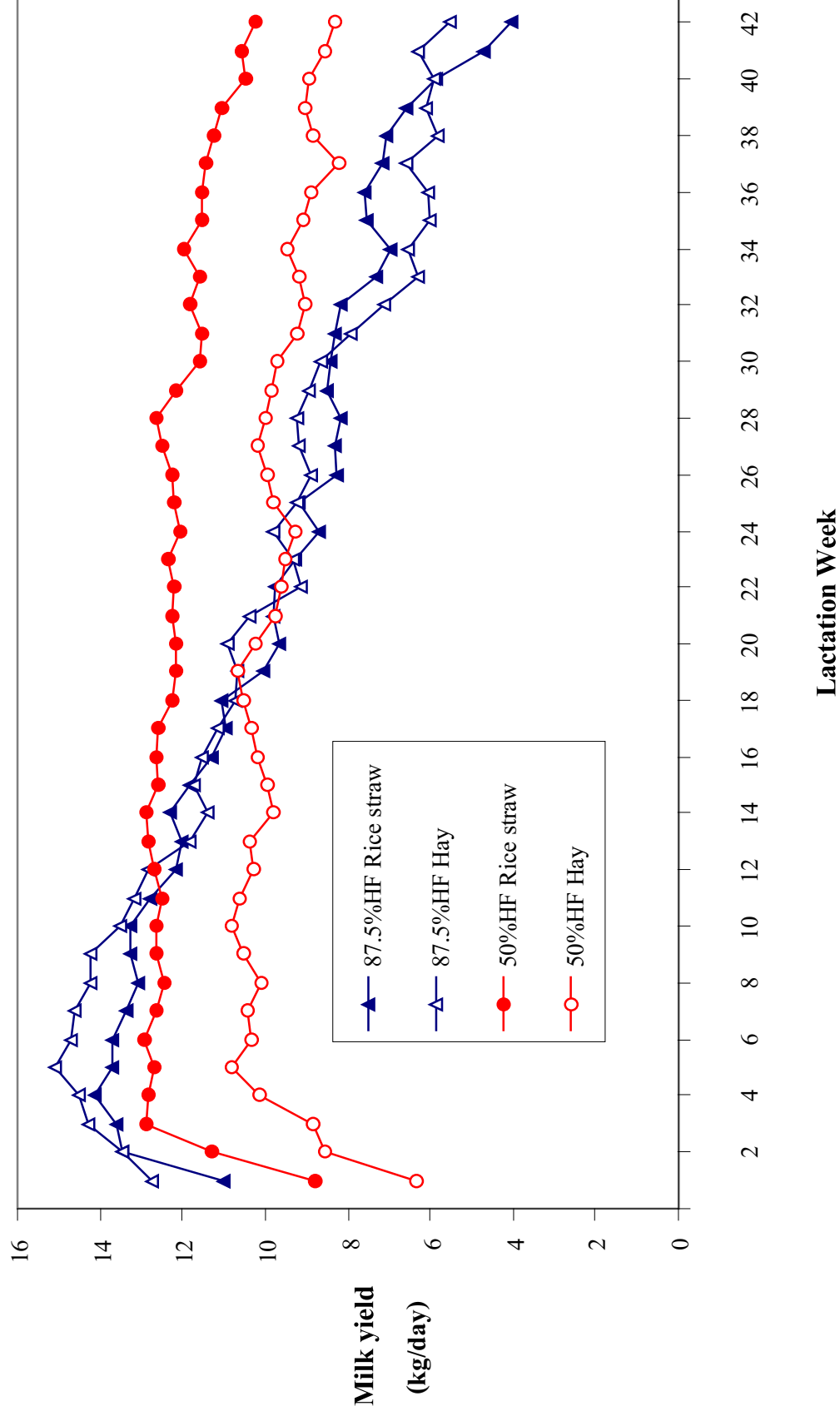


Fig. 1 Average weekly milk yield (kg) measured throughout lactation in crossbred Holstein Friesian (87.5%HF, 50%HF) feeding on either hay or urea treated rice straw.

Comparative studies of glucose metabolism in vivo in crossbred Holstein cattle feeding on different types of roughage at different stages of lactation

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Abstract

An experiment was carried out to study the glucose kinetics of crossbred Holstein cattle feeding on either hay or 5% urea treated rice straw during early lactation (30 days post partum), mid-lactation (120 days post partum) and late lactation (210 days post partum). In all 16 first lactating crossbred Holstein cattle were selected for the experiment including eight animals of two breed types, Holstein Friesian x Red Sindhi (50:50 = 50%HF) and Holstein Friesian x Red Sindhi (87.5:12.5 = 87.5%HF). They were divided into four groups of four animals each with two groups of each 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. In early lactating 87.5%HF animals feeding on either hay or urea treated rice straw, the high milk yields and lactose secretion were related to glucose uptake by the udder and udder blood flow as compared with those of 50%HF animals. The marked decreases in udder blood flow, glucose uptake, lactose secretion and milk yield were apparent in mid- and late lactation of both groups of 87.5%HF animals. In contrast to both groups of 50%HF animals, no significant changes in udder blood flow, udder glucose uptake, lactose secretion and milk yields were apparent throughout the course of lactation. Total glucose entry rate using 3-³H] glucose infusion, recycling of carbon glucose and plasma glucose clearance significantly increased during late lactation for 50%HF and 87.5%HF animals feeding on urea treated rice straw. The utilization rates of glucose using [U-¹⁴C] glucose infusion were not significantly different among groups of animals and periods of lactation. It can be concluded that 87.5%HF animals have the genetic potential for a higher milk yield but a shorter peak yield and poorer persistency in comparison with 50%HF animals. Changes in the utilization of glucose by the mammary gland for milk production in both groups of crossbred animals during feeding on either hay or urea treated rice straw would be dependent on changes in intramammary factors.

Key words: Crossbred Holstein cattle; Glucose turnover rate; Udder glucose uptake;

Urea treated rice straw

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1. Introduction

The major problem for dairy farming in tropical countries is the low milk yield of indigenous dairy cattle. The fastest way to improve the dairy potential of dairy cattle is to introduce inheritance from *Bos taurus* dairy breeds. Exotic *Bos taurus* breeds have higher milk production, but they have inherent disadvantageous traits for a low heat tolerance (Maust et al., 1972). *Bos indicus* cattle have a low genetic potential for milk production but are well adapted to the high ambient temperature. Crossbreeding has therefore 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. However, 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, although some information on the physiological performance of different types of crossbred dairy cattle has been reported (Chaiyabutr et al., 1977; Nakamura et al., 1986).

It is recognized that ruminants fed with low quality roughage cannot supply sufficient nutrients to meet maintenance requirements. One of the problems which may limit milk production of dairy cattle in the tropics 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. To overcome the livestock feed problem, several chemicals such as urea have been used to improve the feeding value of low quality roughage (Klopfenstein, 1978). An improvement in rice straw by treating with urea to help animals survive during periods of scarcity has been reported (Jayasuriya and Perera, 1982; Promma et al., 1994). There is less information on the responses of bodily functions in lactating animals after prolonged feeding with urea treated rice straw as roughage.

It is known that the onset of lactation in dairy cows is associated with the demand for utilization of glucose from fetal development to milk production (Chaiyabutr et al., 1998). Large quantities of glucose are removed by the mammary glands for synthesis of lactose, the major osmotic component of milk (Annison and Linzell, 1964). An increase of milk yield can be achieved only by increasing the rate of lactose synthesis. The lactating udder utilizes most of the glucose entering the circulation of ruminants and irreversible glucose loss from plasma is highly correlated with lactose output (Bickerstaffe et al., 1974; Horsfield et al., 1974). The role of glucose in regulating milk secretion has also been demonstrated in the isolated perfused udder (Hardwick et al., 1961). Transport of glucose in the mammary gland is a rate-limiting step for milk synthesis which has been shown to be related to glucose transporters in the acinar cell (Burnool et al., 1990). The provision of glucose in a ruminant is entirely by endogenous

synthesis from non-carbohydrate sources by gluconeogenesis (Bergman, 1973). Blood glucose concentration could be influenced by both feed quality and quantity (McClure, 1977a, 1977b). Despite several studies, the utilization of glucose in the udder related to bodily glucose metabolism in crossbred dairy cattle during feeding with different types of roughage remains unknown. Knowledge and understanding of glucose metabolism in whole animals and in the mammary glands of crossbred animals at different stages of lactation may help to identify animals for potential high yielding ability and may assist in improving crossbred dairy cattle management. Therefore, the present experiment was conducted to obtain the above information on whether the responses in glucose metabolism are the same in both breeds of cattle feeding on different types of roughage. In the present glucose kinetic study, both (U-¹⁴C)-glucose and (3-³H)-glucose infusions in crossbred animals were studied at different stages of lactation: early, mid- and late lactation, as they were fed either hay or urea treated rice straw through the period of the experiment.

2. Materials and methods

2.1 Animals and management.

Sixteen first lactating crossbred Holstein cattle were selected for the experiments. These animals consisted of eight animals of two breed types, Holstein Friesian x Red Sindhi (50:50 = 50%HF) and Holstein Friesian x 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 $34\pm1^{\circ}\text{C}$ and the minimum temperature at night was $26\pm1^{\circ}\text{C}$. Before parturition, animals were individually fed a concentrate of an average of 4.0 kg/day (DM basis) and roughage to maintain the body condition score at three until calving. 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 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 x 21 day system of urea treatment. After 21 days, the treated rice straw with 5% urea was offered to the animals.

2.2 Experimental procedures.

Three consecutive periods of experiments were carried out in each group. Period 1 began 30 days postpartum (early lactation). Period 2 began 120 days postpartum (mid-lactation) and period 3 began 210 days postpartum (late lactation). Animals were fed the same ration through the completion of period 3. In all periods of experiments, the glucose turnover rate, mammary udder blood flow, udder glucose uptake, milk yield and lactose output were measured. Animals were normally milked at around 0600 h and 1700 h. On the day of the experiment, a blood sample was taken in the morning (~1100 h) from the jugular vein into the heparinized tube. Plasma from this sample was kept at -40°C until insulin and glucagon concentrations were measured. Milk secretion was recorded by hand milking in the afternoon and the measurement of udder blood flow was carried out. Animals were weighed after collecting the milk sample.

On the day before the experiment began in each lactating period, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm) were inserted into either the left or right subcutaneous abdominal vein (milk vein) by using a intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, U.K.) under local anesthesia. This was done in standing animals for the measurement of mammary udder blood flow and for collection of venous blood. The tip of the catheter was positioned near the sigmoid flexure anterior to the point at which the vein leaves the udder. The other catheter was positioned downstream about 20 cm from the first one. The catheter for isotope infusion was inserted into an ear vein under local anesthesia. All catheters were flushed with sterile heparinized normal saline and were left in place during the experiment.

2.3 Glucose turnover measurements.

Glucose kinetic studies of each animal in each lactating periods was carried out as described previously by Chaiyabutr et al. (1998). Briefly, at about 1100h a priming dose of radioactive glucose in 20 ml of sterile NSS containing 60 $\mu\text{Ci}(3\text{-}^3\text{H})$ glucose and 40 $\mu\text{Ci}(\text{U-}^{14}\text{C})$ glucose was administered intravenously via the ear vein catheter and followed by a constant infusion of 1 ml/min of sterile saline (0.9%) containing 2 $\mu\text{Ci}(\text{U-}^{14}\text{C})$ glucose and 3 $\mu\text{Ci}(3\text{-}^3\text{H})$ glucose for 4h (Peristaltic pump; EYLA Model 3). During the final 1 hour (1400-1500h) of infusion, three sets

of blood samples were collected at 20 min. intervals. A venous blood sample was collected from the milk vein via a catheter while an arterial blood sample was collected from the coccygeal artery by venipuncture with a #21 needle. Blood samples in heparinized tubes were kept in crushed ice for chemical studies.

2.4 Udder blood flow measurements.

Udder blood flow measurements were performed in duplicate. Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion as described by Chaiyabutr et al. (1997). In brief, a dye (T-1824) was dissolved in sterile normal saline and diluted to a concentration of 100 mg/L. The solution was infused by a peristaltic pump (Gilson Medical electronics) at a constant rate of 85 ml/min into the milk vein for 1 min which could produce adequate mixing of dye with blood. Before infusion, blood was drawn from downstream in the milk vein as a pre-infusion sample. About 10 seconds after starting the infusion, 10 ml of blood was drawn from downstream in the milk vein at a constant rate into a heparinized tube. Two consecutive plasma samples were taken during each dye infusion at about 5 min intervals. Blood flow of half of the udder was calculated from plasma samples using the equation derived by Thompson and Thomson (1977). Quarter milking showed that the yields of the two halves of the udder were similar. Udder blood flow was therefore calculated by doubling the flow measured in one milk vein (Bickerstaffe et al., 1974). Packed cell volume was measured after centrifugation of the blood in a microcapillary tube.

2.5 Chemical methods.

All chemical and biochemical enzymes were obtained from Sigma Chemical Co. Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase. The concentration of milk lactose was determined by spectrophotometry (Teles et al., 1978). Radiochemicals for (U-¹⁴C)-glucose and (3-³H)-glucose were obtained from the Radiochemical Center, Amersham Bucks, U.K. The isotopes were dissolved in sterile pyrogen free saline (0.9% NaCl). The specific activity of labeled plasma glucose was determined by the method described by Chaiyabutr and Buranakarl (1989). The radioactivity in blood bicarbonate was measured by acidifying 2 ml of blood with an equal volume of 6% perchloric acid. The ¹⁴CO₂ was liberated and trapped as K¹⁴CO₃ in a plastic cup which contained 0.1 ml 40% KOH. Plasma insulin concentration was measured using a radioimmunosay (RIA) kit (Coat-a Count®

Insulin, Diagnostic Products, Los Angeles, CA, USA.). Plasma glucagon concentration was measured using a RIA kit (Glucagon double antibody, Diagnostic Products, Los Angeles, CA, USA.).

2.6 Calculations.

The glucose turnover rate in the whole animal (T), expressed as $\mu\text{mol}/\text{min}$, was calculated from the equation

$$T = I/G_A, \quad (1)$$

where I = rate of infusion of ($\text{U-}^{14}\text{C}$) glucose or ($3\text{-}^3\text{H}$) glucose ($\mu\text{Ci}/\text{min}$) and G_A = specific activity of ^{14}C - or ^3H -glucose in arterial plasma at equilibrium ($\mu\text{Ci}/\mu\text{mol}$).

Recycling of glucose carbon in the whole animal, expressed as % glucose turnover, was calculated from the equation

$$\text{Recycling} = (T_3 - T_{14}) \times 100 / T_3, \quad (2)$$

where T_3 = total turnover rate of glucose calculated from ($3\text{-}^3\text{H}$) glucose and T_{14} = turnover rate of glucose calculated from ($\text{U-}^{14}\text{C}$) glucose.

Glucose clearance in the whole animal (C_G) expressed as ml of plasma cleared of body glucose per minute was calculated from the equation

$$C_G = T_3 / P_A, \quad (3)$$

where T_3 = turnover rate of glucose calculated from ($3\text{-}^3\text{H}$) glucose ($\mu\text{mol}/\text{min}$) and P_A = arterial plasma glucose concentration ($\mu\text{mol}/\text{ml}$).

Uptake of glucose by the udder (U_M), expressed as $\mu\text{mol}/\text{min}$, was calculated from the equation

$$U_M = Q_P \times (P_A - P_V), \quad (4)$$

where Q_P = udder plasma flow (ml/min), P_A = concentration of glucose in coccygeal arterial plasma ($\mu\text{mol}/\text{ml}$) and P_V = concentration of glucose in mammary venous plasma ($\mu\text{mol}/\text{ml}$).

The extraction ratio (%) of glucose by the udder was calculated by dividing the arteriovenous difference ($P_A - P_V$) by arterial plasma glucose concentration (P_A).

Glucose oxidation for non-mammary tissue was estimated from the CO_2 produced by the animal. The ratio of specific radioactivity of arterial blood bicarbonate relative to that of arterial

blood glucose- C^{14} was measured. The arterial blood CO_2 for calculated specific radioactivity was measured by a blood gas analyzer (238PH/blood gas analyzer, Ciba Corning).

2.7 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 lactating periods in each group using the paired t-test. Mean values are presented as mean $\pm$ SD.

3. Results

3.1 Changes in glucose metabolism during course of lactation (Table 2)

The glucose turnover rate in crossbred Holstein was determined by making simultaneous estimates of the total glucose entry rate using 3- $[^3H]$ glucose infusion and utilization rate of glucose using $[U-^{14}C]$ glucose infusion. All values of glucose turnover rates in different periods of lactation for all groups of crossbred animals are expressed as absolute values or as a function of metabolic body size. In early lactation, there were no significant differences of the total glucose entry rate and glucose carbon recycling among groups of crossbred animals feeding on either hay or urea treated rice straw. However, in mid- and late lactation of 50%HF and 87.5%HF animals feeding on urea treated rice straw, the total glucose turnover rates and recycling of carbon glucose were markedly higher than those of crossbred HF animals feeding on hay as roughage ($P < 0.05$). Comparing for the early lactating period in the same group, both 50%HF and 87.5%HF animals feeding on urea treated rice straw showed significant increases in the total glucose turnover rate ($P < 0.05$), recycling of carbon glucose ($P < 0.05$) and plasma glucose clearance ($P < 0.01$) during late lactation, whereas there were no significant changes for both groups of crossbred animals feeding on hay. There were no significant differences of utilization rates of glucose in comparison among groups of animals and periods of lactation. The ratios of the specific radioactivity of arterial blood bicarbonate relative to that of arterial blood $[C^{14}]$ glucose, which indicated the rate of glucose oxidation in early lactating period, were significantly higher in 87.5%HF than in 50%HF fed either hay or urea treated rice straw. This result may indicate that the rate of glucose oxidation of 87.5%HF was higher than that of 50%HF animals in the early lactating period. As lactation advanced, the rate of glucose

oxidation increased in both groups of animals feeding on urea treated rice straw, while a decreasing trend was apparent in both groups of animals feeding on hay.

3.2 Changes in arterial plasma glucose concentration, udder glucose uptake, plasma insulin concentration and plasma glucagon concentration (Table 3)

In the early lactating period, the plasma glucose concentrations of both groups of 50%HF animals were significantly higher ($P<0.05$) than those of 87.5%HF animals fed either hay or urea treated rice straw. During advanced lactation, the same trends were observed for the reductions of plasma glucose concentration in mid- and late lactating periods in all groups of crossbred animals. In early lactation, glucose uptake by the udder of 87.5%HF animals were significantly higher ($P<0.05$) than those of 50%HF animals fed either hay or urea treated rice straw. The extraction ratios for glucose across the mammary gland during early and mid-lactation of both groups of 87.5%HF animals were significantly higher ($P<0.05$) than those of 50%HF animals, except during the late lactating period when they decreased for both groups of 87.5%HF animals. The values of arteriovenous concentration differences of glucose across the mammary gland approximately maintained the same levels for all experimental groups. Mean average values of glucose uptake by the udder relative to the total glucose turnover rate in both groups of 87.5%HF animals were higher than those of 50%HF animals fed either hay or urea treated rice straw. These values decreased during advanced lactation for all groups of experimental animals.

The concentration of insulin and glucagon in plasma of crossbred animals is also presented in Table 3. Insulin concentrations were not different among groups of crossbred animals and were not altered among periods of lactation in the same groups. In the early lactating period, glucagon concentrations in plasma were not significantly different among groups of crossbred animals feeding on either hay or urea treated rice straw. A trend toward increased plasma glucagon concentrations during mid- and late lactation were observed in both 50%HF and 87.5%HF animals feeding on urea treated rice straw.

3.3 Changes in udder blood flow, milk yield and lactose secretion (Table 4)

In 87.5%HF animals, feeding on either hay or urea treated rice straw, mammary blood flow and milk yield initially showed significantly higher levels ($P<0.05$) in early lactation than those of 50%HF animals. No difference in the ratio of mammary blood flow to the rate of milk secretion was observed among groups of crossbred animals in the early lactating period. In mid- and late lactation, both mammary blood flow and milk yield showed a proportional decrease from the early lactating period in both groups of 87.5%HF animals. However, for 50%HF animals feeding on either hay or urea treated rice straw, the trends for persistency were observed

as for udder blood flow and milk yield. No statistical interaction between the period of lactation and type of roughage was detected in 50%HF animals. Milk lactose yields in early lactation of 87.5%HF animals feeding on either hay or urea treated rice straw were significantly higher ($P < 0.05$) than those of 50%HF animals. The values of milk lactose concentration showed no differences among groups of crossbred animals or among periods of lactation in the same group.

4. Discussion

It is known that glucose is used primarily by the udder for milk lactose synthesis. Lactose secretion determines milk secretion as a whole by water following lactose down an osmotic gradient (Linzell and Peaker, 1971). In the early lactating 87.5%HF animals feeding on either hay or urea treated rice straw, the high milk yields were related to high lactose yields when compared to those of 50%HF animals. In this period, the high lactose output and milk yield have also been shown to be associated with the high body glucose turnover rate. This result is in agreement with the observation of Horsfield et al. (1974) for a correlation between glucose irreversible loss from plasma and lactose output in high milk yield cows. However, the existence of such high rates of both lactose secretion and milk yield in early lactating 87.5%HF animals would be primarily related to an increase in glucose uptake by the udder.

In the present study, changes in the rate of glucose uptake by the udder of both crossbred animals feeding on either hay or urea treated rice straw seem to be dependent on both mammary blood flow and activity of the mammary epithelial cells during the course of lactation. The high mammary glucose uptake in early lactating 87.5%HF animals was dependent upon neither the arterial plasma glucose level nor the mammary glucose arteriovenous concentration differences, which confirms a previous conclusion that mammary glucose uptake is determined mainly by mammary blood flow (Linzell, 1974). During early and mid-lactation, the extraction ratios of glucose by the mammary gland in both groups of 87.5%HF animals was higher than those of 50%HF animals feeding on either hay or urea treated rice straw. The average values of glucose uptake by the udder relative to the total glucose turnover rate of 87.5%HF animals were also higher than those of 50%HF animals in all periods of lactation. These results indicate that 87.5%HF animal had a higher activity of the mammary secretory cells than 50%HF animals. However, during late lactation, both groups of 87.5%HF animals showed reductions in the mammary extraction ratio and arteriovenous concentration differences of glucose which were associated with the decrease in mammary blood flow. The decline in mammary blood flow may be the result of a negative feedback response to reduced demand for substrates by the udder which would be the consequence of a decrease in the amount of mammary secretory tissue

(number of mammary cells) in this period. A possible decrease in the secretory activity of mammary cells during declining lactation is supported by the marked increase in the ratio of mammary blood flow to the rate of milk secretion during late lactation in both groups of 87.5%HF animals fed either hay or urea treated rice straw. Another possible explanation for the decrease in mammary blood flow during late lactation in 87.5%HF animals might be a reduction of the production of vasodilator agent in the mammary gland during advanced lactation (Linzell, 1974). In contrast to lactating 50%HF animals feeding on either hay or urea treated rice straw, no significant changes in the rates of udder blood flow, udder glucose uptake, lactose secretion and milk yield were apparent throughout the course of lactation. This indicates that 50%HF animals can maintain the amount and functional integrity of the secretory tissue during feeding on either hay or urea treated rice straw. The different results for 50 and 87.5%HF animals without reduction in feed intake can be attributed to a difference in inherent characteristics of the animals.

The studies on body glucose kinetics in dual isotope experiments are able to determine both the utilization and total entry rates of glucose turnover. All values of glucose turnover rates in all groups are within the range reported in high milk yield cows of comparable metabolic body size (Bickerstaffe et al., 1974), suggesting animals were synthesizing enough glucose for normal metabolism. In ruminants, the provision of glucose is almost entirely by gluconeogenesis from non-carbohydrate precursors (Bergman, 1973). Blood glucose concentrations are influenced by feed quality (McClure, 1977b) and quantity (Fisher et al., 1975). In the present studies, both 87.5%HF and 50%HF animals were fed either hay or urea treated rice straw while they were maintained on a similar concentration intake throughout all lactating periods. If availability of exogenous glucose absorption from the digestive tract is assumed to remain constant throughout lactating periods in all groups, the relatively constant plasma glucose concentrations among periods of lactation in all groups indicate that steady state conditions between the rate of utilization and the rate of gluconeogenesis existed in the body pool of glucose. A marked increase of the total entry rate of glucose turnover during the transition period from early to mid- and late lactation was apparent in both 87.5%HF and 50%HF animals feeding on urea treated rice straw as roughage, whereas there were no apparent differences in the glucose utilization rates among groups of animals. These results indicate that in maintenance, crossbred animals feeding on urea treated rice straw resynthesize more glucose than those animals feeding on hay. It is clear that the increase in the total turnover rate in late lactating animals feeding on urea treated rice straw is not due to an increase in glucose uptake by the udder. A marked increase in the plasma glucose clearance was noted when lactation advanced. In contrast to both 87.5%HF and 50%HF animals feeding on hay as roughage, plasma glucose clearance was maintained at

similar values during the course of lactation. These different results also suggest that crossbred animals feeding on urea treated rice straw had a higher body glucose metabolism than animals feeding on hay.

There was no apparent evidence for an increase in the rate of glucose uptake by the udder relative to the whole body glucose turnover rate during advanced lactation in the same crossbred HF animals feeding on urea treated rice straw. This suggests that other non-mammary tissues of animals increased the rate of glucose utilization. An increase in the rate of glucose utilization by non-mammary tissue could be calculated when the glucose uptake was subtracted from the total glucose turnover rate. Another indication of an increased rate of glucose utilization by the non-mammary tissue was the increase in the specific radioactivity of blood bicarbonate relative to that of blood glucose, suggesting an increased rate of glucose oxidation. An increase in glucose carbon recycling during advanced lactation in crossbred animals feeding on urea treated rice straw was associated with an increase in the total glucose turnover rate. The reasons for a change in the pattern of glucose metabolism and conservation of glucose carbon in late lactating HF animals normally feeding on urea treated rice straw are still unknown. It is known that an increase in glucose turnover rate is related to an increase in gluconeogenesis in ruminants (Lindsay, 1971), which is subject to both nutritional and hormonal controls. The high values of the total entry rate of glucose turnover in animals feeding on urea treated rice straw during lactation advanced may reflect a sufficient intake of glucose precursor rather than maintenance. The increase in crude proteins in the urea treated rice straw is non-protein nitrogen (Table 1), and it has been shown that crossbred Holstein animals can utilize this efficiently (Promma et al., 1994). This nutritional effect would therefore be expected to involve glucogenic amino acid which would be related to increased gluconeogenesis. However, other effects may be brought about by changes in endocrine status that influence glucose metabolism rather than by elevating a nutrient efficiency per se. In the present study, insulin concentrations in the plasma were not altered among groups of animals, which confirms the previous conclusion that insulin does not appear to alter the metabolism of glucose in ruminants (Laarveld et al., 1985). An increase in the circulating glucagon concentrations during mid- and late lactation in both groups of crossbred HF animals feeding on urea treated rice straw may stimulate increased gluconeogenesis. Interactions probably exist so that the increased supply of glucogenic amino acids elicits secretion of glucagon which in turn stimulates gluconeogenesis (Danfaer, 1994).

In conclusion, the results obtained from this study provide evidence for the suggestion that there were no differences in general physiological performance in the same crossbred cattle fed either hay or urea treated rice straw. The 87.5%HF animals have the genetic potential for a high milk yield but with a shorter peak yield and poorer persistency in comparison with 50%HF

animals. The difference between 50% and 87.5%HF animals for changes of extramammary glucose metabolism could not be interpreted conclusively in terms of changes in the utilization of glucose by the mammary glands in crossbred HF animals feeding on either hay or urea treated rice straw. The difference in the regulation of milk production relating to the utilization of glucose by the mammary gland between 50%HF and 87.5%HF animals is dependent on changes in intramammary factors. Several mechanisms controlling the utilization of glucose within the mammary gland may involve, for example, growth hormones facilitating the transport of glucose into the mammary cell (Mephram et al., 1990), or specific glucose transporters in mammary cell membranes (Burnol et al., 1990; Shennan and Beechey, 1995). Additional experiments on 50%HF and 87.5%HF animals are necessary to describe more precisely their metabolic peculiarities and, in particular, the intracellular translocation of glucose in the mammary gland.

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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).

Table 2

Aspects of glucose metabolism in different periods of lactation of crossbred Holsteins fed with hay and urea treated rice straw (n=4 in each group)

	Period	Hay+concentration		Urea treated rice straw+concentration	
	of lactation	HF:RS (87.5:12.5)	HF:RS (50:50)	HF:RS (87.5:12.5)	HF:RS (50:50)
Glucose turnover rate					
(3- ³ H) glucose (μmol/min)	Early	5662.6±695.5 ^a	4965.6±564.2 ^a	4713.5±804.5 ^a	5115.1±567.6 ^a
	Mid	4587.5±1198.8 ^b	5514.8±803.6 ^{ab}	5225±1081.7 ^{ab}	6481.2±988.6 ^a
	Late	4602.8±900.9 ^{c*}	5766.1±669.0 ^{bc}	6657.5±1313.1 ^{ab**}	7453.9±862.4 ^{a*}
(U- ¹⁴ C)glucose (μmol/min)	Early	4712.3±747.5 ^a	3980.4±399.1 ^a	4471.2±751.9 ^a	3911.8±726.5 ^a
	Mid	3874.3±757.4 ^a	3696.1±270.8 ^a	3755.4±540.9 ^a	4301.4±390.8 ^a
	Late	3793.0±475.6 ^{ab}	3669.8±331.8 ^b	4186.3±691.3 ^{ab}	4451.3±409.6 ^a
(3- ³ H) glucose (μmol/min/kg ^{0.75})	Early	68.5±7.5 ^{ab}	65.6±5.8 ^{ab}	58.8±10.9 ^b	73.5±3.7 ^a
	Mid	52.8±7.1 ^b	69.3±7.7 ^{ab}	61.7±13.5 ^b	81.3±12.9 ^a
	Late	57.0±11.1 ^c	68.3±8.1 ^{bc}	78.2±15.2 ^{ab}	88.7±9.9 ^a
(U- ¹⁴ C)glucose (μmol/min/kg ^{0.75})	Early	56.9±7.3 ^a	52.6±3.4 ^a	48.2±7.3 ^a	52.8±8.4 ^a
	Mid	44.9±3.0 ^b	46.5±1.8 ^b	44.2±5.1 ^b	53.9±5.7 ^a
	Late	46.9±4.9 ^{ab}	43.4±2.8 ^b	49.1±7.7 ^{ab}	53.1±6.2 ^a
Glucose-C recycling (%)	Early	16.8±7.5 ^a	19.7±1.9 ^a	16.1±6.8 ^a	23.8±6.7 ^a
	Mid	15.2±5.5 ^b	32.1±9.8 ^{ab}	25.6±17.8 ^{ab}	33.2±4.4 ^{a*}
	Late	16.6±7.2 ^b	35.9±6.8 ^{a*}	35.8±12.4 ^{a*}	40.1±3.6 ^{a**}
Plasma glucose clearance (ml/min)	Early	1702.1±409.9 ^a	1127.5±197.8 ^b	1333.4±211.9 ^{ab}	1288.6±70.3 ^b
	Mid	1471.2±417.8 ^a	1486.8±358.6 ^a	1534.7±130.5 ^a	1618.3±59.0 ^{a**}
	Late	1296.2±177.1 ^c	1488.7±106.2 ^{bc}	1749.1±262.2 ^{b**}	2122.8±244.1 ^{a**}
Arterial blood ¹⁴CO₂/ ¹⁴C glucose	Early	1.09±0.13 ^a	0.64±0.04 ^b	0.74±0.13 ^b	0.69±0.14 ^b
	Mid	0.75±0.19 ^a	0.81±0.13 ^a	0.81±0.26 ^a	0.81±0.12 ^a
	Late	0.79±0.20 ^{ab}	0.54±0.05 ^b	0.87±0.25 ^a	0.75±0.05 ^{ab}

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).

Table 3

Arterial plasma glucose concentration, udder glucose uptake, plasma insulin concentration and plasma glucagon concentration in different periods of lactation of crossbred Holsteins fed with hay and urea treated rice straw (n=4 in each group)

	Period of	Hay+concentration		Urea treated rice straw+concentration	
	lactation	HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Glucose					
Arterial concentrate (μmol/ml)	Early	3.40±0.53 ^b	4.46±0.42 ^a	3.54±0.19 ^b	4.16±0.13 ^a
	Mid	3.15±0.46 ^b	3.77±0.35 ^{ab*}	3.39±0.56 ^{ab}	3.99±0.45 ^a
	Late	3.54±0.40 ^a	3.86±0.19 ^{a*}	3.79±0.33 ^a	3.52±0.17 ^{a*} _*
A-V (μmol/ml)	Early	0.74±0.04 ^a	0.83±0.12 ^a	0.90±0.15 ^a	0.78±0.05 ^a
	Mid	0.71±0.08 ^a	0.77±0.08 ^a	0.83±0.19 ^a	0.69±0.04 ^a
	Late	0.66±0.08 ^b	0.81±0.08 ^a	0.76±0.09 ^{ab}	0.71±0.09 ^{ab}
Extraction ratio (%)	Early	22.1±3.1 ^{ab}	18.6±1.9 ^b	25.5±3.9 ^a	18.8±1.5 ^b
	Mid	23.2±4.3 ^{ab}	20.6±2.5 ^{ab}	24.5±4.5 ^a	17.5±2.5 ^b
	Late	18.7±0.6 ^a	21.0±1.9 ^{a*}	20.0±1.9 ^{a*}	20.2±3.3 ^a
Udder uptake(μmol/min)	Early	3877±1000 ^a	2230±291 ^b	3113±830 ^{ab}	2618±386 ^b
	Mid	2482±483 ^a	2157±275 ^a	2225±169 ^a	2450±437 ^a
	Late	2451±604 ^{a*}	2195±454 ^a	2158±591 ^{a*}	2640±479 ^a
Udder glucose uptake/ Glucose turnover (%)	Early	68.1±16.8 ^a	45.4±7.7 ^b	69.1±21.3 ^{ab}	51.9±12.1 ^{ab}
	Mid	55.3±9.8 ^a	39.4±4.9 ^b	44.8±10.0 ^{ab*}	38.6±9.8 ^b
	Late	53.3±8.2 ^a	37.9±4.6 ^b	32.9±8.4 ^{b*}	35.6±6.9 ^{b*}
Insulin (μU/ml)	Early	16.94±10.87 ^a	18.93±5.57 ^a	20.37±10.36 ^a	27.13±15.65 ^a
	Mid	21.45±9.46 ^a	25.07±18.43 ^a	25.97±6.96 ^a	20.75±6.56 ^a
	Late	17.13±4.71 ^a	17.63±4.82 ^a	25.00±1.86 ^a	28.25±16.35 ^a
Glucagon (pg/ml)	Early	29.5±5.4 ^a	59.9±17.7 ^a	80.0±52.5 ^a	74.6±39.0 ^a
	Mid	46.6±6.2 ^b	105.3±46.7 ^{ab}	118.8±45.3 ^{a*}	131.9±43.2 ^{a*}
	Late	53.5±35.7 ^c	97.7±12.3 ^{ab**}	77.5±6.1 ^{bc}	124.5±29.3 ^{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).

Table 4

Udder blood flow, milk yield and lactose secretion in different periods of lactation of crossbred Holsteins fed with hay and urea treated rice straw (n=4 in each group)

	Period of lactation	Hay+concentration		Urea treated rice straw+concentration	
		HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Udder blood flow (ml/min)	Early	7160±1807 ^a	3887±543 ^b	4619±1149 ^b	4314±575 ^b
	Mid	4745±836 ^a	4090±398 ^a	3843±872 ^a	5068±1054 ^a
	Late	5026±724 ^{ab}	3942±500 ^b	3995±883 ^b	5371±932 ^a
Milk yield (ml/min)	Early	13.73±3.11 ^a	7.62±0.8 ^b	11.46±4.10 ^{ab}	8.96±1.09 ^b
	Mid	7.64±1.12 ^{a*}	7.31±0.9 ^a	8.13±0.64 ^a	8.56±1.71 ^a
	Late	7.02±0.48 ^{ab*}	7.27±0.6 ^{ab}	6.37±0.84 ^{b*}	8.51±1.74 ^a
Udder blood flow/Milk yield	Early	519±24 ^a	508±19 ^a	413±53 ^a	480±23 ^a
	Mid	620±37 ^{a*}	563±51 ^{ab}	476±129 ^b	594±66 ^{ab}
	Late	713±63 ^{a*}	540±38 ^b	624±87 ^{ab*}	634±25 ^{ab}
Lactose in milk (mmol/100 ml)	Early	13.42±0.23 ^a	12.79±0.41 ^a	13.48±0.18 ^a	13.49±0.47 ^a
	Mid	13.40±0.21 ^a	13.15±0.25 ^a	13.49±0.44 ^a	13.44±0.29 ^a
	Late	13.13±0.51 ^a	13.15±0.46 ^a	13.31±0.47 ^a	13.00±0.32 ^a
Milk lactose secretion (μmol/min)	Early	1845.7±438.8 ^a	976.3±122 ^b	1543.8±544.6 ^{ab}	1210.6±156.8 ^b
	Mid	1025.7±165.4 ^{a*}	958.9±110 ^a	1096.5±59.5 ^a	1149.4±225.4 ^a
	Late	921.9±77.6 ^{ab*}	955.7±72 ^{ab}	846.9±101.4 ^{b**}	1104.4±211.9 ^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} Mean values within a row indicated with different superscripts are significantly different (P<0.05).

(Running head : Milk urea and allantoin with the forage type in crossbred Holstein cattle)

Urea and allantoin in milk of crossbred Holstein cattle feeding on different types of roughage

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ABSTRACT : The present study was carried out to quantify excretions of urea and allantoin in milk of 16 crossbred Holstein Friesian (HF) during feeding with different types of roughage and to evaluate milk urea and milk allantoin concentration as estimators of nutritional status and microbial protein synthesis in the rumen, respectively. The experimental animals consisted of eight animals of two breed types, 50%HF and 87.5% animals, which were divided into four groups of 4 animals each. Animals in each group from the same breed type were fed with either pangola hay or urea treated rice straw as the source of roughage throughout experiment. Plasma and milk samples were obtained from three consecutive periods of lactation (early, mid and late lactation). Concentrations of urea, allantoin and other metabolites in milk and plasma were determined. The high correlation between the milk urea concentration and plasma urea concentration was apparent in all periods of lactation of both 50%HF and 87.5%HF animals feeding on different types of roughage. Concentrations of both plasma and milk urea of animals feeding on urea treated rice straw were higher than those animals fed with hay. In all groups of HF crossbred animals, the type of roughage and the level of feeding have been shown to have no effects on the concentration of milk allantoin in all periods of lactation. Breed differences were in general small and non-significant for both plasma and milk urea concentrations but were significant for the plasma glucose concentration. The mean average plasma glucose concentration of 50%HF animals had a tendency to be higher than for 87.5%HF animals during feeding different types of roughage. The values of the plasma protein and milk protein concentrations in all groups were not affected by feeding on either hay or urea treated rice straw among all periods of lactation. No significant difference was apparent for either urea-N or allantoin-N excreted in milk by

50%HF animals throughout all periods of lactation. Milk yield rather than the concentration of either urea or allantoin in milk appeared to be an important factor affecting their excretion in milk. In view of this it seems reasonable to assume that the relatively stable levels of both milk urea and milk allantoin concentrations indicate a constant level of feeding and synthesis of microbial protein in the rumen during feeding on either hay or urea treated rice straw as a source of roughage.

Key words: milk urea, milk allantoin, urea treated rice straw, crossbred Holstein

INTRODUCTION

One of factors which limit milk production of tropical dairy cattle is an inadequate supply of forages during the dry, summer months. They are fed mainly on crop residues such as rice straw which has a low nutritive value. It is recognized that ruminants fed with low quality roughage cannot supply sufficient nutrients to meet maintenance requirements. To overcome the livestock feed problem, several chemicals such as urea have been used to improve the feeding value of low quality roughage (Klopfenstein, 1978). The importance of an adequate nutrient supply for dairy cattle to maintain high production is well recognized. Nitrogen economy in the nutrition of ruminants is a problem of animal production. It is closely correlated with the utilization of endogenous urea nitrogen, a final product of the catabolic process of nitrogen metabolism. The plasma concentration of urea in ruminants is an indicator which is affected by dietary factors, especially the amount of protein ingested and the protein-energy ratio (Lewis, 1957; Waldo, 1968). The close correlation between the concentrations of urea in blood and milk has been demonstrated (Ide et al. 1966; Oltner and Wiktorsson, 1983; Roseler et al. 1990). So the study of milk urea concentrations has also been used as an indicator of nutritional status. Although milk urea accounts for the main fraction of the nitrogen in the non-protein nitrogen (NPN) compounds of milk in dairy cattle, another NPN fraction in milk is allantoin. This metabolite is the end product of purine metabolism which is derived from nucleic acid metabolism in the rumen microbes of ruminants (Rys et al. 1975). Few data are available on the study of nutritional status concerning bacterial N-formation in the rumen of crossbred dairy cattle feeding on different types of roughage. In the present study, concentrations of both urea and allantoin in milk were determined in crossbred Holstein cattle feeding on different types of roughage in

different periods of lactation. It is hoped that the results will make a useful contribution to the evaluation of milk urea and milk allantoin concentration as indicators of nutritional status and microbial protein synthesis in the rumen of crossbred lactating dairy cattle feeding on either hay or urea treated rice straw as a source of roughage.

MATERIALS AND METHODS

Animals and management

Sixteen first lactating crossbred dairy cattle were selected for the experiments. These animals consisted of eight animals of two breed types, Holstein Friesian x Red Sindhi (50:50 = 50%HF) and Holstein Friesian x 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 and tethered in individual stalls and fed twice daily. The maximum temperature in the shed at noon was $34\pm1^{\circ}\text{C}$ and minimum temperature at night was $26\pm1^{\circ}\text{C}$. Animals individually received an average of 3-5 kg/day of roughage in combination with the same concentrated mixture (7-10 kg/day) to maintain a moderate body condition score (2.5, scale = 1 to 5). The chemical composition of feeds is presented in Table 1. Each day, the food was given into equal portions at about 06.00 h and 17.00 h when animals were milked. Animals had free access to water and a lick block of minerals throughout the experiment.

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 x 21 day system of urea treatment. After 21 days, the rice straw treated with 5% urea was offered to the animals.

Experimental procedures

Three consecutive periods of experiments were carried out in each group. Period 1 was designed to begin 30 days postpartum (early stage of lactating period). Period 2 began 120 days postpartum (middle stage of lactating period). Period 3 began 210 days postpartum (later stage of lactating period). Animals were fed the same ration from before parturition

through the completion of period 3. The dry matter intake of each animal was determined by measuring both the concentrate and roughage offered and refused each day. Animals were normally milked at around 0600 h and 1700 h by a milking machine. On the day of the experiment, milk secretion was recorded by hand milking in the afternoon. During collection, milk was weighed and subsamples were kept on ice and stored at -20°C until analysis for urea, allantoin and protein concentrations. A blood sampling was carried out before milking from the external jugular vein into a heparinized tube at around 11.00 h. On the day of experiment of each period, two aliquot milk samples were taken from each cow and kept in a refrigerator until they were analyzed. To ensure that no deterioration of the milk occurred, a drop of formaldehyde (per 20 ml of milk) was added.

Analytical method

Subsamples of roughage (pangola hay or urea treated rice straw) and concentrate were dried, ground and retained for chemical analysis. Dry matter (DM) was determined by oven drying at 70°C ; crude protein was determined by Kjeldahl method according to AOAC (1981). Neutral detergent fiber (NDF) and acid detergent fiber were assessed using procedures of Goering and Van Soest (1970). The crude protein concentration in milk was determined by means of the micro-Kjeldahl method ($\text{N} \times 6.38$). The plasma protein concentration was determined by the method of Lowry et al. (1951). Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase. To determine the urea and allantoin content in milk, the fat from the milk was removed by centrifugation and the protein with 10% TCA was analyzed for urea and 5% uranyl acetate for allantoin. The allantoin analysis of the fat and protein-free milk was carried out by a colorimetric method according to Young and Conway (1942). Urea concentration in plasma and the fat and protein-free milk was determined by the diacetylmonoxime method (Coulombe and Favreau, 1963).

Statistics

All the results were statistically analyzed by analysis of variance (ANOVA); the significant differences between groups and treatments were compared by Duncan's multiple range test. Values were compared among periods of lactation in each group using the paired t-test. Mean values are presented as mean $\pm$ S.D.

RESULTS

Dietary intakes

The data in Table 1 show the differences of the chemical composition between pangola hay and urea treated rice straw. Compared with pangola hay, urea treated rice straw samples contained more crude protein, more acid detergent fiber (ADF) and less neutral detergent fiber (NDF). The daily intakes of hay and NDF for both groups of crossbred HF animals were significantly higher than for animals fed urea treated rice straw but the total concentrate, and roughage organic matter, and crude protein intakes were not significantly different between 50%HF and 87.5%HF animals fed either hay or urea treated rice straw in all periods of lactation (Table 2).

The concentrations of urea, protein, glucose and allantoin in plasma and milk

The overall means for the concentration of urea and protein in the plasma and milk at different periods of lactation of both 50%HF and 87.5%HF animals are given in Table 3. The correlation between the milk urea concentration (x) and the plasma urea concentration (y) for all periods of lactation of both 50%HF and 87.5%HF animals was $y = 8.85 + 0.477x$ ($r = 0.585$, $P < 0.001$, $n = 48$). However, both plasma urea and milk urea concentrations of 50%HF and 87.5%HF animals feeding on urea treated rice straw were significantly higher than for 87.5%HF animals fed with hay. The concentrations of both plasma and milk urea concentration of 50%HF animals were relatively higher than those of 87.5%HF animals during feeding with the same roughage. Comparing among periods of lactation in 50%HF and 87.5%HF animals fed either hay or urea treated rice straw, the milk urea concentration had a tendency to reduce in the first month of lactation. The values of the plasma protein, milk protein and milk allantoin concentrations were not significantly affected by the feeding on different types of roughage among all periods of lactation for both 50%HF and 87.5%HF animals. The plasma glucose concentration varied among breeds, periods of lactation and treatments. The mean average plasma glucose concentration of 50%HF animals had a tendency to be higher than that of 87.5%HF animals during feeding either hay or urea treated rice straw in all lactating periods. In the early period of lactation, the plasma glucose concentration of 50%HF animals fed either hay or urea treated rice straw was significantly higher in comparison with 87.5%HF animals, but declined thereafter in later periods of lactation. The plasma glucose concentration significantly decreased during middle and later periods of lactation of 50%HF animals feeding on both types of roughage whereas it slightly

increased in 87.5%HF animals. In all groups of HF crossbred animals, the type of roughage and the level of feeding have been shown to have no effect on the concentration of milk allantoin in all periods of lactation.

Milk yield and excretion of milk urea-N and milk allantoin-N

The data in Table 4 show the milk yield and amount of urea-N and allantoin-N excretion in milk per day. The daily milk yield of 87.5%HF animals fed either hay or urea treated rice straw was significantly higher than those of 50%HF animals in the early period of lactation, but it markedly decreased thereafter in the later periods. There were no differences in milk yield among periods of lactation of 50%HF animals feeding on either hay or urea treated rice straw. Because of the high milk yield in the early period of lactation of 87.5%HF animals fed either hay or urea treated rice straw, the daily excretion of both urea-N and allantoin-N increased in the same order as milk yield in comparison with later periods of lactation. No significant differences were apparent for either urea-N and allantoin-N excreted in milk in 50%HF animals fed either hay or urea treated rice straw throughout all periods of lactation.

Urea-N and allantoin-N fractions in the total nitrogen content in milk

Table 5 shows the relative percentages of the measured N-fractions in the total nitrogen content in milk. There were no effects from unbalanced protein nutrition on the percentage of these two nitrogen fractions in milk among periods of lactation of both 50%HF and 87.5%HF animals feeding on hay or urea treated rice straw. The fractions of urea-N percentage significantly increased in 87.5%HF animals fed urea treated rice straw when compared with the same breed animals fed with hay. The allantoin-N fraction of the milk nitrogen was constant at around 0.4% in all periods of lactation of both 50%HF and 87.5%HF animals feeding on either hay or urea treated rice straw.

DISCUSSION

The present results provide information on the excretions of urea and allantoin in milk of crossbred HF animals feeding on different types of roughage. The high correlation ($P < 0.001$) between the milk urea concentration and plasma urea concentration were apparent in all periods of lactation of both 50%HF and 87.5%HF animals feeding on either hay or urea treated rice straw. This result is in agreement with previous observations that there is a very close relation (over 94%) between the urea concentration in blood and in milk and the daily milking represents an average blood urea level (Ide et al.1966, Oltner and Wiktorsson, 1983).

The urea concentration of milk is therefore a suitable indicator of the animal's metabolism regarding ammonia and urea.

Several studies indicate that many factors could affect the milk urea concentration in dairy cattle. A rise in milk urea concentration has been suggested to be due to an excessive crude protein supply and deficiency of energy but not to an increase in the milk protein concentration, while a drop is found only when there is a protein deficiency (Lewis, 1957). In the present study, milk and plasma protein concentrations including milk urea concentration, remained constant throughout all periods of lactation and these values were in normal ranges for lactating cows (Kirchgeßner and Kreuzer, 1986). This result implies that animals in each group received an adequate protein diet and a constant level of feeding. It is probable that animals were fed with similar rations consisting of concentrate mixture and roughage according to their levels of body condition and milk yield throughout all periods of lactation. No differences in the total amount of organic matter and crude protein intakes among groups of crossbred animals with different periods of lactation were noted. Both of these are indices of a constant level of energy status (Rowlands, 1980). In view of this it seems reasonable to assume that the level of milk urea concentration in the present study was not affected by an unbalanced protein and energy supply. However, it has been shown that the plasma and/or milk urea levels were high in both 50%HF and 87.5%HF animals feeding on urea treated rice straw in comparison with the same crossbred animals fed with hay. The reason for the rise in the concentration of plasma urea coinciding with a rise in the milk urea would depend more on the high content of urea supply in roughage than on the total amount of crude protein consumed. Animals feeding on urea treated rice straw would have increased non-protein nitrogen availability in the rumen environment (Huque and Chowdhury, 1997). Dissociation of urea into ammonia and carbon dioxide will appear in the rumen by the action of microbial urease. This ammonia is used in microbial protein synthesis in the rumen, but it is also absorbed from the rumen and other parts of the digestive tract into the portal blood stream and resynthesized into urea in the liver (Lewis et al. 1957), which can be attributed to an elevation in plasma urea level. Urea excretion in milk would be dependent on the blood urea concentration through the concentration gradient. The maintenance of the constant concentration gradient between urea in the blood and milk indicates that urea would move passively by a diffuse process into milk. In order to clarify this process, the role of urea passage from the blood into the milk should be further studied.

Both plasma and milk urea concentrations were found to be higher in 50%HF animals than 87.5%HF animals feeding either on hay or urea treated rice straw. The differences in these results may be explained, in part, on the basis of the difference in the breeds of animals used. Genetic differences may cause variations in the utilization of the feed without any changes in total organic matter and crude protein intakes. The feed efficiency in the 50%HF animals has been suggested to be better than in 87.5%HF animals (Chaiyabutr et al. 1997). The present result is in agreement with the observation of Kitchenham and Rowlands (1976), who found slightly higher urea levels in a group of crossbred animals than in pure breeds. However, breed effects were also marked for plasma glucose concentration. In the early period of lactation, 87.5%HF animals feeding on either hay or urea treated rice straw showed a lower level in the plasma glucose concentration than those of 50%HF animals. This reflects the high body utilization of glucose in 87.5%HF, which probably resulted from the high milk yield causing the high rate of mammary glucose drain in the early period of lactation. These results also confirmed the finding for different types of cows that the plasma glucose levels were low in high yielding cows (Miller et al. 1978). Plasma glucose concentrations of 50%HF animals feeding on either hay or urea were higher in the early periods of lactation and then decreased thereafter without changes in feed intake. This suggests that changes in endocrine status and other signals, rather than nutritional effect per se, might involve glucose metabolism in a different manner in 50%HF animals.

The present results provide further evidence that the milk urea concentration was lower during the first month of lactation than the later periods of lactation in all groups of crossbred HF animals. These results are in agreement with several other reports (Bruckental et al. 1980; Emanuelson et al. 1993). It might be due to the fact that during late pregnancy, the foetus has a pronounced drive to grow and may utilize amino acids more effectively. The consequence of this will be reduced deamination and urea formation in the liver, which will affect the plasma urea level during the transition period from the last month of gestation to the first month of lactation. Another possible explanation is the inability of cattle to ingest sufficient feed in the early period of lactation which might induce suboptimal function of the ruminal flora. However, the possibility of a nitrogen conserving mechanism causing reduction of milk urea concentration in the early period of lactation was also noted (Oldham, 1984). In the present result, an alteration in milk urea concentration was not consistent with the result of the milk allantoin concentration which varied little among periods of lactation and breeds of

animals feeding on either hay or urea treated rice straw. Allantoin is known to be the main end product from the breakdown of purine in the ruminant (Rys, et al. 1975). Many investigations have reported a close correlation between allantoin and the formation of microbial protein in the rumen, based on the high nucleic acid concentration of bacterial cells. An increase in dry matter intake and energy intake have been shown to be positively correlated with the amount of allantoin secreted in milk in dairy cows and excretion of allantoin in milk depends on the allantoin concentration in the plasma (Giesecke, et al. 1994). Thus, in the present study animals were all kept in the same environment and given equal amounts of dry matter intake during feeding on either hay or urea treated rice straw. This probably explains why hardly any effect of the level of feed intake on milk allantoin was found. Therefore, the constant microbial activity and hence the production of microbial protein and allantoin was apparent. In the present study, an increase in the excretion of both urea-N and allantoin-N in milk did not match the concentration of both urea and allantoin in milk because of changes in milk yield, especially in the early period of lactation in 87.5%HF animals fed either hay or urea treated rice straw. This result suggests that milk yield, rather than urea or allantoin concentration in milk, appeared to be an important factor affecting their excretion in milk. From the present data it can be concluded that feeding either 50%HF and 87.5%HF animals with either hay or urea treated rice straw as a source of roughage has no effect on the nutritional status and rumen microbial protein synthesis throughout the lactating period.

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Table 1. Chemical composition (%DM) of feeds used.

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

Concentrate formulation: ingredient fresh weight (kg/100 kg) consisted of soy bean meal (30 kg), cotton seeds (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).

Table 2. Total daily intake of the concentrate, roughage, organic matter (OM), crude protein and NDF.

	Period of Lactation	Hay+concentrate		Urea treated rice straw+concentrate	
		HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Daily intake (kg/d)					
Concentrate	Early	7.05±1.74 ^a	8.41±0.30 ^a	7.58±1.55 ^a	8.25±0.45 ^a
	Mid	8.51±1.51 ^{ab}	6.56±1.67 ^b	10.05±0.86 ^a	9.05±0.07 ^a
	Late	6.90±2.76 ^b	7.04±1.35 ^{ab}	10.18±0.82 ^a	8.90±0.10 ^{ab}
Roughage	Early	4.00±1.28 ^a	3.47±0.49 ^{ab}	2.47±0.24 ^b	2.75±0.22 ^b
	Mid	3.43±0.72 ^a	3.64±0.42 ^a	2.60±0.27 ^b	2.29±0.32 ^b
	Late	3.79±0.44 ^b	4.47±0.26 ^a	2.64±0.18 ^c	2.95±0.17 ^c
Organic Matter	Early	10.25±0.49 ^{ab}	11.05±0.55 ^a	9.44±1.01 ^b	10.07±0.51 ^{ab}
	Mid	11.11±1.41 ^{ab}	9.46±1.44 ^b	11.65±0.72 ^a	10.49±0.26 ^{ab}
	Late	9.92±2.29 ^a	10.66±1.51 ^a	11.80±0.66 ^a	10.86±0.11 ^a
Crude protein	Early	1.43±0.25 ^b	1.65±0.06 ^{ab}	1.61±0.19 ^{ab}	1.71±0.09 ^a
	Mid	1.66±0.26 ^{bc}	1.33±0.28 ^c	2.02±0.14 ^a	1.82±0.03 ^{ab}
	Late	1.39±0.47 ^c	1.45±0.25 ^{bc}	2.04±0.13 ^a	1.85±0.03 ^{ab}
NDF	Early	5.27±0.53 ^a	5.23±0.23 ^a	3.92±0.34 ^b	4.23±0.22 ^b
	Mid	5.23±0.63 ^a	4.84±0.44 ^a	4.64±0.22 ^{ab}	4.16±0.21 ^b
	Late	5.06±0.54 ^{ab}	5.65±0.60 ^a	4.70±0.15 ^b	4.55±0.14 ^b

^{a-c} Mean values with different superscripts within the same row are significantly different at P<0.05.

Table 3. The concentrations of urea, protein and glucose in plasma and the concentration of protein, urea and allantoin in milk of crossbred Holstein cattle feeding on different types of roughage.

	Period of Lactation	Hay+concentrate		Urea treated rice straw+concentrate	
		HF:RS (87.5:12.5)	HF:RS (50:50)	HF:RS (87.5:12.5)	HF:RS (50:50)
Plasma urea (mg/100 ml)	Early	13.37±2.29 ^b	16.58±2.66 ^{ab}	18.41±2.01 ^a	19.13±0.7 ^a
	Mid	14.43±2.97 ^c	15.99±0.53 ^{bc}	17.77±3.54 ^b	26.55±1.85 ^a
	Late	14.39±3.42 ^c	18.69±2.08 ^{ab}	19.17±2.84 ^{bc}	25.19±1.59 ^a
Plasma protein (gm/100 ml)	Early	7.89±0.34 ^a	7.00±0.14 ^b	7.83±0.41 ^a	7.44±0.15 ^{ab}
	Mid	7.97±0.49 ^a	7.57±0.34 ^a	7.59±0.47 ^a	7.55±0.65 ^a
	Late	8.23±0.37 ^a	7.17±0.20 ^b	7.78±0.49 ^{ab}	7.69±0.43 ^{ab}
Plasma glucose (mg/100 ml)	Early	61.25±9.46 ^b	80.24±7.65 ^a	63.65±3.58 ^b	74.97±2.36 ^a
	Mid	56.77±8.31 ^b	67.82±6.25 ^{ab*}	61.09±10.00 ^{ab}	72.68±7.37 ^a
	Late	63.72±7.28 ^a	69.55±3.44 ^{a*}	68.31±5.92 ^{a*}	63.27±3.12 ^{a*}
Milk protein (gm/100 ml)	Early	3.11±0.12 ^a	3.27±0.15 ^a	3.01±0.19 ^a	3.59±0.18 ^a
	Mid	3.34±0.31 ^a	3.44±0.30 ^a	3.28±0.30 ^a	3.68±0.13 ^a
	Late	3.31±0.16 ^b	3.61±0.19 ^{ab}	3.43±0.29 ^b	3.85±0.23 ^a
Milk urea (mg/100 ml)	Early	14.03±6.02 ^b	19.31±4.03 ^{ab}	21.97±3.09 ^a	19.58±5.06 ^{ab}
	Mid	15.77±5.40 ^b	19.49±5.17 ^{ab}	23.77±2.79 ^a	23.91±1.39 ^a
	Late	14.84±5.26 ^b	22.36±2.56 ^{a*}	24.26±1.90 ^a	24.73±4.61 ^{a**}
Milk allantoin (mg/100 ml)	Early	6.66±0.71 ^a	5.97±0.25 ^a	6.06±0.66 ^a	5.99±0.39 ^a
	Mid	6.20±0.48 ^a	6.02±0.33 ^a	5.76±0.40 ^a	6.12±0.57 ^a
	Late	5.28±0.22 ^b	5.45±0.37 ^{ab}	5.86±0.48 ^a	5.67±0.26 ^{ab}

P-values by paired t-test: *P<0.05, ** P<0.01 with respect to the early period of lactation in each group.

^{a-c} Mean values with different superscripts within the same row are significantly different at P<0.05

Table 4. Excretion of urea-N and allantoin-N in milk and milk yield of crossbred Holstein cattle feeding on different types of roughage.

	Period of Lactation	Hay+concentrate		Urea treated rice straw+concentrate	
		HF:RS (87.5:12.5)	HF:RS (50:50)	HF:RS (87.5:12.5)	HF:RS (50:50)
Milk yield (kg/d)	Early	19.76±4.47 ^a	10.98±1.17 ^b	16.51±5.92 ^{ab}	12.91±1.58 ^b
	Mid	11.00±1.61 ^{a*}	10.52±1.34 ^a	11.72±0.93 ^a	12.33±2.46 ^a
	Late	10.11±0.69 ^{ab*}	10.47±0.81 ^{ab}	9.18±1.21 ^{b*}	12.26±2.51 ^a
Urea-N (mg/d)	Early	1295.6±644.7 ^a	990.5±252.8 ^a	1710.1±692.1 ^a	1151.3±160.1 ^a
	Mid	780.5±194.0 ^c	956.9±283.1 ^{bc}	1303.5±208.7 ^{ab}	1370.3±247.5 ^a
	Late	587.9±141.5 ^{c*}	1085.6±58.4 ^b	1040.5±175.4 ^b	1380.6±139.6 ^a
Allantoin-N (mg/d)	Early	578.8±156.2 ^a	287.2±36.5 ^b	448.5±206.2 ^{ab}	337.4±36.5 ^b
	Mid	297.7±44.4 ^{a*}	276.3±29.7 ^a	296.3±40.7 ^a	332.9±87.7 ^a
	Late	233.6±19.2 ^{b*}	249.1±18.8 ^{ab}	236.6±47.1 ^b	303.0±57.9 ^a

P-values by paired t-test: *P<0.05, with respect to the early period of lactation in each group.

^{a-c} Mean values with different superscripts within the same row are significantly different at P<0.05.

Table 5. Urea-N and allantoin-N fractions in the total nitrogen concentration of milk of crossbred Holstein cattle feeding on different types of roughage.

N-fractions	Period of Lactation	Hay+concentrate		Urea treated rice straw+concentrate	
		HF:RS (87.5:12.5)	HF:RS (50:50)	HF:RS (87.5:12.5)	HF:RS (50:50)
Urea-N (%)	Early	1.36 $\pm$ 0.62 ^b	1.77 $\pm$ 0.45 ^{ab}	2.18 $\pm$ 0.31 ^a	1.59 $\pm$ 0.47 ^{ab}
	Mid	1.42 $\pm$ 0.48 ^b	1.72 $\pm$ 0.55 ^{ab}	2.16 $\pm$ 0.33 ^a	1.94 $\pm$ 0.07 ^{ab}
	Late	1.14 $\pm$ 0.32 ^b	1.85 $\pm$ 0.26 ^a	2.10 $\pm$ 0.19 ^a	1.92 $\pm$ 0.39 ^a
Allantoin-N (%)	Early	0.48 $\pm$ 0.07 ^a	0.41 $\pm$ 0.02 ^a	0.45 $\pm$ 0.07 ^{ab}	0.38 $\pm$ 0.01 ^b
	Mid	0.42 $\pm$ 0.05 ^a	0.40 $\pm$ 0.04 ^a	0.40 $\pm$ 0.06 ^a	0.38 $\pm$ 0.04 ^a
	Late	0.36 $\pm$ 0.01 ^a	0.34 $\pm$ 0.01 ^a	0.39 $\pm$ 0.06 ^a	0.33 $\pm$ 0.03 ^a

^{a-b} Mean values with different superscripts within the same row are significantly different at P<0.05.

COMPARISON OF PLASMA LEVELS OF HORMONES (GROWTH HORMONE, PROLACTIN, INSULIN, GLUCAGON, CORTISOL, THYROXINE, TRIIODOTHYRONINE, PROGESTERONE, AND ESTRADIOL) AND METABOLITES OF CROSSBRED HOLSTEIN CATTLE FEEDING ON DIFFERENT TYPES OF ROUGHAGE AT LATE PREGNANCY AND DIFFERENT STAGES OF LACTATION ¹

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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 x Red Sindhi (50:50 = 50%HF) and Holstein Friesian x 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 (T₃), 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 (T₄) 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 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.

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 (1,2).

During pregnancy, mammary growth has been known to be a prerequisite for satisfactory lactation (3) 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 (4). 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 reviewed (5). It has been realized that during late pregnancy, lactogenesis occurs concurrently with mammary development and many hormones are needed for maximal stimulation of lactogenesis (5,6). 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 x Red Sindhi (50:50 = 50%HF) and Holstein Friesian x 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 $34\pm 1^{\circ}\text{C}$ and the minimum temperature at night was $26\pm 1^{\circ}\text{C}$. 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 x 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°C. Plasma from the venous blood samples were kept in aliquots at -40°C until hormone concentrations were assayed. An arterial plasma samples were kept at -40°C 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°C until time of hormone assays.

Radio immuno 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 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/1µl) 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 x 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 Gamma 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 divided in aliquots and store at -20°C.

Assay protocol of bGH. Plasma samples (0.1 ml) were pipetted in disposable plastic tubes (10 x 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 then vortexed and incubated at refrigerator temperature (4°C) 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°C. Following this incubation, appropriately diluted 0.5 ml of sheep purified anti-rabbit gamma 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°C. The supernatant was decanted and assay tubes were kept inverted on the absorbent paper. The assay tubes were subsequently counted in Auto Gamma 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 ngGH/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, $\text{C}_{16}\text{-C}_{18}$) 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. (7). 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 $\pm$ 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 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 -hydroxybutyrate (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 this transformation is very likely, since plasma T_4 has been shown to transform to T_3 in

tissue before it becomes biologically active (8). 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 (9), 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 (10). 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 87.5%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 (11) and to feed effect (12). 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 (13). At the onset of lactation, the decreases in the sensitivity of adipose tissue to insulin has been reported (14). Evidence for adipocytes becoming insulin resistant during lactation has also been found with laboratory species with both *in vitro* and *in vivo* studies (15). During late pregnancy, mammary growth and foetus development could account for energy deficit 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 (16). 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 (17). 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 (18). 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 it 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 (19, 20). 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 (21, 22). 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 (23).

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 (24, 25). Furthermore, it has been shown that circulating prolactin in cattle can be raised or lowered by day length (26) or ambient temperature (27).

The levels of β -hydroxybutyrate (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 chronically acting growth hormones through the period of lactation.

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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).

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 $\pm$ 13.6 ^a	81.5 $\pm$ 29.9 ^a	96.8 $\pm$ 18.0 ^a	93.5 $\pm$ 19.8 ^a
	Early lactation	93.8 $\pm$ 41.5 ^a	131.5 $\pm$ 16.4 ^{a†}	107.2 $\pm$ 8.2 ^a	133.0 $\pm$ 44.7 ^a
	Mid lactation	111.0 $\pm$ 28.0 ^a	120.5 $\pm$ 21.2 ^a	118.7 $\pm$ 17.5 ^a	142.5 $\pm$ 28.1 ^a
	Late lactation	107.0 $\pm$ 32.0 ^b	111.5 $\pm$ 7.4 ^{ab*}	122.7 $\pm$ 19.0 ^{ab}	141.2 $\pm$ 19.5 ^a
Thyroxine (T4) (ug/100 ml)	Late pregnancy	3.00 $\pm$ 0.68 ^b	3.93 $\pm$ 0.49 ^{ab}	4.33 $\pm$ 0.95 ^a	3.63 $\pm$ 0.88 ^{ab}
	Early lactation	3.29 $\pm$ 0.41 ^a	4.15 $\pm$ 0.29 ^a	4.05 $\pm$ 0.93 ^a	3.83 $\pm$ 0.90 ^a
	Mid lactation	4.09 $\pm$ 1.16 ^a	3.88 $\pm$ 0.68 ^a	3.78 $\pm$ 0.85 ^a	3.50 $\pm$ 0.59 ^a
	Late lactation	3.98 $\pm$ 0.95 ^a	3.78 $\pm$ 0.67 ^a	3.41 $\pm$ 0.68 ^a	3.64 $\pm$ 0.98 ^a
Prolactin (ng/ml)	Late pregnancy	3.94 $\pm$ 0.39 ^a	9.36 $\pm$ 5.42 ^a	4.83 $\pm$ 1.15 ^a	4.08 $\pm$ 2.47 ^a
	Early lactation	4.80 $\pm$ 1.23 ^{ab}	9.83 $\pm$ 7.48 ^a	5.83 $\pm$ 2.16 ^{ab}	3.56 $\pm$ 1.52 ^b
	Mid lactation	4.93 $\pm$ 1.52 ^a	9.68 $\pm$ 6.27 ^a	7.27 $\pm$ 5.56 ^a	3.80 $\pm$ 0.63 ^a
	Late lactation	7.38 $\pm$ 3.16 ^a	11.48 $\pm$ 7.98 ^a	9.65 $\pm$ 6.01 ^a	4.10 $\pm$ 0.88 ^a
Growth hormone (ng/ml)	Late pregnancy	10.72 $\pm$ 4.95 ^{ab}	15.80 $\pm$ 4.56 ^a	6.18 $\pm$ 1.55 ^b	14.53 $\pm$ 4.07 ^a
	Early lactation	15.10 $\pm$ 6.39 ^a	9.45 $\pm$ 4.27 ^{ab}	9.00 $\pm$ 1.50 ^{ab}	8.62 $\pm$ 1.37 ^b
	Mid lactation	11.30 $\pm$ 0.93 ^a	8.20 $\pm$ 3.98 ^a	6.90 $\pm$ 2.94 ^a	9.33 $\pm$ 4.67 ^a
	Late lactation	9.12 $\pm$ 3.81 ^a	9.27 $\pm$ 3.19 ^a	6.70 $\pm$ 0.79 ^a	10.25 $\pm$ 4.23 ^a
Cortisol (ng/ml)	Late pregnancy	10.3 $\pm$ 11.0 ^{ab}	23.5 $\pm$ 14.5 ^{ab}	7.5 $\pm$ 2.8 ^b	30.2 $\pm$ 20.1 ^a
	Early lactation	19.8 $\pm$ 18.4 ^a	29.9 $\pm$ 24.9 ^a	8.6 $\pm$ 6.9 ^a	28.6 $\pm$ 18.6 ^a
	Mid lactation	7.5 $\pm$ 3.4 ^a	25.7 $\pm$ 25.4 ^a	4.0 $\pm$ 1.9 ^a	25.0 $\pm$ 19.1 ^a
	Late lactation	6.8 $\pm$ 6.9 ^b	26.8 $\pm$ 22.9 ^{ab}	5.7 $\pm$ 7.0 ^b	45.0 $\pm$ 19.2 ^a

P-values by paired t-test. [†] 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 (μ U/ml)	Late pregnancy	9.40 $\pm$ 3.82 ^a	14.93 $\pm$ 9.09 ^a	15.30 $\pm$ 4.46 ^a	18.17 $\pm$ 13.10 ^a
	Early lactation	16.94 $\pm$ 10.87 ^a	18.93 $\pm$ 5.57 ^a	20.37 $\pm$ 10.36 ^a	27.13 $\pm$ 15.65 ^a
	Mid lactation	21.45 $\pm$ 9.46 ^a	25.07 $\pm$ 18.43 ^a	25.97 $\pm$ 6.96 ^a	20.75 $\pm$ 6.56 ^a
	Late lactation	17.13 $\pm$ 4.71 ^a	17.63 $\pm$ 4.82 ^a	25.00 $\pm$ 1.86 ^a	28.25 $\pm$ 16.35 ^a
Glucagon (pg/ml)	Late pregnancy	31.8 $\pm$ 11.3 ^a	48.6 $\pm$ 19.8 ^a	51.8 $\pm$ 22.7 ^a	65.5 $\pm$ 46.4 ^a
	Early lactation	29.5 $\pm$ 5.4 ^a	59.9 $\pm$ 17.7 ^a	80.0 $\pm$ 52.5 ^a	74.6 $\pm$ 39.0 ^a
	Mid lactation	46.6 $\pm$ 6.2 ^b	105.3 $\pm$ 46.7 ^{ab}	118.8 $\pm$ 45.3 ^{a*}	131.9 $\pm$ 43.2 ^{a*}
	Late lactation	53.5 $\pm$ 35.7 ^c	97.7 $\pm$ 12.3 ^{ab**}	77.5 $\pm$ 6.1 ^{bc}	124.5 $\pm$ 29.3 ^{a*}
Progesterone (ng/ml)	Late pregnancy	4.30 $\pm$ 1.60 ^a	5.70 $\pm$ 1.10 ^a	3.40 $\pm$ 1.10 ^a	3.20 $\pm$ 1.50 ^a
	Early lactation	0.16 $\pm$ 0.08 ^{a††}	0.15 $\pm$ 0.09 ^{a††}	0.61 $\pm$ 1.02 ^{a†}	0.10 $\pm$ 0.01 ^{a††}
	Mid lactation	0.10 $\pm$ 0.01 ^b	2.70 $\pm$ 2.30 ^a	1.35 $\pm$ 1.46 ^{ab}	0.15 $\pm$ 0.06 ^b
	Late lactation	1.15 $\pm$ 1.25 ^{ab}	3.76 $\pm$ 3.84 ^a	3.40 $\pm$ 0.80 ^{ab}	0.56 $\pm$ 0.37 ^b
Estradiol (pg/ml)	Late pregnancy	129.8 $\pm$ 56.2 ^a	171.3 $\pm$ 79.8 ^a	115.0 $\pm$ 56.1 ^a	180.0 $\pm$ 81.69 ^a
	Early lactation	12.0 $\pm$ 6.2 ^{a†}	17.5 $\pm$ 5.7 ^{a†}	16.0 $\pm$ 7.9 ^{a†}	19.1 $\pm$ 7.8 ^{a†}
	Mid lactation	9.3 $\pm$ 6.8 ^b	18.7 $\pm$ 13.1 ^{ab}	14.5 $\pm$ 7.5 ^b	25.5 $\pm$ 9.2 ^a
	Late lactation	10.5 $\pm$ 2.3 ^b	23.6 $\pm$ 13.5 ^{ab}	18.0 $\pm$ 9.9 ^{ab}	28.3 $\pm$ 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} Mean values within a row indicated with different superscripts are significantly different (P<0.05).

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)
Glucose ($\mu\text{mol/ml}$)	Late pregnancy	3.67 $\pm$ 0.32 ^a	4.06 $\pm$ 0.59 ^a	3.49 $\pm$ 1.04 ^a	3.60 $\pm$ 0.81 ^a
	Early lactation	3.40 $\pm$ 0.53 ^b	4.46 $\pm$ 0.42 ^a	3.54 $\pm$ 0.19 ^b	4.16 $\pm$ 0.13 ^a
	Mid lactation	3.15 $\pm$ 0.46 ^b	3.77 $\pm$ 0.35 ^{ab}	3.39 $\pm$ 0.56 ^{ab}	3.99 $\pm$ 0.45 ^a
	Late lactation	3.54 $\pm$ 0.40 ^a	3.86 $\pm$ 0.19 ^a	3.79 $\pm$ 0.33 ^a	3.52 $\pm$ 0.17 ^a
Free fatty acid (C16-18) ($\mu\text{mol/l}$)	Late pregnancy	369.5 $\pm$ 83.0 ^b	526.7 $\pm$ 135.3 ^{ab}	393.7 $\pm$ 90.3 ^{ab}	573.3 $\pm$ 165.6 ^a
	Early lactation	302.0 $\pm$ 111.3 ^a	314.4 $\pm$ 115.8 ^a	317.5 $\pm$ 171.5 ^a	446.5 $\pm$ 223.5 ^a
	Mid lactation	260.7 $\pm$ 191.7 ^a	375.1 $\pm$ 191.3 ^a	200.7 $\pm$ 50.3 ^a	298.8 $\pm$ 146.4 ^a
	Late lactation	182.5 $\pm$ 62.4 ^b	350.4 $\pm$ 129.3 ^a	237.9 $\pm$ 76.0 ^{ab}	288.8 $\pm$ 117.7 ^{ab}
β -hydroxybutyrate ($\mu\text{mol/l}$)	Late pregnancy	680.2 $\pm$ 153.6 ^a	646.0 $\pm$ 140.3 ^a	539.0 $\pm$ 160.4 ^a	800.5 $\pm$ 569.9 ^a
	Early lactation	648.0 $\pm$ 51.8 ^a	508.0 $\pm$ 125.6 ^{ab}	397.3 $\pm$ 68.4 ^b	536.7 $\pm$ 195.9 ^{ab}
	Mid lactation	563.0 $\pm$ 154.2 ^a	563.7 $\pm$ 64.6 ^a	432.5 $\pm$ 119.9 ^a	517.7 $\pm$ 102.9 ^a
	Late lactation	531.3 $\pm$ 79.6 ^a	523.0 $\pm$ 67.0 ^{ab}	408.0 $\pm$ 74.2 ^c	431.3 $\pm$ 19.5 ^{bc}
Lactate ($\mu\text{mol/l}$)	Late pregnancy	124.2 $\pm$ 21.2 ^a	138.0 $\pm$ 47.1 ^a	188.0 $\pm$ 66.1 ^a	138.3 $\pm$ 52.9 ^a
	Early lactation	134.5 $\pm$ 54.0 ^a	114.0 $\pm$ 20.8 ^a	95.4 $\pm$ 24.7 ^a	127.4 $\pm$ 46.3 ^a
	Mid lactation	146.4 $\pm$ 35.7 ^a	96.6 $\pm$ 29.1 ^b	119.4 $\pm$ 23.1 ^{ab}	108.2 $\pm$ 30.2 ^{ab}
	Late lactation	110.3 $\pm$ 28.4 ^{ab}	96.5 $\pm$ 21.3 ^{ab}	91.2 $\pm$ 15.5 ^b	124.3 $\pm$ 19.3 ^a
Milk yield (kg/d)	Early lactation	19.76 $\pm$ 4.47 ^a	10.98 $\pm$ 1.17 ^b	16.51 $\pm$ 5.92 ^{ab}	12.91 $\pm$ 1.58 ^b
	Mid lactation	11.00 $\pm$ 1.61 ^{a*}	10.52 $\pm$ 1.34 ^a	11.72 $\pm$ 0.93 ^a	12.33 $\pm$ 2.46 ^a
	Late lactation	10.11 $\pm$ 0.69 ^{ab*}	10.47 $\pm$ 0.81 ^{ab}	9.18 $\pm$ 1.21 ^{b*}	12.26 $\pm$ 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} Mean values within a row indicated with different superscripts are significantly different (P<0.05).

Mammary blood flow and regulation of substrate supply for milk synthesis in crossbred Holstein cattle feeding on different types of roughage

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INTRODUCTION

It is known that lactating mammary gland is dependent upon its blood supply to provide substrates at appropriate rates to sustain milk synthesis. The rate of substrates supplying to mammary gland is determined by substrate concentration in the plasma and mammary blood flow. There is evident that substrate supply to the mammary gland is often inadequate to maintain the maximum rate of milk synthesis (Linzell and Mephram 1974). The mammary gland may be producing milk at a rate below its potential. However, the rate of milk production depends on function of number of secretory cells and their metabolic activity. Mammary growth during pregnancy has been known to be a prerequisite for lactation. It has been known that prolonged undernutrition of heifers in early lactation resulted in impairment of lactational ability throughout the course of lactation (Broster et al., 1969) Little is known about function of mammary tissue and the utilization of substrate in the mammary gland in different types of crossbred cattle. Therefore, the present experiment was conducted to obtain more informations on whether different types of crossbred Holstein cattle show any differences of mammary uptake of substrates during pregnancy and lactation in animals feeding on either hay or urea treated rice straw. The purpose of the present experiment was performed by using techniques for measuring mammary blood flow and combining these with measurement of arteriovenous concentration differences for the mammary uptake of substrates.

MATERIALS AND METHODS

Animals and Management. Sixteen pregnant, crossbred Holstein heifers, 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 x Red Sindhi (50:50 = 50%HF) and Holstein Friesian x 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 $34\pm 1^{\circ}\text{C}$ and the minimum temperature at night was $26\pm 1^{\circ}\text{C}$. Before parturition, animals were individually fed a concentrate of an average of 4.0 kg/day (DM basis) and roughage to maintain a moderate 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 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 with dry straw (5 kg urea dissolved in 100 litres water per 100 kg dry rice 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 x 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 a heparinized tube. Blood samples in heparinized tubes were kept in crushed ice and

then centrifuged at 3000 rpm for 30 min at 4°C. Plasma from the venous blood samples was kept in aliquots at -40°C until hormone concentrations were assayed. Arterial plasma samples were kept at -40°C for chemical studies. Milk yield was recorded by a milking machine in each lactating period of the study.

Udder blood flow measurements. Udder blood flow measurements were performed in duplicate. Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion as described by Chaiyabutr et al. (1997). In brief, a dye (T-1824) was dissolved in sterile normal saline and diluted to a concentration of 100 mg/L. The solution was infused by a peristaltic pump (Gilson Medical electronics) at a constant rate of 85 ml/min into the milk vein for 1 min which could produce adequate mixing of dye with blood. Before infusion, blood was drawn from downstream in the milk vein as a pre-infusion sample. About 10 seconds after starting the infusion, 10 ml of blood was drawn from downstream in the milk vein at a constant rate into a heparinized tube. Two consecutive plasma samples were taken during each dye infusion at about 5 min intervals. Blood flow of half of the udder was calculated from plasma samples using the equation derived by Thompson and Thomson (1977). Quarter milking showed that the yields of the two halves of the udder were similar. Udder blood flow was therefore calculated by doubling the flow measured in one milk vein (Bickerstaffe et al., 1974). Packed cell volume was measured after centrifugation of the blood in a microcapillary tube.

Metabolite Determinations. Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase. Plasma free fatty acid (FFA, C₁₆-C₁₈) concentrations were measured by using gas chromatography (Shimazu GC-7AG Gas Chromatograph) in comparison with the internal standard. The internal standard of triheptadecanoate and heptadecanoic acid was used for estimation of plasma triacylglycerol and FFA respectively as described by Thompson et al. (1975). Plasma β -hydroxybutyrate concentrations were assayed using an enzymatic reaction in the presence of β -hydroxybutyrate dehydrogenase (Sigma Chemical Co.). Plasma acetate concentrations were determined by chromatographic method. Plasma glycerol concentrations were determined by enzymatic method. Plasma lactate concentrations were assayed using an enzymatic reaction in the presence of lactate dehydrogenase

(Sigma Chemical Co.). Plasma and milk concentrations for sodium and potassium were determined by flame photometry, chloride by chloridometer, calcium by cresolphthalein complexone and inorganic phosphorus by molybdate and methyl p-amonophenol sulfate method.

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 $\pm$ SD.

RESULTS

Changes in udder blood flow and milk yield (Table 2).

Udder blood flow were nearly three times higher in lactating period animals than those in late pregnant period in both 50%HF and 87.5%HF feeding on either hay or urea treated rice straw. During early lactation, mammary blood flow and milk yield of both groups of 87.5%HF animals feeding on either hay or urea treated rice straw were higher than those of 50%HF animals ($P < 0.05$). In mid- and late lactation, both mammary blood flow and milk yield showed a proportional decreased from early lactating period of in both groups 87.5%HF animals. In 50%HF animals feeding on either hay or urea treated rice straw, the trends for persistency were observed as for udder blood flow and milk yield through the experimental periods.

Arterial plasma concentration, arteriovenous concentration differences and mammary uptakes of glucose and acetate (Table 3).

The mean arterial plasma glucose concentration remained stable throughout periods of study in each group. However, the plasma glucose level in 50%HF animals feeding on either hay or urea treated rice straw was higher than in the 87.5%HF animals in all periods of experiment. During late pregnancy in all groups, the arteriovenous differences and extraction ratio of glucose across the mammary gland markedly lowered approximately 4 fold; while the mean arterial plasma concentration, arteriovenous concentration differences and extraction ratio for acetate showed no significant differences in comparison to early lactating period. The net mammary uptake of glucose and acetate in late pregnancy markedly lowered approximately 10

and 4 times respectively in comparison to early lactating period in all groups. In mid- and late lactation, the mammary uptake for glucose and acetate in both groups of 87.5%HF animals showed a decrease from early lactating period. The trends for constancy were observed as for mammary uptake of glucose and acetate in 50%HF animals through the course of lactation.

Arterial plasma concentration, arteriovenous concentration differences and mammary uptakes of β -hydroxybutyrate and glycerol (Table 4).

The mean arterial plasma concentrations for β -hydroxybutyrate and free glycerol remained stable throughout experimental periods in each group. During late pregnancy, the arteriovenous differences and extraction ratio of β -hydroxybutyrate and free glycerol across the mammary gland markedly lowered which coincided with a lower net uptake by the mammary gland in comparison to early lactating period. In mid- and late lactation, the mammary uptake for β -hydroxybutyrate in both groups of 87.5%HF animals showed a decrease from the early lactating period whereas no appearances were observed for free glycerol. The net mammary uptakes for β -hydroxybutyrate and glycerol in 50%HF animals remained constant through the course of lactation.

Arterial plasma concentration, arteriovenous concentration differences and mammary uptakes of free fatty acid, triacylglycerol and lactate (Table 5 and Table 6).

The mean arterial plasma concentrations for free fatty acid, triacylglycerol (C_{16} to C_{18}) and lactate were higher in late pregnancy compared with early lactation in 50%HF and 87.5%HF animals feeding on either hay or urea treated rice straw. The values of arteriovenous differences and the net uptake by the mammary gland for FFA and lactate were variable during the pregnant and lactating periods in all groups. During late pregnancy, the arteriovenous differences, extraction ratio and net uptake of triacylglycerol across the mammary gland markedly lowered in comparison to early lactating period. There were no significant differences of arteriovenous differences, extraction ratio and net uptake of triacylglycerol during lactation advance in both groups of 50%HF and 87.5%HF animals.

Arterial plasma and milk concentrations of sodium, potassium, chloride, calcium and inorganic phosphorus (Table 7 and Table 8).

The mean arterial plasma electrolyte concentrations were in similar ranges at the different periods of study and different groups of animals. During lactation, the compositions of the aqueous phase of milk electrolytes of sodium, potassium, chloride, calcium and inorganic phosphorus were not affected by feeding on different types of roughage and different types of crossbred HF animals.

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).

Table 2. Udder blood flow and milk secretion during late pregnancy and different stages of lactation.

	Period of experiment	Hay+concentration		Urea treated rice straw+concentration	
		HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Udder blood flow (ml/min)	Pregnant	2696± 265 ^a	1034± 354 ^b	1876± 363 ^b	1396± 406 ^b
	Early	7160± 1807 ^a	3887± 543 ^b	4619± 1149 ^b	4314± 575 ^b
	Mid	4745± 836 ^a	4090± 398 ^a	3843± 872 ^a	5068± 1054 ^a
	Late	5026± 724 ^{ab}	3942± 500 ^b	3995± 883 ^b	5371± 932 ^a
Milk secretion (ml/min)	Early	13.7± 3.1 ^a	7.6± 0.8 ^b	11.5± 4.1 ^{ab}	8.9± 1.1 ^b
	Mid	7.6± 1.1 ^a	7.3± 0.9 ^a	8.1± 0.6 ^a	8.6± 1.7 ^a
	Late	7.0± 0.5 ^{ab*}	7.3± 0.6 ^{ab}	6.4± 0.8 ^{b*}	8.5± 1.7 ^a

P-values by paired t-test. *P<0.05 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. Arterial plasma concentrations, mammary arteriovenous differences and mammary uptake for glucose and acetate during late pregnancy and different stages of lactation.

	Period of	Hay+concentration		Urea treated rice straw+concentration	
	experiment	HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Glucose :					
Arterial concentrate (μmol/ml)	Pregnant	3.67±0.32 ^a	4.06±0.59 ^a	3.49±1.04 ^a	3.60±0.81 ^a
	Early	3.40±0.53 ^b	4.46±0.42 ^a	3.54±0.19 ^b	4.16±0.13 ^a
	Mid	3.15±0.46 ^b	3.77±0.35 ^{ab}	3.39±0.56 ^{ab}	3.99±0.45 ^a
	Late	3.54±0.40 ^a	3.86±0.19 ^a	3.79±0.33 ^a	3.52±0.17 ^a
A-V (μmol/ml)	Pregnant	0.16±0.60 ^a	0.32±0.20 ^a	0.22±0.07 ^a	0.20±0.07 ^a
	Early	0.74±0.04 ^b	0.83±0.12 ^{ab}	0.90±0.15 ^a	0.78±0.05 ^{ab}
	Mid	0.71±0.08 ^a	0.77±0.08 ^a	0.83±0.19 ^a	0.69±0.04 ^a
	Late	0.66±0.08 ^b	0.81±0.08 ^a	0.76±0.09 ^{ab}	0.71±0.09 ^{ab}
Extraction (%)	Pregnant	4.2±1.5 ^a	8.3±6.6 ^a	6.4±2.2 ^a	5.5±1.2 ^a
	Early	22.1±3.1 ^{ab}	18.6±1.9 ^b	25.5±3.9 ^a	18.8±1.5 ^b
	Mid	23.2±4.3 ^{ab}	20.6±2.5 ^{ab}	24.5±4.5 ^a	17.5±2.5 ^b
	Late	18.7±0.6 ^a	21.0±1.9 ^a	20.0±1.9 ^a	20.2±3.3 ^a
Udder Uptake (μmol/min)	Pregnant	301±108 ^a	254±226 ^a	291±91 ^a	188±65 ^a
	Early	3877±1000 ^a	2230±291 ^b	3113±830 ^{ab}	2618±386 ^b
	Mid	2482±483 ^a	2157±275 ^a	2225±169 ^a	2450±437 ^a
	Late	2451±604 ^a	2195±454 ^a	2158±591 ^a	2640±479 ^a
Acetate :					
Arterial concentrate (μmol/l)	Pregnant	977±121 ^a	915±226 ^a	1117±428 ^a	810±261 ^a
	Early	713±201 ^a	691±146 ^a	919±452 ^a	811±189 ^a
	Mid	736±203 ^a	821±211 ^a	963±477 ^a	712±220 ^a
	Late	966±105 ^a	714±144 ^a	893±275 ^a	664±235 ^a
A-V (μmol/l)	Pregnant	467±78 ^a	326±259 ^a	675±255 ^a	407±163 ^a
	Early	418±123 ^a	377±117 ^a	541±336 ^a	481±238 ^a
	Mid	441±128 ^a	403±218 ^a	581±322 ^a	330±108 ^a
	Late	506±97 ^a	380±132 ^a	483±117 ^a	378±228 ^a
Extraction (%)	Pregnant	49±14 ^a	34.3±23.5 ^a	62±16 ^a	50.1±9.8 ^a
	Early	59.5±14.7 ^a	54.2±11.6 ^a	55.3±10.0 ^a	56.9±18.2 ^a
	Mid	60.8±12.1 ^a	47.9±21.8 ^a	58.2±5.8 ^a	47.0±9.0 ^a
	Late	52.9±13.0 ^a	52.6±14.3 ^a	51.7±6.7 ^a	55.6±16.9 ^a
Udder Uptake (μmol/min)	Pregnant	902.1±136.4 ^a	257.7±273.6 ^b	931.0±404.1 ^a	390.2±201.4 ^{ab}
	Early	2186.7±742.2 ^a	1050.3±325.1 ^a	2427.7±1797.8 ^a	1677.3±990.6 ^a
	Mid	1512.7±291.6 ^a	1126.1±637.1 ^a	1733.1±1158.8 ^a	1250.3±527.9 ^a
	Late	1861.4±488.2 ^a	1034.6±388.8 ^b	1329.9±564.3 ^{ab}	1517.4±1198.6 ^a

^{a,b} Mean values within a row indicated with different superscripts are significantly different (P<0.05).

Table 4. Arterial plasma concentrations, mammary arteriovenous differences and mammary uptake for Beta-hydroxybutyrate and glycerol during late pregnancy and different stages of lactation.

	Period of	Hay+concentration		Urea treated rice straw+concentration	
	experiment	HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Beta- hydroxybutyrate :					
Arterial concentrate (μmol/l)	Pregnant	680.2±153.6 ^a	646.0±140.3 ^a	539.0±160.4 ^a	800.5±569.9 ^a
	Early	648.0±51.8 ^a	508.0±125.6 ^{ab}	397.3±68.4 ^b	536.7±195.9 ^{ab}
	Mid	563.0±154.2 ^a	563.7±64.6 ^a	432.5±119.9 ^a	517.7±102.9 ^a
	Late	531.3±79.6 ^a	523.0±67.0 ^{ab}	408.0±74.2 ^c	431.3±19.5 ^{bc}
A-V (μmol/l)	Pregnant	69.5±42.5 ^a	61.7±51.1 ^a	84.7±33.8 ^a	80.7±25.6 ^a
	Early	235.7±65.6 ^a	165.3±44.5 ^a	155.7±34.5 ^a	217.7±103.1 ^a
	Mid	213.5±78.4 ^{ab}	240.7±34.3 ^a	130.7±22.7 ^b	211.5±56.2 ^{ab}
	Late	199.3±53.4 ^a	216.3±47.3 ^a	160.0±5.9 ^a	212.7±22.5 ^a
Extraction (%)	Pregnant	9.8±4.3 ^a	8.8±6.3 ^a	17.4±8.5 ^a	10.0±11.0 ^a
	Early	35.9±7.3 ^a	32.9±7.5 ^a	39.5±7.5 ^a	39.8±6.2 ^a
	Mid	38.2±10.7 ^{ab}	42.6±2.3 ^a	31.4±6.3 ^b	40.7±5.2 ^{ab}
	Late	37.5±7.7 ^b	41.6±8.6 ^{ab}	40.3±7.9 ^{ab}	49.3±3.6 ^a
Udder Uptake (μmol/min)	Pregnant	131.6±73.0 ^a	48.0±51.5 ^a	112.2±41.8 ^a	77.2±33.7 ^a
	Early	1217.5±380.7 ^a	462.8±125.7 ^b	635.1±237.7 ^b	706.6±272.6 ^b
	Mid	749.8±330.7 ^a	678.9±154.4 ^{ab}	372.4±133.6 ^b	785.3±240.6 ^a
	Late	716.7±126.5 ^{ab}	591.7±194.2 ^{ab}	455.6±110.8 ^b	801.7±168.8 ^a
Glycerol :					
Arterial concentrate (μmol/l)	Pregnant	92.7±22.9 ^a	89.0±48.3 ^a	84.0±31.5 ^a	144.0±22.8 ^a
	Early	61.3±20.2 ^a	111.2±52.0 ^a	74.0±25.0 ^a	95.2±33.0 ^a
	Mid	59.5±20.6 ^a	117.7±39.7 ^a	42.0±28.9 ^a	112.5±30.4 ^a
	Late	54.0±22.0 ^a	106.0±33.8 ^a	92.0±9.4 ^a	107.2±27.7 ^a
A-V (μmol/l)	Pregnant	-7.5±14.0 ^a	4.7±34.2 ^a	-22.0±33.6 ^a	-41.7±25.3 ^a
	Early	10.3±9.9 ^a	8.7±4.2 ^a	9.2±16.6 ^a	3.7±17.2 ^a
	Mid	11.0±15.4 ^a	9.2±18.7 ^a	5.2±8.3 ^a	9.2±9.2 ^a
	Late	13.5±7.8 ^a	9.5±4.4 ^a	6.7±18.2 ^a	20.2±11.8 ^a
Extraction (%)	Pregnant	-11.4±18.0 ^a	-5.4±37.4 ^a	29.3±35.2 ^a	-29.6±16.6 ^a
	Early	16.0±11.4 ^a	8.3±3.1 ^a	9.5±24.8 ^a	0.1±16.6 ^a
	Mid	17.4±27.6 ^a	5.4±13.8 ^a	18.4±17.3 ^a	9.5±8.1 ^a
	Late	26.5±17.2 ^a	9.1±3.1 ^a	6.7±20.9 ^a	18.1±6.7 ^a
Udder Uptake (μmol/min)	Pregnant	-16.5±28.6 ^a	9.1±27.1 ^a	-24.2±40.4 ^a	-40.4±32.6 ^a
	Early	50.1±40.8 ^a	25.2±13.3 ^a	32.3±70.6 ^a	7.6±54.5 ^a
	Mid	42.6±59.1 ^a	22.8±50.4 ^a	15.1±19.6 ^a	35.4±34.6 ^a
	Late	50.1±34.0 ^a	25.0±10.5 ^a	21.8±57.9 ^a	74.6±35.3 ^a

^{a,b,c} Mean values within a row indicated with different superscripts are significantly different (P<0.05).

Table 5. Arterial plasma concentrations, mammary arteriovenous differences and mammary uptake for free fatty acid and triacylglycerol during late pregnancy and different stages of lactation.

	Period of	Hay+concentration		Urea treated rice straw+concentration	
	experiment	HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Free fatty acid (C16-18) :					
Arterial concentrate (μmol/l)	Pregnant	369.5±83.0 ^b	526.7±135.3 ^{ab}	393.7±90.3 ^{ab}	573.3±165.6 ^a
	Early	302.0±111.3 ^a	314.4±115.8 ^a	317.5±171.5 ^a	446.5±223.5 ^a
	Mid	260.7±191.7 ^a	375.1±191.3 ^a	200.7±50.3 ^a	298.8±146.4 ^a
	Late	182.5±62.4 ^b	350.4±129.3 ^a	237.9±76.0 ^{ab}	288.8±117.7 ^{ab}
A-V (μmol/l)	Pregnant	-74.6±118.1 ^a	90.4±204.5 ^a	-67.2±37.7 ^a	-20.7±39.5 ^a
	Early	37.8±59.2 ^a	-121.4±198.8 ^a	-34.3±26.8 ^a	23.6±77.4 ^a
	Mid	-5.3±56.9 ^a	6.53±106.3 ^a	-24.5±34.3 ^a	-45.7±39.5 ^a
	Late	-37.1±25.2 ^{ab}	14.2±60.8 ^a	-86.9±41.7 ^a	15.7±56.7 ^a
Extraction (%)	Pregnant	-25±36 ^a	12±29 ^a	-19±14 ^a	-4±7 ^a
	Early	9±24 ^a	-40±61 ^a	-13±13 ^a	1±13 ^a
	Mid	-15±30 ^a	-4±29 ^a	-16±24 ^a	-20±25 ^a
	Late	-19±8 ^{ab}	2±16 ^a	-39±23 ^b	2±24 ^a
Udder Uptake (μmol/min)	Pregnant	-224.6±339.1 ^a	56.5±134.7 ^a	-124.9±81.9 ^a	-17.4±40.6 ^a
	Early	239.0±337.5 ^a	-391.5±661.9 ^a	-391.5±661.9 ^a	61.5±235.2 ^a
	Mid	-15.0±354.7 ^a	-5.5±287.4 ^a	-5.5±287.4 ^a	-143.3±118.1 ^a
	Late	-176.1±87.9 ^a	45.6±192.7 ^a	45.6±192.7 ^a	61.2±175.8 ^a
Triacylglycerol (C16-18) :					
Arterial concentrate (μmol/l)	Pregnant	239.3±145.6 ^a	80.6±41.4 ^{ab}	204.5±69.7 ^{ab}	195.6±118.4 ^{ab}
	Early	63.6±27.9 ^c	69.6±15.1 ^{bc}	111.4±13.2 ^a	96.7±20.0 ^{ab}
	Mid	108.6±64.1 ^a	83.9±37.1 ^a	80.5±16.0 ^a	88.4±40.2 ^a
	Late	82.4±34.4 ^b	71.4±7.5 ^b	134.7±13.1 ^a	62.9±10.2 ^b
A-V (μmol/l)	Pregnant	10.3±16.2 ^a	25.0±39.9 ^a	8.4±14.9 ^a	-7.0±28.9 ^a
	Early	52.7±26.9 ^{ab}	36.8±20.3 ^{ab}	68.8±16.0 ^{ab}	71.7±15.8 ^a
	Mid	81.3±48.9 ^a	65.7±31.1 ^a	52.4±13.5 ^a	64.4±25.2 ^a
	Late	53.5±25.2 ^{ab}	55.1±11.9 ^{ab}	84.7±30.3 ^a	41.1±5.5 ^b
Extraction (%)	Pregnant	3.1±3.2 ^a	20.2±31.1 ^a	3.9±6.4 ^a	6.9±30.8 ^a
	Early	79.9±9.9 ^a	50.1±23.5 ^a	62.8±16.8 ^a	73.9±2.6 ^a
	Mid	73.3±9.4 ^a	76.5±7.2 ^a	64.5±8.8 ^a	74.8±10.5 ^a
	Late	64.6±10.9 ^a	76.5±10.2 ^a	62.1±19.7 ^a	65.7±6.0 ^a
Udder Uptake (μmol/min)	Pregnant	28.9±47.3 ^a	29.0±22.8 ^a	40.7±40.5 ^a	-9.1±36.0 ^a
	Early	258.9±113.0 ^a	103.1±61.7 ^a	269.9±69.9 ^a	236.3±47.8 ^a
	Mid	298.9±203.0 ^a	179.6±81.3 ^a	147.1±54.7 ^a	229.0±61.9 ^a
	Late	186.8±64.6 ^a	150.2±46.0 ^a	245.4±120.8 ^a	153.1±24.4 ^a

^{a,b,c} Mean values within a row indicated with different superscripts are significantly different (P<0.05).

Table 6. Arterial plasma concentrations, mammary arteriovenous differences and mammary uptake for lactate during late pregnancy and different stages of lactation.

		Hay+concentration		Urea treated rice straw+concentration	
	Period of experiment	HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Lactate :					
Arterial concentrate (μmol/l)	Pregnant	124±21 ^a	138±52 ^a	188±66 ^a	138±47 ^a
	Early	135±54 ^a	127±46 ^a	95±25 ^a	114±34 ^a
	Mid	146±36 ^a	108±30 ^{ab}	119±23 ^{ab}	114±21 ^b
	Late	110±28 ^{ab}	124±19 ^a	91±15 ^b	97±29 ^{ab}
A-V (μmol/l)	Pregnant	-0.5±6.9 ^a	-25.3±10.2 ^a	7.3±33.3 ^a	-5.5±3.1 ^a
	Early	2.0±2.0 ^b	2.0±2.0 ^b	1.0±2.0 ^a	1.0±2.0 ^b
	Mid	1.0±1.0 ^b	2.0±1.0 ^b	1.1±2.0 ^a	2.0±1.0 ^b
	Late	2.0±1.0 ^b	2.0±1.0 ^b	8.0±2.0 ^a	2.0±3.0 ^b
Extraction (%)	Pregnant	-0.6±5.9 ^a	-20.3±9.9 ^b	4.3±16.7 ^a	-2.9±2.7 ^a
	Early	11.6±6.3 ^b	11.9±13.3 ^b	99.5±16.4 ^a	6.0±14.2 ^b
	Mid	8.5±5.8 ^c	23.4±8.5 ^b	93.4±14.6 ^a	18.9±4.3 ^{bc}
	Late	15.3±5.0 ^b	20.0±10.1 ^b	91.9±10.1 ^a	13.1±17.7 ^b
Udder Uptake (μmol/min)	Pregnant	-0.6±13.7 ^a	-5.8±3.8 ^a	11.8±51.5 ^a	-16.3±12.3 ^a
	Early	104.1±116.4 ^b	43.9±49.9 ^b	387.9±172.5 ^a	42.4±53.5 ^b
	Mid	62.9±44.1 ^b	60.8±26.5 ^b	386.8±115.5 ^a	70.6±32.4 ^b
	Late	95.1±56.8 ^b	55.9±39.4 ^b	297.5±112.9 ^a	55.5±74.0 ^b

^{a,b,c} Mean values within a row indicated with different superscripts are significantly different ($P < 0.05$).

Table 7. Arterial plasma concentrations for sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{++}) and inorganic phosphorus (Pi) during late pregnancy and different stages of lactation.

	Period of experiment	Hay+concentration		Urea treated rice straw+concentration	
		HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Plasma Na^+ (mM)	Pregnant	133.0 $\pm$ 1.8 ^a	135.0 $\pm$ 4.1 ^a	132.0 $\pm$ 2.2 ^a	135.5 $\pm$ 3.4 ^a
	Early	129.8 $\pm$ 2.2 ^b	133.7 $\pm$ 0.9 ^a	132.3 $\pm$ 1.2 ^{ab}	135.0 $\pm$ 2.8 ^a
	Mid	130.0 $\pm$ 2.1 ^a	131.5 $\pm$ 2.5 ^a	132.0 $\pm$ 2.4 ^a	133.3 $\pm$ 1.5 ^a
	Late	128.8 $\pm$ 1.9 ^b	130.0 $\pm$ 2.7 ^{ab}	132.0 $\pm$ 2.7 ^{ab}	133.0 $\pm$ 1.4 ^a
Plasma K^+ (mM)	Pregnant	3.8 $\pm$ 0.3 ^{ab}	3.3 $\pm$ 0.3 ^c	3.5 $\pm$ 0.3 ^{bc}	4.0 $\pm$ 0.2 ^a
	Early	3.5 $\pm$ 0.1 ^b	3.4 $\pm$ 0.1 ^b	3.6 $\pm$ 0.2 ^b	3.9 $\pm$ 0.2 ^a
	Mid	3.6 $\pm$ 0.2 ^b	3.4 $\pm$ 0.2 ^b	3.8 $\pm$ 0.3 ^b	4.2 $\pm$ 0.3 ^a
	Late	3.6 $\pm$ 0.3 ^b	3.8 $\pm$ 0.2 ^{ab}	3.7 $\pm$ 0.2 ^{ab}	3.9 $\pm$ 0.2 ^a
Plasma Cl^- (mM)	Pregnant	105.5 $\pm$ 4.2 ^a	98.8 $\pm$ 3.8 ^b	104.5 $\pm$ 3.8 ^{ab}	105.7 $\pm$ 2.7 ^a
	Early	104.0 $\pm$ 6.8 ^a	99.5 $\pm$ 5.6 ^a	99.0 $\pm$ 3.8 ^a	100.0 $\pm$ 3.6 ^a
	Mid	110.0 $\pm$ 4.7 ^a	98.5 $\pm$ 4.6 ^b	105.0 $\pm$ 6.9 ^{ab}	97.8 $\pm$ 4.6 ^b
	Late	101.5 $\pm$ 3.4 ^a	104.7 $\pm$ 5.3 ^a	99.3 $\pm$ 6.7 ^a	100.3 $\pm$ 5.4 ^a
Plasma Ca^{++} (mg%)	Pregnant	9.17 $\pm$ 1.06 ^a	8.93 $\pm$ 0.49 ^a	9.41 $\pm$ 0.82 ^a	9.64 $\pm$ 0.53 ^a
	Early	8.73 $\pm$ 0.78 ^a	9.32 $\pm$ 0.43 ^a	8.68 $\pm$ 0.69 ^a	9.46 $\pm$ 0.56 ^a
	Mid	9.40 $\pm$ 0.24 ^{ab}	9.78 $\pm$ 1.03 ^a	8.52 $\pm$ 0.51 ^b	9.35 $\pm$ 0.47 ^{ab}
	Late	9.27 $\pm$ 0.58 ^a	9.93 $\pm$ 0.83 ^a	9.06 $\pm$ 0.72 ^a	9.47 $\pm$ 0.55 ^a
Plasma P (mg%)	Pregnant	6.28 $\pm$ 1.39 ^a	6.07 $\pm$ 1.38 ^a	6.97 $\pm$ 0.71 ^a	6.35 $\pm$ 0.71 ^a
	Early	5.46 $\pm$ 0.58 ^a	6.75 $\pm$ 0.53 ^a	5.83 $\pm$ 1.26 ^a	6.18 $\pm$ 1.02 ^a
	Mid	6.17 $\pm$ 1.02 ^a	6.66 $\pm$ 1.07 ^a	6.59 $\pm$ 0.81 ^a	7.28 $\pm$ 0.44 ^a
	Late	6.72 $\pm$ 0.42 ^a	6.58 $\pm$ 1.14 ^a	6.72 $\pm$ 0.95 ^a	7.16 $\pm$ 0.48 ^a

^{a,b,c} Mean values within a row indicated with different superscripts are significantly different ($P < 0.05$).

Table 8. Milk concentrations for sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{++}) and inorganic phosphorus (Pi) during late pregnancy and different stages of lactation.

	Period of experiment	Hay+concentration		Urea treated rice straw+concentration	
		HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Milk Na^+ (mM)	Early	53.3 $\pm$ 6.6 ^b	69.0 $\pm$ 2.4 ^a	54.0 $\pm$ 2.4 ^b	54.0 $\pm$ 4.2 ^b
	Mid	59.3 $\pm$ 3.8 ^a	64.5 $\pm$ 15.5 ^a	56.7 $\pm$ 4.5 ^a	57.7 $\pm$ 6.2 ^a
	Late	58.5 $\pm$ 1.7 ^{ab}	66.0 $\pm$ 4.2 ^a	61.5 $\pm$ 5.2 ^{ab}	56.3 $\pm$ 7.9 ^b
Milk K^+ (mM)	Early	36.5 $\pm$ 3.2 ^a	35.8 $\pm$ 3.2 ^a	39.2 $\pm$ 0.8 ^a	38.5 $\pm$ 3.6 ^a
	Mid	41.9 $\pm$ 4.4 ^a	39.4 $\pm$ 6.2 ^a	39.4 $\pm$ 4.5 ^a	39.8 $\pm$ 4.2 ^a
	Late	39.3 $\pm$ 4.5 ^{ab}	41.4 $\pm$ 1.8 ^a	38.7 $\pm$ 4.4 ^{ab}	35.3 $\pm$ 2.9 ^b
Milk Cl^- (mM)	Early	32.0 $\pm$ 2.4 ^b	49.0 $\pm$ 7.6 ^a	35.5 $\pm$ 4.0 ^b	35.3 $\pm$ 4.5 ^b
	Mid	32.3 $\pm$ 1.5 ^b	44.3 $\pm$ 7.9 ^a	28.5 $\pm$ 1.7 ^b	32.3 $\pm$ 6.6 ^b
	Late	33.0 $\pm$ 5.4 ^{ab}	39.7 $\pm$ 6.2 ^a	31.7 $\pm$ 5.5 ^{ab}	30.0 $\pm$ 4.2 ^b
Milk Ca^{++} (mg%)	Early	101.0 $\pm$ 24.0 ^a	105.0 $\pm$ 11.8 ^a	99.0 $\pm$ 27.4 ^a	112.0 $\pm$ 9.0 ^a
	Mid	68.0 $\pm$ 26.7 ^a	101.0 $\pm$ 10.1 ^a	92.0 $\pm$ 34.9 ^a	97.0 $\pm$ 20.7 ^a
	Late	95.0 $\pm$ 9.1 ^a	121.0 $\pm$ 10.9 ^a	101.0 $\pm$ 31.2 ^a	118.0 $\pm$ 11.0 ^a
Milk P (mg%)	Early	51.0 $\pm$ 20.6 ^a	52.0 $\pm$ 13.1 ^a	55.0 $\pm$ 7.2 ^a	68.0 $\pm$ 10.0 ^a
	Mid	64.0 $\pm$ 5.0 ^a	60.0 $\pm$ 8.4 ^a	60.0 $\pm$ 14.5 ^a	57.0 $\pm$ 2.9 ^a
	Late	66.0 $\pm$ 9.6 ^a	65.0 $\pm$ 7.5 ^a	62.0 $\pm$ 15.6 ^a	68.0 $\pm$ 5.7 ^a

^{a,b} Mean values within a row indicated with different superscripts are significantly different ($P < 0.05$).

DISCUSSION

The conclusions in the present studies for the mammary uptake of substrates are not based on changes in arteriovenous concentration differences and extraction ratio between late pregnancy and lactating periods of both groups of crossbred HF animals. The rate of blood flow to the udder was a major determinant of rate of substrate supply. In the present results, there were markedly changes in average blood flow to the udder during the transition period from pregnancy to lactation. Changes of substrate across the udder should account for changes in mammary blood flow. The low values of both arteriovenous concentration differences and extraction ratio of glucose during late pregnancy in all groups indicates that the rate of glucose uptake by the mammary gland seemed to be dependent on both mammary blood flow and the activity of the mammary epithelial cell. A marked increase of both mammary extraction ratio and uptake of glucose during lactation in all groups would be an evidence for the high demand of glucose by the mammary gland for milk synthesis.

It has been known that volatile fatty acid in the form of acetate are the major of source energy of normal fed ruminants. In the present study, mammary arteriovenous concentration differences and extraction ratio of acetate during late pregnancy and lactation were unchanged in all groups. Its low rate of mammary uptakes for acetate during pregnancy was critically dependent upon rate of blood flow. Circulating β -hydroxybutyrate arise mainly from rumen butyrate in the fed animal (Leng and West, 1969). In the present result, lower levels of arteriovenous concentration differences and extraction ratio of β -hydroxybutyrate across the mammary gland were apparent with no change in the arterial plasma concentration during late pregnancy indicate that the utilization by the mammary tissue was not obvious in the late pregnant period of both 50%HF and 87.5%HF feeding on either hay or urea treated rice straw. Although the greater energy requirement of the late pregnant animals resulting in increased hepatic ketogenesis due to greater mobilization of fat reserves (Schultz, 1974).

In the present experiment, the mean values for arterial plasma concentration of free fatty acid and triacylglycerol increased in the late pregnancy of both crossbred HF animals and were more sensitive to alteration than other blood substrate, this phenomenon has been proposed as an indication of under-nutrition (Reid and Hinks,

1962). However the mobilization of body fat in late pregnancy occurred in response to hormonal secretion was also noted (Lindsay, 1973). Thus it should be regarded as a physiological phenomenon, not a consequence of under-nutrition in 50%HF and 87.5%HF animals feeding on either hay or urea treated rice straw. The measurement of arteriovenous differences of FFA across the mammary gland together with mammary blood flow did not provide a quantitative estimation of their total uptake by mammary tissue, since there is the release of FFA into venous blood due to triacylglycerol hydrolysis during the uptake of plasma triacylglycerol as in lactation (West et al., 1967). However, the changes in arteriovenous differences, the extraction ratio and net uptake of FFA would be suggested that the utilization of FFA occurs during the development of the mammary gland in normal late pregnancy. The net uptake of triacylglycerol by the mammary gland significantly increased in lactating period in comparison to the late pregnancy in all groups. It is possible that changes for releasing of FFA which are a result of changes of enzymatic activity of lipoprotein lipase in the mammary tissue. This enzyme activity has been reported to be higher in lactating bovine mammary tissue relative to pregnant tissue (Shirley et al., 1973).

In the present experiment, arterial plasma lactate levels were variable in all crossbred HF animals. Circulating lactate level measured as a monitor of excitement because it is known to increase under conditions of stress (Marple et al., 1969) and it might not occur in this trained animals during the experiment. The variable uptake of circulating lactate by the mammary gland in both 50%HF and 87.5%HF animals is difficult to explain. Thus, during lactation in each group, amounts of lactate would be neither absorbed nor excreted by the mammary gland of crossbred lactating animals.

The composition of aqueous phase of milk was not affected by feeding on different types of roughage in either 50%HF or 87.5%HF animals. The mechanism of ion transport in the mammary cell would be proposed as either occurring by transcellular route or a paracellular route (Linzell and Peaker, 1971). In the present results, all values of ion compositions in milk were in similar manners. These results might indicate that no disturbances of ion pumps activity in intracellular route were apparent at different periods of lactation and different groups of animals.

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Running head (Short Title) : Utilization of glucose in the mammary gland

Key Words: crossbred Holstein cattle, glucose metabolism, mammary gland, roughage, lactation

Comparative studies on the utilization of glucose in the mammary gland of crossbred Holstein cattle feeding on different types of roughage during different stages of lactation^a

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**Comparative studies on the utilization of glucose in the mammary gland
of crossbred Holstein cattle feeding on different types of roughage
during different stages of lactation**

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ABSTRACT : The present experiment was carried out to study the utilization of glucose in the mammary gland of crossbred Holstein cattle during feeding with different types of roughage. Sixteen first lactating crossbred Holstein cattle which comprised eight animals of two breed types, Holstein Friesian x Red Sindhi (50x50 = 50%HF) and Holstein Friesian x Red Sindhi (87.5x12.5 = 87.5%HF). They were divided into four groups of 4 animals each of the same breed. The utilization of glucose in the mammary gland was determined by measuring rates of glucose uptake and the incorporation of glucose into milk components in both groups of 50% HF and 87.5% HF animals feeding on either hay or urea treated rice straw. In early lactation, there were no significant differences of the total glucose entry rate and glucose carbon recycling among groups of crossbred animals feeding on either hay or urea treated rice straw. During lactation advance, the total glucose turnover rates and recycling of carbon glucose of crossbred HF animals feeding on urea treated rice straw were markedly higher than those of crossbred HF animals feeding on hay as roughage, whereas there were no significant changes for both groups of crossbred animals feeding on hay. The percentages and values of non-mammary glucose utilization showed an increase during lactation advance in the same group of both 50% HF and 87.5% HF animals. The percentage of glucose uptake for utilization in the synthesis of milk lactose by the mammary gland was approximately 62% for both groups of 87.5% HF and by approximately 55% for both groups of 50% HF animals feeding on either hay or urea treated rice straw. Intracellular glucose 6-phosphate metabolized via the pentose phosphate pathway accounted for the NADPH (reducing equivalent) of fatty acid synthesis in the mammary gland being higher in 87.5% HF animals during mid- lactation. A large proportion of metabolism of glucose via the Embden-Meyerhof pathway in the mammary gland was more apparent in both groups of 50% HF animals than those of 87.5% HF animals during early and mid- lactation while it markedly increased for both groups of 87.5% HF animals during late lactation. It can be concluded that utilization of glucose in the mammary gland occurs in a different manner for 50% HF and

87.5% HF animals feeding on either hay or urea treated rice straw. The glucose utilization for biosynthetic pathways in the mammary gland of 50% HF animals is maintained in a similar pattern throughout the periods of lactation. A poorer lactation persistency in both groups of 87.5% HF animals occurs during lactation advance, which is related to a decrease in the lactose biosynthetic pathway.

Key words : crossbred Holstein cattle, glucose metabolism, mammary gland, roughage, lactation

INTRODUCTION

It is known that dairy herds in tropical countries are mixed exotic breeds and crossbreeds. Exotic Bos taurus breeds have higher milk production but they also have inherent disadvantageous traits. They have low heat tolerance with a higher heat load which causes a decrease in milk production (Maust et al., 1972). Bos indicus cattle have low genetic potential for milk production but are well adapted to the environment. Therefore, exotic Bos taurus breeds are used mainly for crossbreeding with native and other Bos indicus cows. However, low milk production of both exotic and crossbred cattle is still the main problem in dairy farming in the tropics. 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. One of the problems which may limit milk production of dairy cattle in the tropics 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. To overcome the livestock feed problem, several chemicals such as urea have been used to improve the feeding value of low quality roughage (Klopfenstein, 1978). An improvement in rice straw by treating with urea to help animals survive during periods of scarcity has been reported (Jayasuriya and Perera, 1982; Promma et al., 1994). The mechanism acting within the body and the mechanism responsible for mammary secretory activity for milk production in different types of lactating crossbred Holstein cattle feeding on urea treated rice straw as a source of roughage are unknown, although some profound biochemical and physiological differences that occur between 50%HF and 87.5%HF animals have been reported (Chaiyabutr et al., 1997; 1998; Nakamura et al., 1986).

Glucose is an important intermediary of metabolism in general and is particularly important for lactation. Glucose is utilized by the mammary gland for the biosynthesis of

lactose, triacylglycerol and citrate. This has been studied in lactating ruminants *in vivo* (Annison and Linzell, 1964; Chaiyabutr et al., 1980) and in the isolated perfused udder (Hardwick et al, 1963). The role of glucose in regulating milk secretion has been formulated in the theory that lactose secretion can draw water osmotically from the inside of the mammary cells to milk (Linzell and Peaker, 1971). This is believed to be a mechanism for increasing milk yield by which bulk water movement occurs into milk. Metabolism of glucose in mammary glands is also important in providing the reducing equivalents required for the *de novo* synthesis of fatty acids (Bauman and Davis, 1975). Few data are available concerning the utilization of glucose and glucose metabolism in the udders of crossbred Holstein dairy cattle *in vivo* during feeding with different types of roughage. Metabolism parameters in different types of crossbred cattle are known to be inherited and are thought to be among the causes of differences in mechanisms of milk secretion. Therefore, the present experiment was conducted to obtain the above information on whether the responses in glucose metabolism and the efficiency of utilization of glucose by the mammary gland are the same in both types of 50% Holstein and 87.5% Holstein cattle feeding on different types of roughage.

MATERIALS AND METHODS

Animals and feed management.

Sixteen first lactating crossbred Holstein cattle were chosen from a herd which comprised eight animals of two breed types, Holstein Friesian x Red Sindhi (50:50 = 50% HF) and Holstein Friesian x 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 $34\pm 1^{\circ}\text{C}$ and the minimum temperature at night was $26\pm 1^{\circ}\text{C}$. Before parturition, animals were individually fed a concentrate of an average of 4.0 kg/day (DM basis) and roughage to maintain the body condition score at three until calving. 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 0600-0700 h and the other half between 1600-1700 h at the time of milking. 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 x 21 day system of urea treatment. After 21 days, the treated rice straw with 5% urea was offered to the animals.

Experimental procedures.

Three consecutive periods of experiments were carried out in each group. Period 1 began 30 days postpartum (early lactation). Period 2 began 120 days postpartum (mid-lactation) and period 3 began 210 days postpartum (late lactation). Animals were fed the same ration through the completion of period 3. In all periods of experiments, the glucose turnover rate, mammary udder blood flow, glucose metabolism in the udder, milk yield and milk composition were measured. Animals were normally milked at around 0600 h and 1700 h. On the day of the experiment, milk secretion was recorded by hand milking in the afternoon and the measurement of udder blood flow was carried out. Animals were weighed after collecting the milk sample.

On the day before the experiment began in each lactating period, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm) were inserted into either the left or right subcutaneous abdominal vein (milk vein) by using a intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, U.K.) under local anesthesia. This was done in standing animals for the measurement of mammary udder blood flow and for collection of venous blood. The tip of the catheter was positioned near the sigmoid flexure anterior to the point at which the vein leaves the udder. The other catheter was positioned downstream about 20 cm from the first one. The catheter for isotope infusion was inserted into an ear vein under local anesthesia. All catheters were flushed with sterile heparinized normal saline and were left in place during the experiment.

Glucose turnover measurements.

The present study on glucose kinetics and efficiency of utilization of glucose by the mammary gland using both (U- ^{14}C)-glucose and (3- ^3H)-glucose infusions in crossbred animals was performed at different stages of lactation: early, mid- and late lactation, as cows were fed either hay or urea treated rice straw through the period of the experiment. Glucose kinetic studies of each animal in each lactating period were carried out as described previously by Chaiyabutr et al. (1998). Briefly, at about 1100h a priming dose of radioactive glucose in 20 ml of sterile NSS containing 60 μCi (3- ^3H) glucose and 40 μCi (U- ^{14}C) glucose was administered intravenously via the ear vein catheter and followed by a constant infusion of 1 ml/min of sterile saline (0.9%) containing 2 μCi (U- ^{14}C) glucose and 3 μCi (3- ^3H) glucose for 4h (Peristaltic pump; EYLA Model 3). During the final 1 hour (1400-1500h) of infusion, three sets of blood samples were collected at 20 min. intervals. A venous blood sample was collected from the milk vein via a catheter while an arterial blood sample was collected from the coccygeal artery by venipuncture with a #21 needle. Blood samples in heparinized tubes were kept in crushed ice for chemical studies. Milk secretion was recorded for the final 1 hour of infusion. Milk samples were used for measurement of radioactive glucose incorporation into other milk components.

Udder blood flow measurements.

Measurements of udder blood flow through half of the udder were performed in duplicate by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion as described by Chaiyabutr et al. (1997). In brief, a dye (T-1824) was dissolved in sterile normal saline and diluted to a concentration of 100 mg/L. The solution was infused by a peristaltic pump (Gilson Medical Electronics) at a constant rate of 85 ml/min into the milk vein for 1 min which could produce adequate mixing of dye with blood. Before infusion, blood was drawn from downstream in the milk vein as a pre-infusion sample. About 10 seconds after starting the infusion, 10 ml of blood was drawn from downstream in the milk vein at a constant rate into a heparinized tube. Two consecutive plasma samples were taken during each dye infusion at about 5 min intervals. Blood flow of half of the udder was calculated from plasma samples using the equation derived by Thompson and Thomson (1977). Quarter milking showed that the yields of the two halves of the udder were similar. Udder blood flow was therefore calculated by doubling the flow measured in one milk vein (Bickerstaffe et al., 1974). Packed cell volume was measured after centrifugation of the blood in a microcapillary tube. Lactating cows were hand milked before start of infusion and milked again before the

final 1 hour (1400-1500) of infusion. Milk was collected during the final 1 hour of infusion for measurement of radioactive glucose incorporation into lactose, milk citrate and milk fat. Milk yield was recorded by weight.

Chemical methods

Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase (Human GmBH, Germany). Plasma triacylglycerol (TG, C₁₆-C₁₈) and plasma free fatty acids (FFA, C₁₆-C₁₈) were measured by using gas chromatography (Shimazu GC-7AG Gas Chromatograph) in comparison with the appropriate internal standard. The internal standards of triheptadecanoate and heptadecanoic acid for estimation of plasma TG and FFA, respectively, were as described by Thomson et al (1979). The specific activity of labelled plasma glucose was determined by the method described by Chaiyabutr and Buranakarl (1989). Radiochemicals for [U-¹⁴C]glucose and [3-³H]glucose were obtained from the Radiochemical Center, Amersham Bucks, U.K. The isotopes were dissolved in sterile pyrogen free saline (0.9% NaCl). The radioactivity in blood bicarbonate was measured by acidifying 2 ml of blood with an equal volume of 6% perchloric acid. ¹⁴CO₂ was liberated and trapped as K¹⁴CO₃ in a plastic cup which contained 0.1 ml 40% KOH.

The concentration of milk lactose was determined by spectrophotometry (Teles et al., 1978). Lactose radioactivity was determined after isolation by the hydrolysis method (Wood et al., 1965). Milk triglyceride fatty acid composition (C₆ to C₁₈) was determined by gas chromatography after extraction by chloroform and methanol (Christopherson and Glass, 1969). Milk fat was isolated by centrifugation at 50,000 g for 1h at 3°C. The solidified top layer of lipid was assayed for radioactivity after extraction by chloroform and methanol. The concentration of milk citrate was determined by spectrophotometry from tricarboxylic acid filtrate (White and Davies, 1963). Citrate radioactivity was determined after isolation by anion exchange chromatography (Hardwick et al., 1963).

Calculations

Glucose turnover in the whole animal (T), expressed as µmol/min, was calculated from the equation

$$T = I/G_A$$

where I = rate of infusion of U- ^{14}C glucose or 3- ^3H glucose ($\mu\text{Ci}/\text{min}$) and G_A = specific activity of ^{14}C - or ^3H -glucose in arterial plasma at equilibrium ($\mu\text{Ci}/\mu\text{mol}$).

Recycling of glucose carbon in the whole animal, expressed as % glucose turnover, was calculated from the equation:

$$\text{Recycling} = (T_3 - T_{14}) \times 100 / T_3$$

where T_3 = reversible turnover of glucose calculated from 3- ^3H glucose and T_{14} = irreversible turnover of glucose calculated from U- ^{14}C glucose.

Glucose clearance in the whole animal (C_G), expressed as ml/min, was calculated from the equation:

$$C_G = T_3 / P_{AG}$$

where T_3 = reversible turnover of glucose calculated from 3- ^3H glucose ($\mu\text{mol}/\text{min}$) and P_{AG} = arterial plasma glucose concentration ($\mu\text{mol}/\text{ml}$).

Uptake of substrates by the udder (U_M), expressed as $\mu\text{mol}/\text{min}$, was calculated from the equation:

$$U_M = Q_P \times (P_A - P_V)$$

where Q_P = udder plasma flow (ml/min), P_A = concentration of substrate in coccygeal arterial plasma ($\mu\text{mol}/\text{ml}$) and P_V = concentration of substrate in mammary venous plasma ($\mu\text{mol}/\text{ml}$).

Milk substrate output (OS), expressed as $\mu\text{mol}/\text{min}$, was calculated from the equation:

$$OS = M \times C_m / 1000$$

where M = milk secretion rate (ml/min) and C_m = concentration of substrate in milk ($\mu\text{mol}/\text{l}$).

Release (R) of $^{14}\text{CO}_2$ into mammary venous blood, expressed as μmol glucose incorporated into CO_2 per min, was calculated from the equation:

$$R_{\text{CO}_2} = Q_B \times (^{14}\text{CO}_{2V} - ^{14}\text{CO}_{2A}) / G_A$$

where Q_B = udder blood flow (ml/min), $^{14}\text{CO}_{2A}$ = arterial blood $^{14}\text{CO}_2$ ($\mu\text{Ci}/\text{ml}$), $^{14}\text{CO}_{2V}$ = mammary venous blood $^{14}\text{CO}_2$ ($\mu\text{Ci}/\text{ml}$) and G_A = specific activity of ^{14}C -glucose in arterial plasma at equilibrium ($\mu\text{Ci}/\mu\text{mol}$).

Incorporation (A) of radioactivity from glucose into milk components was calculated from the equation:

$$A = M_A / G_A \times t$$

where A = incorporation of radioactivity from glucose into milk components ($\mu\text{mol}/\text{min}$), M_A = total activity of ^3H or ^{14}C in the milk components (μCi), G_A = specific activity of ^{14}C - or ^3H -glucose in arterial plasma at equilibrium ($\mu\text{Ci}/\mu\text{mol}$) and t = time of infusion (min).

This value of A probably underestimates incorporation of radioactivity from glucose into milk constituents by using G_A . During the early part of the infusion, the specific radioactivity of plasma glucose is likely to be below that determined at equilibrium.

Requirement of NADPH for fatty acid synthesis (P) in the mammary gland, expressed as $\mu\text{mol}/\text{min}$, was calculated from the equation:

$$P_{\text{NADPH}} = \Sigma[\text{FFA}_n \times (n-2)]$$

where n = chain length of the fatty acid (6 to 16) and FFA_n = output in milk of fatty acid chain length n ($\mu\text{mol}/\text{min}$).

Values for FFA_n were calculated from all medium chain length fatty acids and 30% of C_{16} -fatty acids (Annison and Linzell, 1964).

Net metabolism of glucose phosphorylation (G_{6p}), expressed as $\mu\text{mol}/\text{min}$, was calculated from the equation:

$$G_{6p} = U_G - L$$

where U_G = mammary glucose uptake ($\mu\text{mol}/\text{min}$) and L = output of lactose in milk ($\mu\text{mol}/\text{min}$).

Net metabolism of glucose (B) to the galactose or glucose moiety of lactose, expressed as $\mu\text{mol}/\text{min}$, was calculated from the equation:

$$B = L$$

where L = output of lactose in milk ($\mu\text{mol}/\text{min}$).

Metabolism of glucose via the pentose phosphate pathway (PC) was calculated from the equation:

$$Y = 3 \text{ PC} / (1 + 2\text{PC})$$

where Y = specific yield of $^{14}\text{CO}_2$ from (1- ^{14}C) glucose via the pentose phosphate pathway (Katz and Wood, 1963).

If the NADPH formed via PC were used exclusively for reductive biosynthesis of fatty acids, the ^3H -incorporation from (3- ^3H) glucose into fatty acids would equal the $^{14}\text{CO}_2$ released from (1- ^{14}C) glucose via the pentose phosphate pathway (Katz et al., 1974). Metabolism of glucose via PC was therefore calculated from the equation:

$$Z = 3 \text{ PC} / (1 + 2\text{PC})$$

where Z = (Total ^3H in milk fatty acid)/ $t \times G_A \times (U_G - L)$

Net metabolism of glucose 6-phosphate via (G_{PC}), expressed as $\mu\text{mol/min}$, was calculated from the equation:

$$G_{PC} = G_{6p} \times PC$$

Net metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway (G_E), expressed as $\mu\text{mol/min}$, was calculated from the equation:

$$G_E = G_{6p} - (B + G_{PC})$$

The $^3\text{H}/^{14}\text{C}$ ratio in the plasma and related products was calculated from the equations:
 $^3\text{H}/^{14}\text{C}$ glucose = $^3\text{H}/^{14}\text{C}$ in plasma glucose relative to a $^3\text{H}/^{14}\text{C}$ ratio of 1 in the infusion,
 $^3\text{H}/^{14}\text{C}$ lactose = $^3\text{H}/^{14}\text{C}$ in milk lactose relative to a $^3\text{H}/^{14}\text{C}$ ratio of 1 in the infusion,
 $^3\text{H}/^{14}\text{C}$ citrate = $^3\text{H}/^{14}\text{C}$ in milk citrate relative to a $^3\text{H}/^{14}\text{C}$ ratio of 1 in the infusion, and
 $^3\text{H}/^{14}\text{C}$ triacylglycerol = $^3\text{H}/^{14}\text{C}$ in milk triacylglycerol relative to a $^3\text{H}/^{14}\text{C}$ ratio of 1 in the infusion.

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 (Duncan, 1955). Values were compared among lactating periods in each group using the paired t-test. Mean values are presented as mean $\pm$ SD.

RESULTS

Glucose turnover, related variables and body weight (Table 2)

The glucose turnover rate in crossbred Holsteins was determined by making simultaneous estimates of the total glucose entry rate using 3- ^3H glucose infusion and the utilization rate of glucose using $[\text{U}-^{14}\text{C}]$ glucose infusion. All values of glucose turnover rates in different periods of lactation for all groups of crossbred animals are expressed as absolute values. In early lactation, there were no significant differences of the total glucose entry rate and glucose carbon recycling among groups of crossbred animals feeding on either hay or urea treated rice straw. However, in mid- and late lactation of 50% HF and 87.5% HF animals feeding on urea treated rice straw, the total glucose turnover rates and recycling of carbon glucose were markedly higher than those of crossbred HF animals feeding on hay as roughage ($P < 0.05$). Comparing for the early lactating period in the same group, both 50% HF and 87.5% HF animals feeding on urea treated rice straw showed significant increases in the total glucose turnover rate ($P < 0.05$), recycling of carbon glucose ($P < 0.05$) and plasma glucose

c l e a r a n c e
($P < 0.01$) during late lactation, whereas there were no significant changes for both groups of crossbred animals feeding on hay. Both absolute values and percentages of utilization of glucose by tissues other than the mammary gland were calculated from the total rate of glucose synthesis and the rate of glucose uptake by the mammary gland. It was higher in both groups of 50% HF than those of 87.5% HF animals feeding on either hay or urea treated rice straw in all stages of lactation. The percentages and values of non-mammary glucose utilization showed an increase during lactation advance in the same group of both 50% HF and 87.5% HF animals. During the course of lactation there were no significant differences of body weight among groups of 87.5%HF and 50%HF animals.

Udder blood flow, milk yield and milk composition (Tables 3, 4)

In 87.5% HF animals feeding on either hay or urea treated rice straw, mammary blood flow and milk yield initially showed significantly higher levels ($P < 0.05$) in early lactation than those of 50% HF animals. Both mammary blood flow and milk yield showed a proportional decrease from the early lactating period in both groups of 87.5% HF animals. However, for 50% HF animals feeding on either hay or urea treated rice straw, the trends for persistency were observed as for udder blood flow and milk yield. The values of milk lactose concentration showed no differences among groups of crossbred animals or among periods of lactation in the same group. In 87.5% HF animals, mean values of milk citrate concentration during early lactation were significantly higher ($P < 0.05$) than those of 50% HF animals feeding on either hay or urea treated rice straw. During lactation advance, the milk citrate concentration decreased in both groups of 87.5% HF animals while it remained constant for 50% HF animals. Milk triacylglycerol concentrations of both groups of 50% HF animals were markedly higher than those of 87.5% HF animals feeding on either hay or urea treated rice straw in all periods of lactation.

Utilization of glucose carbon in the udder (Table 5)

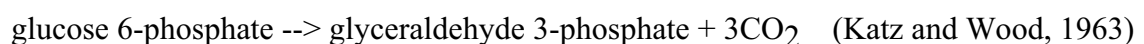
A low milk lactose secretion and citrate secretion during early lactation were apparent in both groups of 50% HF animals when compared to those of 87.5% HF animals feeding on either hay or urea treated rice straw. These differences were primarily due to differences in milk secretion rates. However, the percentage of utilization of glucose carbon for synthesis of milk lactose was not significantly different between 87.5% HF and 50% HF animals. The

utilization of glucose carbon for synthesis of milk citrate for 87.5% HF animals was markedly higher than that of 50% HF during early and mid-lactation. However, in both groups of 87.5% HF, the utilization of glucose carbon for synthesis of milk citrate decreased from that in the early lactating period, while the trends for persistency throughout lactating periods were observed in both groups of 50% HF animals feeding on either hay or urea treated rice straw. The utilization of glucose for synthesis of milk triacylglycerol was significantly lower ($P < 0.05$) in 87.5% HF during early lactation when compared to 50% HF animals while it increased during lactation advance. The ^3H from C-3 of glucose was recovered in milk fat. The major portion of this ^3H was associated with the fatty acid fraction of the saponified triacylglycerol. Less than 2% of radioactive carbon was present in triacylglycerol in both groups of 50% HF animals feeding on either hay or urea treated rice straw. The amount of ^{14}C -glucose incorporated to CO_2 in the venous blood varied among different stages of lactation in both groups of crossbred HF animals.

Rates of pathways of glucose metabolism in the udder (Table 6)

Data for glucose metabolism via the pentose phosphate pathway show that the incorporation of ^3H from $[3-^3\text{H}]\text{glucose}$ into fatty acids and the flux through the pentose phosphate pathway during mid-lactation was lower in both groups of 50% HF animals when compared to those of 87.5% HF animals. The flux was calculated to be 261 and 83 $\mu\text{mol/min}$ for 87.5% HF and 50% HF animals feeding on hay, respectively, and 253 and 149 $\mu\text{mol/min}$ for 87.5% HF and 50% HF animals feeding on urea treated rice straw, respectively. Correction of the lower $^3\text{H}/^{14}\text{C}$ ratio likely to be present in intracellular glucose 6-phosphate gave flux values of 299 and 118 $\mu\text{mol/min}$ for 87.5% HF and 50% HF animals feeding on hay, respectively, and 314 and 220 $\mu\text{mol/min}$ for 87.5% HF and 50% HF animals feeding on urea treated rice straw, respectively. All of these values declined during late lactation.

The results of the net metabolism of glucose 6-phosphate via the pentose phosphate pathway (PC) has been calculated according to the equation:



Complete metabolism of one molecule of glucose 6-phosphate according to this equation would require three cycles of the pentose phosphate pathway. Therefore, the flux through the pathway should be three times the net rate of glucose metabolized in the pentose phosphate pathway. From the results during mid-lactation of individual animals, mean values of 254 and 63 $\mu\text{mol/min}$ for 87.5% HF and 50% HF animals feeding on hay respectively and 255 and 90

$\mu\text{mol/min}$ for 87.5% HF and 50% HF animals feeding on urea treated rice straw, respectively, of the intracellular glucose phosphorylated by the mammary gland were calculated to be completely metabolized via the pentose phosphate pathway. The percentages of net metabolism of glucose 6-phosphate via the pentose phosphate pathway of both groups of 87.5% HF was significantly higher when compared to those of 50% HF animals. The percentages of metabolism of glucose 6-phosphate to the galactose moiety of lactose were slightly higher in 87.5% HF when compared to 50% HF animals and during lactation advance, these values decreased in both groups of 87.5% HF while it remained constant for 50% HF animals. Metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway was calculated either in terms of as absolute values or the proportion of glucose metabolized, which was markedly higher in both groups of 50% HF, while it markedly increased for 87.5% HF animals during late lactation.

NADPH production from glucose (Table 7)

It can be calculated from the milk fat composition and output in the present experiment that the requirements for NADPH for fatty acid synthesis varied among groups of animals and among periods of lactation. During mid- lactation, the NADPH formation from glucose accounted for 32% to 42% of that required for fatty acid synthesis *de novo* in the mammary gland of 87.5% HF, in comparison to values of 11% to 15% for 50% HF animals feeding on either hay or urea treated rice straw ($P < 0.05$).

Milk fatty acid concentrations (Table 8)

During early lactation, the milk fatty acid concentrations with a chain length of C_6 to C_{18} for both groups of 50% HF animals were significantly higher than those of 87.5% HF animals feeding on either hay or urea treated rice straw ($P < 0.05$). During mid- and late lactation, similar concentrations were maintained as in early lactation for both groups of 50% HF animals. There was considerable variation with advanced lactation in the levels of milk fatty acid concentration of both groups of 87.5% HF animals. During mid- and late lactation, the milk fatty acid concentration, particularly with a chain length of C_{16} to C_{18} , increased to the same level as that in 50% HF animals.

The $^3\text{H}/^{14}\text{C}$ ratios in glucose and related products (Table 9)

The $^3\text{H}/^{14}\text{C}$ ratio in arterial plasma glucose was lower than that of the infusion in both groups of crossbred HF cattle. These values were not different among groups of animals, indicating some recycling of glucose-C in the whole animal. A slight decrease in the $^3\text{H}/^{14}\text{C}$ ratio was seen in milk lactose in both groups of 50% HF animals, whereas the $^3\text{H}/^{14}\text{C}$ ratio of milk triacylglycerol was slightly higher in both groups of 87.5% HF animals. The ^3H and ^{14}C from glucose were also shown to be incorporated into milk citrate. The $^3\text{H}/^{14}\text{C}$ ratio of milk citrate was slightly higher in 87.5% HF during early lactation.

DISCUSSION

The present results and those of Chaibabutr et al. (1997) indicate that both 50% HF and 87.5% HF animals feeding on urea treated rice straw as roughage did not show any under-nutritional effects in comparison to those fed with hay in different periods of lactation. However, the milk yields in both groups of 50% HF animals feeding on either hay or urea treated rice straw was significantly lower than those of 87.5% HF in the early period of lactation. The milk secretion of both groups of 50% HF animals was not dependent on the blood glucose level, since the plasma glucose concentration of 50% HF has been shown to be slightly higher than that of both groups of 87.5% HF animals (Chaibabutr et al., 1998). The udder blood flow showed significant differences between 50% HF and 87.5% HF animals in early lactation while the ratio of udder blood flow to the rate of milk yield was not different. This might support the previous conclusion from a study in cows or goats by Linzell (1973) which found that milk secretion was shown to be related to the mammary blood flow. However, it has been reported that the arteriovenous differences of blood glucose across the udder remained constant over a wide range of arterial concentration in both types of crossbred HF animals (Chaibabutr et al., 1998). The low milk yield in both groups of 50% HF animals was related to a low lactose yield but was not related to the lactose concentration in milk when compared to those of 87.5% HF animals. These results can be attributed to a difference in the activity of the mammary epithelial cells between 50% HF and 87.5% HF animals.

Glucose is known to be used for the synthesis of lactose and other milk components in the process of milk synthesis (Linzell and Peaker, 1971; Bauman and Davis, 1975). Measurement of glucose kinetics in both types of crossbred HF animals feeding on either hay or urea treated rice straw in the present studies gave similar results. Values of glucose turnover rates were not different among groups of crossbred cattle. Values for irreversible turnover of $[\text{U-}^{14}\text{C}]$ glucose in the low milk yield of 50% HF cattle in the present study are

within the range reported in high milk yield cows of comparable body weight (Bickerstaffe et al., 1974). The reversible turnover of [3-³H]glucose may represent the total glucose turnover rate as the ³H is not recycled from products of partial glucose degradation (Katz et al., 1965). Thus one way of estimating ¹⁴C-recycling is by simultaneously injecting [3-³H]glucose and [U-¹⁴C]glucose as in the present experiments. The increased recycling of glucose-C during lactation advances in both 50% HF and 87.5% HF animals suggests that a constant level of tricarbon units originally derived from glucose is again reincorporated into glucose. However, these values are slightly higher in both groups of 50% HF than in 87.5% HF animals feeding on either hay or urea treated rice straw. This indicates a slightly greater dependence on glucose metabolites for glucose resynthesis in 50% HF animals. This phenomenon might be related to the higher level of the plasma glucose concentration in 50% HF compared to 87.5% HF animals which has been previously reported (Chaiyabutr et al. 1998). It has been postulated that both types of crossbred HF animals in the present study may metabolize total body glucose to other metabolites in the same manner and return all metabolites for glucose resynthesis (Ballard et al., 1969). Gluconeogenesis in ruminants has been known to be the main source of glucose production (Lindsay, 1970). In the present studies, animals were maintained on a similar concentrate intake. Relatively constant plasma glucose concentrations in each group of crossbred HF animals indicate that steady state conditions between the rate of irreversible loss of glucose and the rate of gluconeogenesis existed in the body pool of glucose.

The synthesis of lactose involves a combination of glucose and UDP-galactose. The UDP-galactose originates from glucose 6-phosphate (Ebner and Schanbacher, 1974). The results in the present study on mammary function indicate that the calculated percentage of metabolism of glucose 6-phosphate to the galactose moiety of lactose in both groups of 87.5% HF was higher than in 50% HF animals in early lactation and declined during lactation advance. In contrast to 50% HF animals, persistency of the percentage of glucose 6-phosphate to the galactose moiety of lactose seemed to be apparent throughout periods of lactation during feeding on either hay or urea treated rice straw. The availability of cytosolic glucose 6-phosphate in the cells of 50% HF animals was found to be sufficient to account for the cytosolic lactose synthesis. The decrease in the metabolism of glucose 6-phosphate to the galactose moiety of lactose as lactation advances in both groups of 87.5% HF animals would affect the lactose synthesis and milk production. In 87.5% HF cattle, a low enzymatic activity for lactose synthesis might be expected to appear in late lactation. However, lactose synthesis

is a complex process (Kuhn et al., 1980). There is still a need for more information to elucidate the changes in enzymatic activity in this particular system between 50% HF and 87.5% HF animals.

The quantitative utilization of the glucose taken up by the mammary gland is used directly in the synthesis of lactose, and in other portions is metabolized via the pentose phosphate pathway, Embden-Meyerhof pathway and the tricarboxylic acid cycle. Glucose carbon was used by the mammary cells to produce lactose, citrate and triacylglycerol for milk secretion. The data obtained for the utilization of glucose carbon for the synthesis of lactose and citrate during early and mid-lactation was lower in both groups of 50% HF in comparison to 87.5% HF animals feeding on either hay or urea treated rice straw. The differences in these results between 50% HF and 87.5% HF without a reduction in feed intake may be explained by the conversion of some glucose into non-essential amino acids which could then be used for milk protein synthesis (Linzell and Mepham, 1968) or lost as venous plasma lactate. An index for this adjustment with a slightly higher level in the milk protein concentration in 50% HF has been noted (Chaiyabutr et al., 1999).

In addition to the use of glucose carbon for milk synthesis, the hydrogen from glucose has been shown to be incorporated into milk fat. Studies *in vitro* have shown that glucose metabolism via the pentose phosphate pathway may not be as important for NADPH production as in the rat. Fatty acid synthesis from acetate can occur in the absence of glucose in sheep mammary-tissue slices (Balmain et al., 1952) and the perfused goat udder (Hardwick et al., 1963). In the present studies, estimates of the contribution of the pentose phosphate pathway in providing NADPH for fatty acid synthesis *in vivo* have been based on the assumption that all the glucose that was oxidized to CO₂ was metabolized via the pentose phosphate pathway. The calculation of the metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway or the pentose phosphate pathway has been estimated in the goat udder *in vivo* (Chaiyabutr et al., 1980). However, few data have been available from the *in vivo* study of crossbred lactating cows. In the present studies glucose 6-phosphate metabolized via the pentose phosphate pathway gave percentage values of 5% to 21% for both types of crossbred HF animals. These estimations are in contrast to experiments in the isolated perfused cow udder by Wood and co-workers (1965), in which about 23% to 30% of the glucose was metabolized via the pentose phosphate pathway. The difference in estimation is probably due to no consideration of the recycling of glucose 6-phosphate which occurs when glucose is metabolized via the pentose cycle in the udder with the consequent loss of

^3H from glucose 6-phosphate (Davis and Bauman, 1974). However, the net proportion of the metabolism of glucose 6-phosphate via the pentose cycle pathway during mid-lactation in 87.5% HF animals was higher than that of 50% HF animals feeding on either hay or urea treated rice straw. Metabolism of glucose via the pentose phosphate pathway yields 2 molecules of NADPH per molecule of glucose, only one of which could be labelled with ^3H in the present experiments. The data presented here provided evidence that 32% to 42% of the NADPH was required during mid-lactation for fatty acid synthesis *de novo* from glucose metabolism in the udder of both groups of 87.5% HF, while 11% to 15% was required in groups of 50% HF animals feeding on either hay or urea treated rice straw, respectively. If there is a common pool of glucose 6-phosphate which is available for both lactose synthesis and pentose phosphate metabolism, then the recycling of glucose 6-phosphate within the udder would result in too low a value for NADPH production from glucose.

The net metabolism of glucose in the pentose phosphate pathway can be calculated from the incorporation of ^3H from [$3\text{-}^3\text{H}$]glucose in fatty acids assuming that the NADPH formed is used exclusively for biosynthesis of fatty acids (Katz et al., 1974). This technique has been used to study the *in vitro* metabolism of rat mammary and adipose tissue (Katz and Wals 1970, 1972; Katz et al., 1966) and was also used for the study of the *in vivo* metabolism of goat mammary tissue (Chaiyabutr et al., 1980). Based on the techniques and calculations of Katz and co-workers (1974) and assuming that cytosolic NADPH is used only for fatty acid synthesis, it has been shown that the glucose phosphorylated by the udder of both groups of 87.5% HF animals was metabolized via the pentose phosphate pathway and was markedly higher than those of 50% HF animals during mid-lactation. In 87.5% HF animals feeding on either hay or urea treated rice straw, a high proportion of the glucose taken up by the udder which was oxidized in the tricarboxylic acid cycle would be apparent in early and mid-lactations. High values of both the proportion and absolute amount of glucose carbon incorporation to milk citrate seen in both groups of 87.5% HF animals is evidence for this. In addition, calculations showed that there was a higher proportion and absolute amount of glucose 6-phosphate metabolized via the Embden-Meyerhof pathway in all periods of lactation in both groups of 50% HF compared to 87.5% HF animals. An increase in the percentage of glucose carbon in milk triacylglycerol of 50% HF animals during early lactation is evidence supporting an increased proportion of glucose 6-phosphate metabolized via the Embden-Meyerhof pathway compared to the pentose phosphate pathway. It has been shown that metabolism of glucose 6-phosphate by the Embden-Meyerhof pathway can result in ^3H

being retained in glycerol if the triose phosphate isomerase reaction is not at equilibrium (Katz and Rognstad, 1976). Metabolism of glucose 6-phosphate by the pentose phosphate pathway usually results in the loss of all ^3H from [$3\text{-}^3\text{H}$]glucose in lactating cows. Therefore, during lactation advance, whether an increased disequilibrium of the triose phosphate isomerase reaction occurs in the udder of 87.5% HF compared to 50% HF animals and causes a higher level of $^3\text{H}/^{14}\text{C}$ ratio in milk triacylglycerol needs to be further investigated. The low metabolism of glucose 6-phosphate seen in early lactation of 50% HF animals feeding on either hay or urea treated rice straw appeared to be due primarily to a low flux through the pentose phosphate pathway and to lactose synthesis, probably reflecting the low milk production in this breed. Tritium and carbon from glucose were also shown to be incorporated into milk citrate. Glucose carbon provided 1.3-2.2% in both groups of 87.5% HF animals and 0.35-0.39% in both groups of 50% HF animals for the carbon skeleton of citrate in the early lactating period. It has been postulated that milk citrate could be synthesized from 2-oxoglutarate via the NADP-dependent isocitrate dehydrogenase reaction (Hardwick, 1965). In addition ^3H is lost to NADPH or water in metabolism via the pentose phosphate pathway or glycolytic pathway, so it is likely that ^3H incorporation into milk citrate was also via NADP ^3H . The ratio of tritium in milk citrate to that of plasma glucose in both types of crossbred HF animals in the present experiments would appear to support this hypothesis. However, the different values of the specific radioactivity of the ^3H in milk citrate were apparent in 50% HF and 87.5% HF animals. It is possible that the incorporation of ^3H into milk citrate may occur in different manners in the exchange reaction of the cytosolic NADP-dependent isocitrate dehydrogenase. Both fatty acid synthesis and the NADP-dependent isocitrate dehydrogenase reaction between 50% HF and 87.5% HF animals may have different mechanisms with a common pool of cytosolic NADPH.

In conclusion, the data presented here represent the estimation *in vivo* of glucose metabolism in the udder and its distribution to lactose synthesis, the pentose phosphate pathway and the Embden-Meyerhof pathway in 50% HF and 87.5% HF animals feeding on either hay or urea treated rice straw. Of the glucose taken up by the udder of both groups of 87.5% HF during mid-lactation, on average 21% and 36% were metabolized completely in the pentose phosphate pathway and contributed to NADPH production, respectively. These rates of metabolism were higher than those in early periods of lactation and these rates were also higher by approximately 4 times than those present in both groups of 50% HF animals. It is probable that during mid-lactation, the metabolism of glucose 6-phosphate increased flux

through the pentose cycle pathway when animals were coming into energy balance. The genetic difference would appear imply that a larger proportion of glucose 6-phosphate is metabolized via the Embden-Mayerhof pathway in 50% HF animals fed either hay or urea treated rice straw. Although we know a great deal of differences that occur between different types of crossbred animals, we do not know the different enzymatic activities including different stages of lactation which affect the rate of metabolic pathways in different breeds. There is still a need for more information, for example, on whether the low enzymatic activity of fructose 1-6 diphosphatase or the higher enzymatic activity of pyruvate dehydrogenase occurs in 50% HF animals throughout the period of lactation or occurs during the transition period to late lactation in 87.5% HF animals which causes an increase in the metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway and tricarboxylic acid cycle.

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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).

Table 2. Glucose turnover rate, related variables and body weight at different stages of lactation of crossbred Holsteins fed with hay or urea treated rice straw.

	Period of	Hay+concentrate		Urea treated rice straw+concentrate	
	lactation	HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Glucose turnover rate					
(3- ³ H) glucose (μmol/min)	Early	5662.6±695.5 ^a	4965.6±564.2 ^a	4713.5±804.5 ^a	5115.1±567.6 ^a
	Mid	4587.5±1198.8 ^b	5514.8±803.6 ^{ab}	5225.0±1081.7 ^{ab}	6481.2±988.6 ^a
	Late	4602.8±900.9 ^{c*}	5766.1±669.0 ^{bc}	6657.5±1313.1 ^{ab**}	7453.9±862.4 ^{a*}
(U- ¹⁴ C)glucose (μmol/min)	Early	4712.3±747.5 ^a	3980.4±399.1 ^a	4471.2±751.9 ^a	3911.8±726.5 ^a
	Mid	3874.3±757.4 ^a	3696.1±270.8 ^a	3755.4±540.9 ^a	4301.4±390.8 ^a
	Late	3793.0±475.6 ^{ab}	3669.8±331.8 ^b	4186.3±691.3 ^{ab}	4451.3±409.6 ^a
Glucose-C recycling (%)	Early	16.8±7.5 ^a	19.7±1.9 ^a	16.1±6.8 ^a	23.8±6.7 ^a
	Mid	15.2±5.5 ^b	32.1±9.8 ^{ab}	25.6±17.8 ^{ab}	33.2±4.4 ^{a*}
	Late	16.6±7.2 ^b	35.9±6.8 ^{a*}	35.8±12.4 ^{a*}	40.1±3.6 ^{a**}
Plasma glucose clearance (ml/min)	Early	1702.1±409.9 ^a	1127.5±197.8 ^b	1333.4±211.9 ^{ab}	1288.6±70.3 ^b
	Mid	1471.2±417.8 ^a	1486.8±358.6 ^a	1534.7±130.5 ^a	1618.3±59.0 ^{a**}
	Late	1296.2±177.1 ^c	1488.7±106.2 ^{bc}	1749.1±262.2 ^{b**}	2122.8±244.1 ^{a**}
Non mammary Glucose utilization (μmol/min)	Early	1784.7±986.0 ^a	2537.2±820.5 ^a	1600.3±957.1 ^a	2669.3±698.2 ^a
	Mid	2106.5±891.9 ^a	3356.7±666.9 ^{ab}	3000.5±1199.9 ^{ab}	4093.7±1127.4 ^b
	Late	2151.7±569.1 ^a	3570.7±354.5 ^b	4499.3±1317.3 ^{bc}	4849.0±938.7 ^c
Non mammary Glucose utilization (%)	Early	31.4±16.6 ^a	50.2±11.6 ^a	33.4±16.6 ^a	51.7±9.6 ^a
	Mid	44.7±9.6 ^a	60.1±5.3 ^b	55.8±10.6 ^{ab}	62.4±9.0 ^b
	Late	46.7±8.1 ^a	62.2±4.5 ^b	66.9±8.2 ^b	64.9±7.2 ^b
Body weight (kg)	Early	360±33 ^a	321±36 ^{ab}	341±21 ^{ab}	309±14 ^b
	Mid	346±47 ^a	342±35 ^a	372±14 ^a	344±18 ^a
	Late	350±38 ^a	371±23 ^a	375±29 ^a	368±28 ^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).

Table 3. Udder blood flow, milk yield and milk components in different stages of lactation of crossbred Holstein cattle fed with hay or urea treated rice straw.

	Period of lactation	Hay+concentrate		Urea treated rice straw+concentrate	
		HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Udder blood flow (ml/min)	Early	7160±1807 ^a	3887±543 ^b	4619±1149 ^b	4314±575 ^b
	Mid	4745±836 ^a	4090±398 ^a	3843±872 ^a	5068±1054 ^a
	Late	5026±724 ^{ab}	3942±500 ^b	3995±883 ^b	5371±932 ^a
Milk yield (kg/d)	Early	19.76±4.47 ^a	10.98±1.17 ^b	16.51±5.92 ^{ab}	12.91±1.58 ^b
	Mid	11.00±1.61 ^{a*}	10.52±1.34 ^a	11.72±0.93 ^a	12.33±2.46 ^a
	Late	10.11±0.69 ^{ab*}	10.47±0.81 ^{ab}	9.18±1.21 ^{b*}	12.26±2.51 ^a
Lactose in milk (mmol/100 ml)	Early	13.42±0.23 ^a	12.79±0.40 ^a	13.48±0.18 ^a	13.49±0.47 ^a
	Mid	13.40±0.21 ^a	13.15±0.25 ^a	13.49±0.44 ^a	13.44±0.29 ^a
	Late	13.13±0.51 ^a	13.15±0.46 ^a	13.31±0.47 ^a	13.00±0.32 ^a
Citrate in milk (mmol/100 ml)	Early	0.841±0.124 ^a	0.618±0.063 ^b	0.811±0.068 ^a	0.590±0.063 ^b
	Mid	0.667±0.112 ^a	0.673±0.092 ^a	0.623±0.159 ^a	0.698±0.074 ^a
	Late	0.580 ±0.039 ^a	0.694±0.054 ^a	0.690±0.254 ^a	0.697±0.119 ^a
Triacylglycerol in milk (mmol/l)	Early	53.71±21.34 ^b	95.34±24.40 ^a	51.74±10.25 ^b	73.97±28.33 ^b
	Mid	55.77±12.22 ^b	83.64±20.89 ^a	61.33±13.77 ^{ab}	79.06±16.72 ^{ab}
	Late	73.37±23.20 ^a	74.46±22.92 ^a	70.50±22.34 ^a	100.43±19.61 ^a

P-values by paired t-test. *P<0.05 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 4. The secretion of milk components at different stages of lactation of crossbred Holstein cattle fed with hay or urea treated rice straw.

	Period of lactation	Hay+concentrate		Urea treated rice straw+concentrate	
		HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Milk lactose secretion ($\mu\text{mol}/\text{min}$)	Early	1845.7 $\pm$ 438.8 ^a	976.3 $\pm$ 122 ^b	1543.8 $\pm$ 544.6 ^{ab}	1210.6 $\pm$ 156.8 ^b
	Mid	1025.7 $\pm$ 165.4 ^{a*}	958.9 $\pm$ 110 ^a	1096.5 $\pm$ 59.5 ^a	1149.4 $\pm$ 225.4 ^a
	Late	921.9 $\pm$ 77.6 ^{ab*}	955.7 $\pm$ 72 ^{ab}	846.9 $\pm$ 101.4 ^{b*}	1104.4 $\pm$ 211.9 ^a
Milk citrate secretion ($\mu\text{mol}/\text{min}$)	Early	113.0 $\pm$ 15.7 ^a	47.2 $\pm$ 7.7 ^b	93.3 $\pm$ 35.7 ^a	52.6 $\pm$ 6.7 ^b
	Mid	51.8 $\pm$ 15.7 ^a	49.2 $\pm$ 10 ^a	50.3 $\pm$ 11.2 ^a	59.5 $\pm$ 12.2 ^a
	Late	40.6 $\pm$ 1.6 ^a	50.5 $\pm$ 5.9 ^a	43.4 $\pm$ 14.6 ^a	60.7 $\pm$ 21.9 ^a
Milk triacylglycerol secretion ($\mu\text{mol}/\text{min}$)	Early	700.1 $\pm$ 148.7 ^a	719.7 $\pm$ 174.1 ^a	584.4 $\pm$ 206.2 ^a	650.9 $\pm$ 191.3 ^a
	Mid	415.9 $\pm$ 37.4 ^c	598.3 $\pm$ 83.8 ^{ab}	495.7 $\pm$ 93.1 ^{bc}	655.8 $\pm$ 34.9 ^a
	Late	507.5 $\pm$ 133.9 ^b	538.8 $\pm$ 149.4 ^b	440.4 $\pm$ 100.4 ^b	831.8 $\pm$ 67.0 ^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).

Table 5. Utilization of glucose carbon in the udder at different stages of lactation of crossbred Holstein cattle fed with hay or urea treated rice straw.

	Period of	Hay+concentrate		Urea treated rice straw+concentrate	
	lactation	HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
[14C]Glucose incorporation (μmol/min) into:					
milk lactose	Early	2189.3±530.2 ^a	1339.4±217 ^b	2000.5±743.8 ^{ab}	1334.0±300.8 ^b
	Mid	1594.5±279.4 ^{a*}	1219.4±356 ^a	1227.3±161.2 ^a	1291.0±354.5 ^a
	Late	1158.8±347.8 ^{a*}	1228.2±358 ^a	873.1±119.8 ^{a*}	1084.0±188.4 ^a
milk citrate	Early	47.80±22.61 ^a	7.69±2.78 ^b	68.64±37.80 ^a	9.21±4.10 ^b
	Mid	35.77±26.85 ^a	5.88±2.71 ^b	12.30±2.18 ^{b*}	7.65±3.73 ^b
	Late	7.60±6.60 ^{a*}	6.50±1.55 ^a	7.14±2.66 ^{a*}	6.06±3.27 ^a
milk triacylglycerol	Early	21.63±8.24 ^a	19.12±6.51 ^a	14.66±9.07 ^a	22.96±9.02 ^a
	Mid	34.13±26.03 ^a	18.64±6.76 ^a	22.22±13.74 ^a	27.75±16.88 ^a
	Late	23.67±11.47 ^a	18.19±6.88 ^a	16.98±10.22 ^a	21.66±12.96 ^a
venous blood CO2	Early	153.94±41.07 ^a	37.88±22.56 ^c	66.06±28.63 ^{bc}	87.60±24.42 ^b
	Mid	98.29±36.99 ^a	69.95±57.41 ^a	49.02±43.08 ^a	61.68±38.97 ^a
	Late	100.46±35.46 ^a	41.26±32.17 ^b	81.05±34.89 ^{ab}	109.52±42.47 ^a
Percentage of glucose carbon appearing as:					
milk lactose	Early	60.3±10.7 ^a	58.5±10.9 ^a	62.2±9.1 ^a	53.2±7.1 ^a
	Mid	63.9±9.5 ^a	53.9±10.0 ^a	55.0±4.3 ^a	53.4±7.3 ^a
	Late	46.8±3.4 ^{ab*}	54.2±9.0 ^a	41.5±5.6 ^{b*}	41.9±5.0 ^{b*}
milk citrate	Early	1.29±0.76 ^{ab}	0.39±0.12 ^b	2.17±1.21 ^a	0.35±0.15 ^b
	Mid	1.34±0.75 ^a	0.27±0.11 ^b	0.54±0.09 ^{b*}	0.30±0.10 ^b
	Late	0.29±0.19 ^{a*}	0.30±0.05 ^a	0.33±0.06 ^{a*}	0.23±0.08 ^a
milk triacylglycerol	Early	0.57±0.21 ^{ab}	0.89±0.39 ^{ab}	0.44±0.22 ^b	0.91±0.28 ^a
	Mid	1.31±0.75 ^a	0.85±0.28 ^a	1.06±0.72 ^a	1.13±0.50 ^a
	Late	1.16±0.68 ^a	0.83±0.26 ^a	0.75±0.34 ^a	0.81±0.43 ^a
venous blood CO2	Early	4.20±1.45 ^a	1.37±1.28 ^b	2.23±1.41 ^{ab}	3.41±1.07 ^{ab}
	Mid	3.93±1.18 ^a	3.16±2.27 ^a	2.21±2.01 ^a	2.67±1.90 ^a
	Late	4.33±2.04 ^a	1.78±1.11 ^b	3.92±2.06 ^{ab}	4.17±1.42 ^{ac}

P-values by paired t-test. *P<0.05 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 ($P < 0.05$).

Table 6. Rates of pathways of glucose metabolism in the udder at different stages of lactation of crossbred Holsteins fed with hay or urea treated rice straw.

	Period of	Hay+concentrate		Urea treated rice straw+concentrate	
	lactation	HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Flux through the pentose phosphate pathway calculated as ³ H incorporation into milk fatty acid (equivalent μmol of glucose/min)					
	Early	236.3±163.6 ^a	108.3±58.9 ^a	106.7±28.5 ^a	180.2±143.5 ^a
	Mid	261.0±157.7 ^a	83.2±45.6 ^a	252.8±140.5 ^a	149.4±94.5 ^a
	Late	206.2±153.6 ^a	120.7±89.6 ^a	82.1±39.8 ^a	112.6±68.6 ^a
Corrected ³ H incorporation into milk fatty acid (equivalent μmol of glucose/min)					
	Early	295.6±225.7 ^a	135.9±75.5 ^a	125.7±26.9 ^a	238.2±191.9 ^a
	Mid	298.9±172.8 ^a	117.6±51.2 ^a	313.6±147.4 ^a	219.8±129.2 ^a
	Late	254.2±193.7 ^a	184.2±137.5 ^a	134.4±87.3 ^a	181.8±109.1 ^a
Net metabolism of glucose 6-phosphate via the pentose phosphate pathway (μmol/min)					
	Early	200.5±168.2 ^a	85.9±52.9 ^a	78.8±24.5 ^a	98.6±59.9 ^a
	Mid	254.4±164.2 ^a	62.9±39.4 ^a	255.5±157.5 ^a	89.7±49.8 ^a
	Late	170.9±138.4 ^a	97.7±79.3 ^a	60.3±29.7 ^a	85.1±55.2 ^a
Net metabolism of glucose 6-phosphate via the pentose phosphate pathway (%)					
	Early	9.8±7.4 ^a	7.5±5.1 ^a	5.3±2.6 ^a	6.6±3.1 ^a
	Mid	20.6±12.4 ^{ab}	5.3±3.5 ^b	21.1±12.5 ^a	6.5±2.9 ^{ab}
	Late	9.9±6.2 ^a	7.8±5.3 ^a	4.6±1.9 ^a	5.4±3.1 ^a
Metabolism of glucose 6-phosphate via the galactose moiety of lactose (%)					
	Early	92.2±13.8 ^a	79.5±16.8 ^a	97.5±20.8 ^a	86.3±5.9 ^a
	Mid	73.0±14.8 ^b	80.0±4.4 ^{ab}	91.0±10.5 ^a	89.0±8.7 ^{ab}
	Late	66.7±23.4 ^{a*}	81.3±20.1 ^a	69.0±17.6 ^{a*}	72.0±12.2 ^a
Metabolism of glucose 6-phosphate via Embden-Meyerhof pathway (μmol/min)					
	Early	-14.4±407.3 ^a	260.9±243.8 ^a	-52.5±385.1 ^a	98.6±92.4 ^a
	Mid	118.8±245.3 ^a	177.1±108.2 ^a	-143.4±96.2 ^b	52.7±106.0 ^{ab}
	Late	430.6±410.2 ^a	180.0±309.4 ^a	404.4±397.9 ^a	346.8±285.9 ^a
Metabolism of glucose 6-phosphate via Embden-Meyerhof pathway (%)					
	Early	-2.3±18.2 ^a	18.9±14.3 ^a	-3.2±22.0 ^a	6.8±5.7 ^a
	Mid	5.9±17.7 ^{ab}	14.4±7.7 ^a	-12.5±8.5 ^b	4.1±8.6 ^{ab}
	Late	23.4±20.6 ^a	9.6±22.5 ^a	26.2±17.5 ^a	21.3±15.5 ^a

P-values by paired t-test. *P<0.05 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 7. NADPH production from glucose in the udder at different stages of lactation of crossbred Holsteins fed with hay or urea treated rice straw.

Period of lactation	Hay+concentrate		Urea treated rice straw+concentrate	
	HF:RS	HF:RS	HF:RS	HF:RS
	(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Requirement of all NADPH for fatty acid synthesis ($\mu\text{mol/min}$)				
Early	1773.06 $\pm$ 296.44 ^a	2053.24 $\pm$ 652.89 ^a	1452.99 $\pm$ 742.15 ^a	2063.16 $\pm$ 808.55 ^a
Mid	1181.16 $\pm$ 124.74 ^b	1565.51 $\pm$ 219.09 ^{ab}	1578.33 $\pm$ 516.27 ^{ab}	1996.28 $\pm$ 177.20 ^a
Late	1425.87 $\pm$ 253.95 ^b	1474.31 $\pm$ 364.64 ^a	1147.57 $\pm$ 404.55 ^b	2474.08 $\pm$ 317.72 ^a
Requirement of all NADPH formation from glucose via the pentose phosphate pathway (%)				
Early	25.8 $\pm$ 15.2 ^a	10.0 $\pm$ 3.3 ^a	16.1 $\pm$ 3.9 ^a	19.5 $\pm$ 16.8 ^a
Mid	42.5 $\pm$ 21.8 ^a	11.1 $\pm$ 6.8 ^b	31.9 $\pm$ 20.5 ^{ab}	14.8 $\pm$ 9.6 ^b
Late	32.1 $\pm$ 26.6 ^a	17.8 $\pm$ 13.4 ^a	14.5 $\pm$ 5.8 ^a	8.9 $\pm$ 5.1 ^a

^{a,b} Mean values within a row indicated with different superscripts are significantly different ($P < 0.05$).

Table 8. Fatty acid composition of milk fat in the udder at different stages of lactation of crossbred Holsteins fed with hay or urea treated rice straw.

Period of lactation	Fatty acid C-chain length ($\mu\text{mol/ml}$ milk)	Hay+concentrate		Urea treated rice straw+concentrate	
		HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5) ^a	(50:50) ^b	(87.5:12.5) ^a	(50:50) ^b
Early lactation	C6	1.25 $\pm$ 0.52 ^a	2.78 $\pm$ 1.01 ^b	1.03 $\pm$ 0.57 ^a	2.52 $\pm$ 1.70 ^b
	C8	0.57 $\pm$ 0.14 ^a	1.25 $\pm$ 0.49 ^b	0.44 $\pm$ 0.28 ^{ac}	1.10 $\pm$ 0.86 ^{ab}
	C10	0.95 $\pm$ 0.16 ^a	2.23 $\pm$ 0.91 ^b	0.72 $\pm$ 0.47 ^{ac}	1.93 $\pm$ 1.58 ^{ab}
	C12	0.98 $\pm$ 0.17 ^a	2.32 $\pm$ 0.79 ^b	0.80 $\pm$ 0.51 ^{ac}	1.80 $\pm$ 1.39 ^{ab}
	C14	3.59 $\pm$ 0.83 ^a	8.08 $\pm$ 2.80 ^b	3.20 $\pm$ 1.73 ^{ac}	6.61 $\pm$ 3.56 ^{ab}
	C16:0	16.40 $\pm$ 5.82 ^a	29.70 $\pm$ 8.56 ^b	16.72 $\pm$ 3.59 ^{ac}	24.69 $\pm$ 8.00 ^{ab}
	C16:1	1.06 $\pm$ 0.87 ^a	1.69 $\pm$ 0.53 ^a	1.02 $\pm$ 0.23 ^a	1.71 $\pm$ 0.36 ^a
	C18:0	13.20 $\pm$ 5.34 ^a	23.05 $\pm$ 5.54 ^b	12.35 $\pm$ 3.55 ^a	14.45 $\pm$ 4.46 ^a
	C18:1	14.88 $\pm$ 8.97 ^{ac}	23.05 $\pm$ 6.16 ^a	14.50 $\pm$ 2.16 ^c	17.53 $\pm$ 5.01 ^{ac}
	C18:2	0.82 $\pm$ 0.38 ^a	1.20 $\pm$ 0.27 ^a	0.96 $\pm$ 0.42 ^a	1.64 $\pm$ 0.47 ^a
	Total	53.71 $\pm$ 21.34 ^a	95.34 $\pm$ 24.40 ^b	51.74 $\pm$ 10.25 ^{ac}	73.97 $\pm$ 26.33 ^{ab}
Mid lactation	C6	1.38 $\pm$ 0.54 ^a	1.87 $\pm$ 0.53 ^a	1.64 $\pm$ 0.65 ^a	2.57 $\pm$ 0.52 ^a
	C8	0.65 $\pm$ 0.21 ^a	0.80 $\pm$ 0.25 ^a	0.77 $\pm$ 0.33 ^a	1.21 $\pm$ 0.26 ^a
	C10	1.10 $\pm$ 0.38 ^a	1.43 $\pm$ 0.49 ^a	1.43 $\pm$ 0.60 ^a	2.23 $\pm$ 0.50 ^a
	C12	1.16 $\pm$ 0.34 ^a	1.51 $\pm$ 0.58 ^a	1.59 $\pm$ 0.63 ^a	2.16 $\pm$ 0.53 ^a
	C14	4.42 $\pm$ 1.33 ^a	6.40 $\pm$ 1.80 ^{ab}	5.85 $\pm$ 2.12 ^{ab}	7.25 $\pm$ 1.37 ^b
	C16:0	19.21 $\pm$ 4.04 ^a	26.28 $\pm$ 6.63 ^a	22.08 $\pm$ 6.04 ^a	25.26 $\pm$ 5.98 ^a
	C16:1	1.00 $\pm$ 0.49 ^a	1.69 $\pm$ 0.41 ^a	1.02 $\pm$ 0.34 ^a	1.15 $\pm$ 0.52 ^a

	C18:0	11.86±2.42 ^a	20.45±5.34 ^b	10.78±3.39 ^a	16.97±6.61 ^{ab}
	C18:1	14.39±3.84 ^a	21.76±5.27 ^a	14.91±4.06 ^a	18.43±3.26 ^a
	C18:2	0.60±0.23 ^a	1.44±0.48 ^b	1.25±0.27 ^b	1.81±0.71 ^b
	Total	55.77±12.22 ^a	83.64±20.89 ^a	61.33±13.77 ^a	79.06±16.72 ^a
Late lactation	C6	1.90±0.62 ^{ab}	3.02±2.91 ^{ab}	1.54±0.81 ^a	3.01±0.54 ^b
	C8	0.82±0.21 ^a	0.92±0.40 ^a	0.67±0.40 ^a	1.38±0.28 ^a
	C10	1.39±0.26 ^a	1.48±0.43 ^a	1.18±0.72 ^a	2.51±0.55 ^a
	C12	1.46±0.26 ^a	1.50±0.50 ^a	1.33±0.77 ^a	2.52±0.54 ^a
	C14	5.83±1.51 ^a	5.74±1.28 ^a	5.01±2.32 ^a	9.02±1.30 ^b
	C16:0	25.33±6.90 ^a	23.21±5.79 ^a	22.81±7.68 ^a	31.96±6.90 ^a
	C16:1	1.54±0.58 ^a	1.46±0.52 ^a	1.20±0.77 ^a	1.78±0.59 ^a
	C18:0	14.87±7.57 ^a	17.43±5.29 ^a	15.81±4.71 ^a	22.00±5.11 ^a
	C18:1	19.33±6.76 ^a	18.53±5.88 ^a	19.62±6.32 ^a	23.88±5.40 ^a
	C18:2	0.89±0.20 ^a	1.17±0.65 ^a	1.41±0.15 ^a	2.37±0.69 ^a
	Total	73.37±23.20 ^a	74.46±22.92 ^a	70.59±22.34 ^a	100.43±19.61 ^a

^{a,b,c} Mean values within a row indicated with different superscripts are significantly different (P<0.05).

Table 9. ³H/¹⁴C ratios in plasma glucose and related products at different stages of lactation of crossbred Holsteins fed with hay or urea treated rice straw.

	Period of lactation	Hay+concentrate		Urea treated rice straw+concentrate	
		HF:RS	HF:RS	HF:RS	HF:RS
		(87.5:12.5)	(50:50)	(87.5:12.5)	(50:50)
Plasma glucose	Early	0.83±0.08 ^a	0.80±0.02 ^a	0.84±0.07 ^a	0.76±0.07 ^a

	Mid	0.86±0.06 ^a	0.69±0.09 ^a	0.74±0.18 ^a	0.67±0.04 ^a
	Late	0.83±0.07 ^a	0.64±0.07 ^a	0.65±0.12 ^a	0.60±0.04 ^a
Milk lactose	Early	0.87±0.06 ^a	0.82±0.02 ^a	0.81±0.08 ^a	0.63±0.10 ^b
	Mid	0.84±0.02 ^a	0.51±0.13 ^b	0.69±0.22 ^a	0.40±0.08 ^{bc}
	Late	0.85±0.08 ^a	0.44±0.02 ^b	0.43±0.12 ^b	0.32±0.09 ^b
Milk triacylglycerol	Early	2.93±0.86 ^a	1.52±0.86 ^a	2.97±2.12 ^a	1.80±0.90 ^a
	Mid	3.45±2.86 ^a	1.04±0.38 ^a	3.17±0.42 ^a	1.32±0.70 ^a
	Late	2.16±0.93 ^a	1.31±0.55 ^a	0.83±0.51 ^a	1.02±0.20 ^a
Milk citrate	Early	3.55±1.03 ^a	0.62±0.25 ^b	1.15±0.32 ^a	0.91±0.41 ^{ab}
	Mid	1.45±0.89 ^a	0.84±0.19 ^a	0.77±0.13 ^a	0.78±0.37 ^a
	Late	1.46±0.12 ^a	0.83±0.32 ^a	0.95±0.24 ^a	0.96±0.62 ^a

^{a,b,c} Mean values within a row indicated with different superscripts are significantly different (P < 0.05).

Figure 1 The metabolic pathway involved in the metabolism of the precursor of milk in different state of lactation of 87.5% HF feeding on hay (The value shown are in micromole/min.)

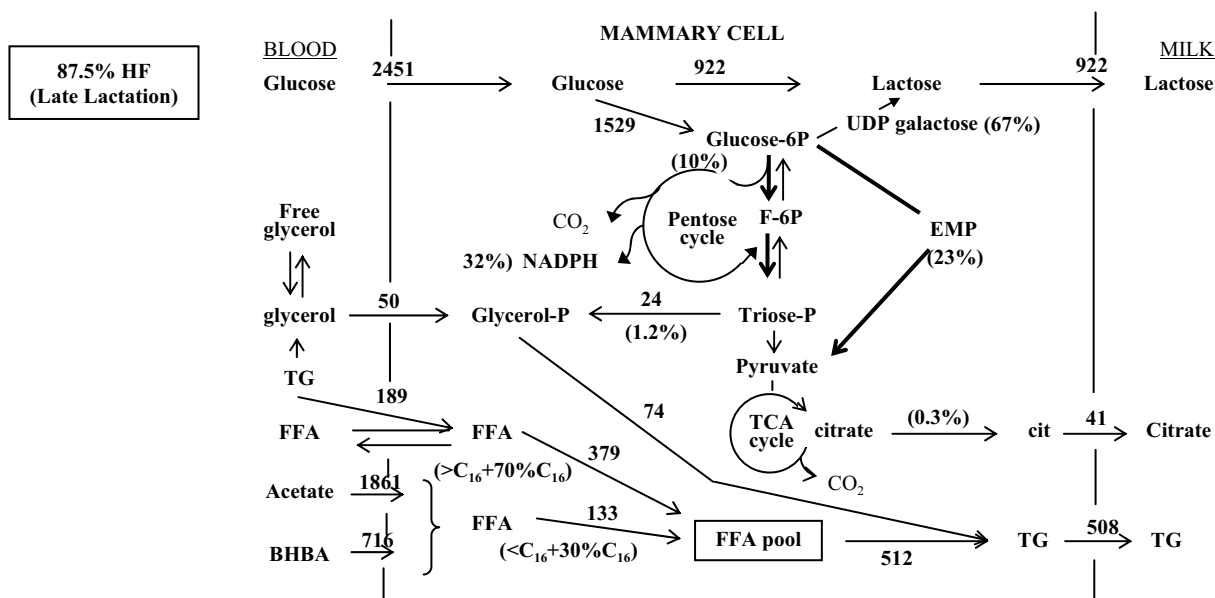
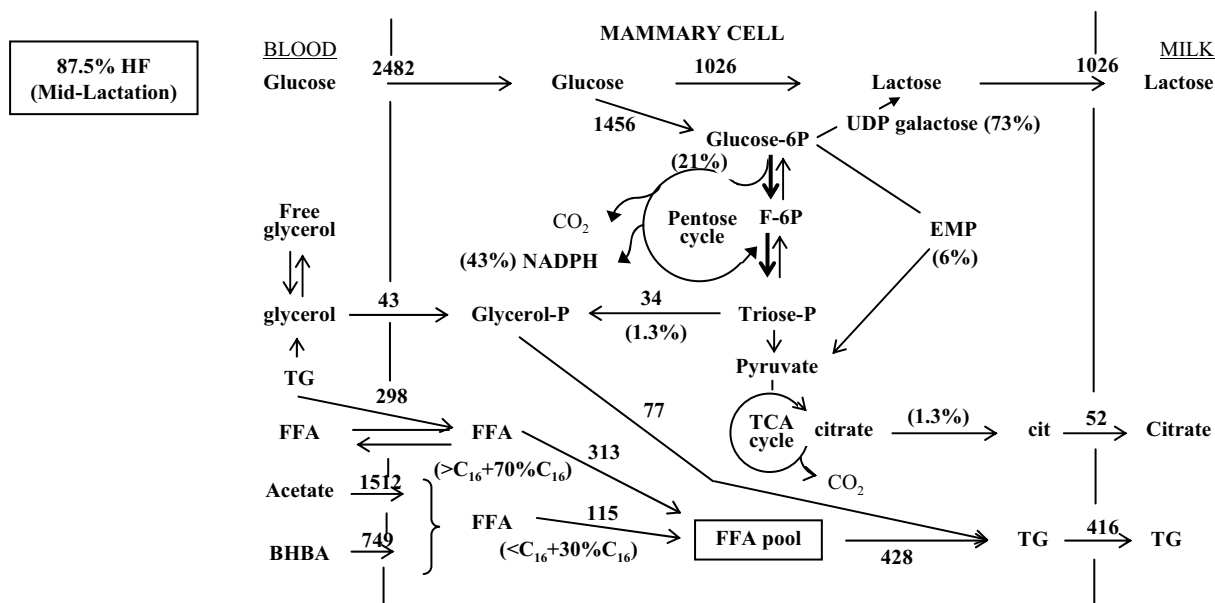
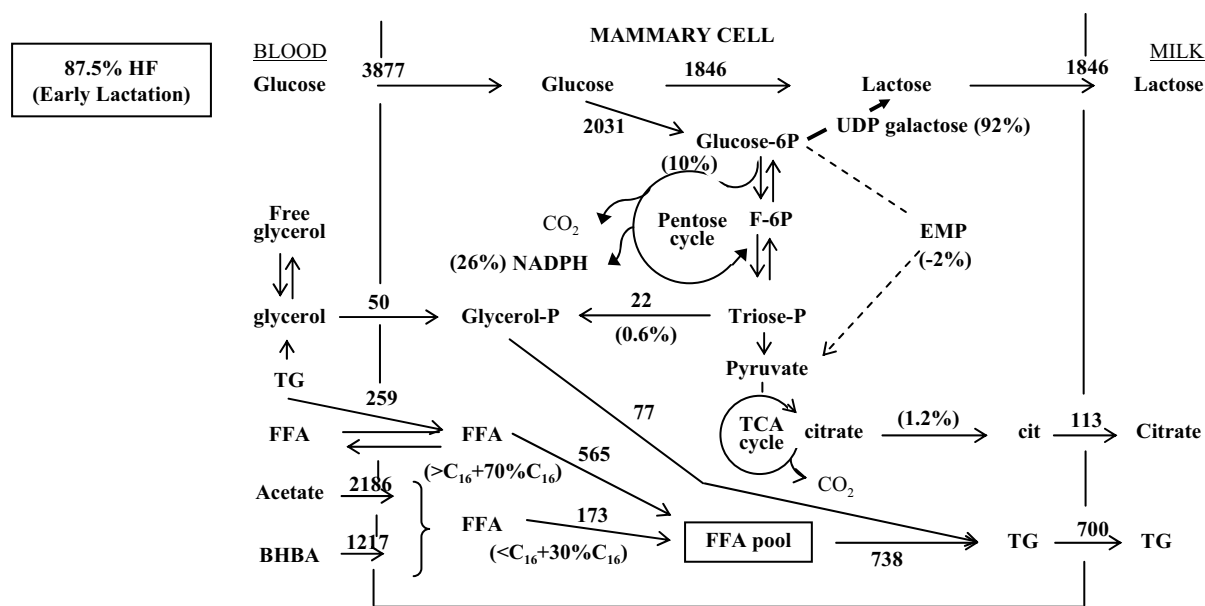


Figure 2 The metabolic pathway involved in the metabolism of the precursor of milk in different state of lactation of 87.5% HF feeding on urea treated rice straw (The value shown are in micromole/min.)

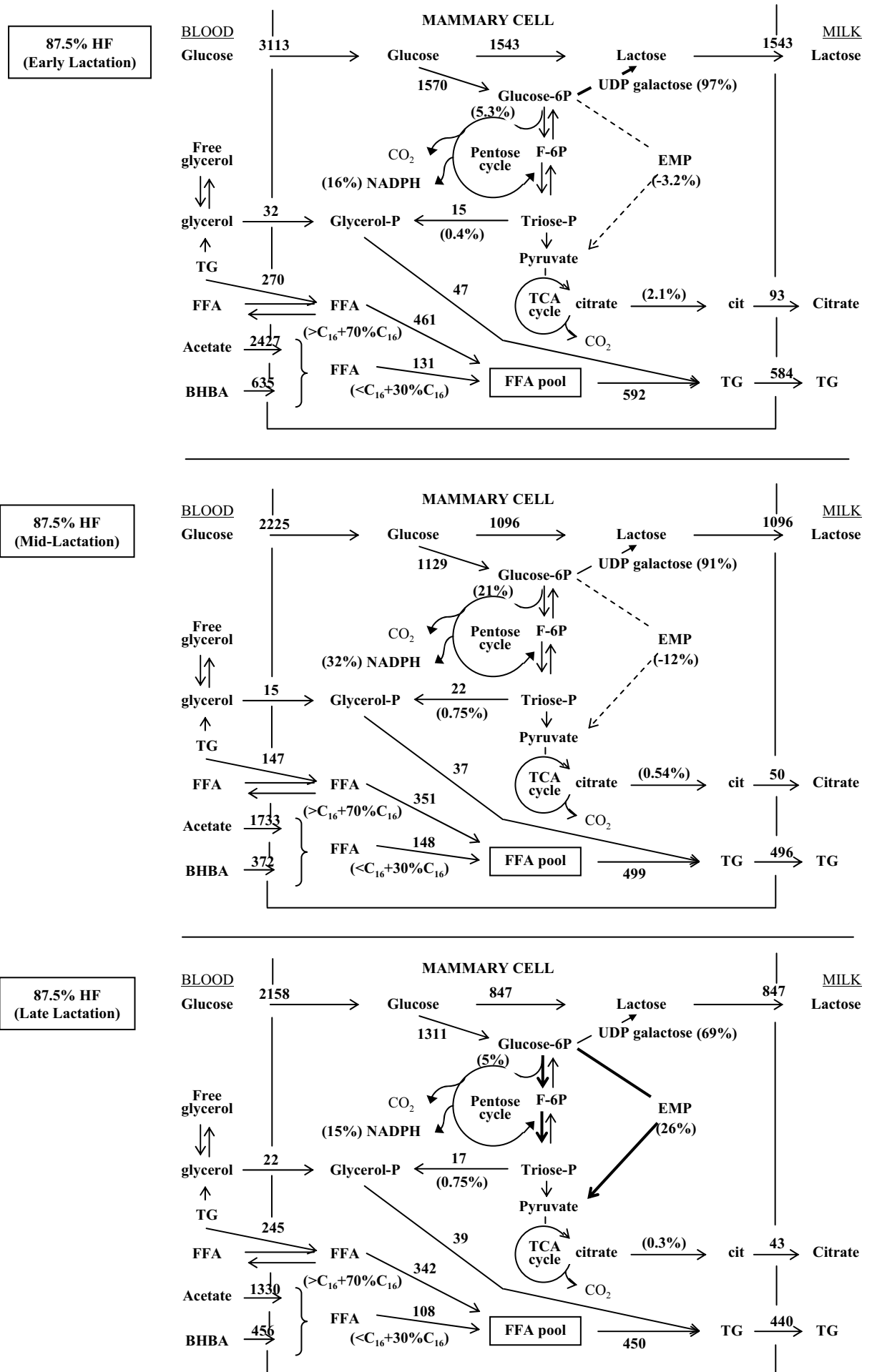


Figure 3 The metabolic pathway involved in the metabolism of the precursor of milk in different state of lactation of 50%HF feeding on hay (The value shown are in micromole/min.)

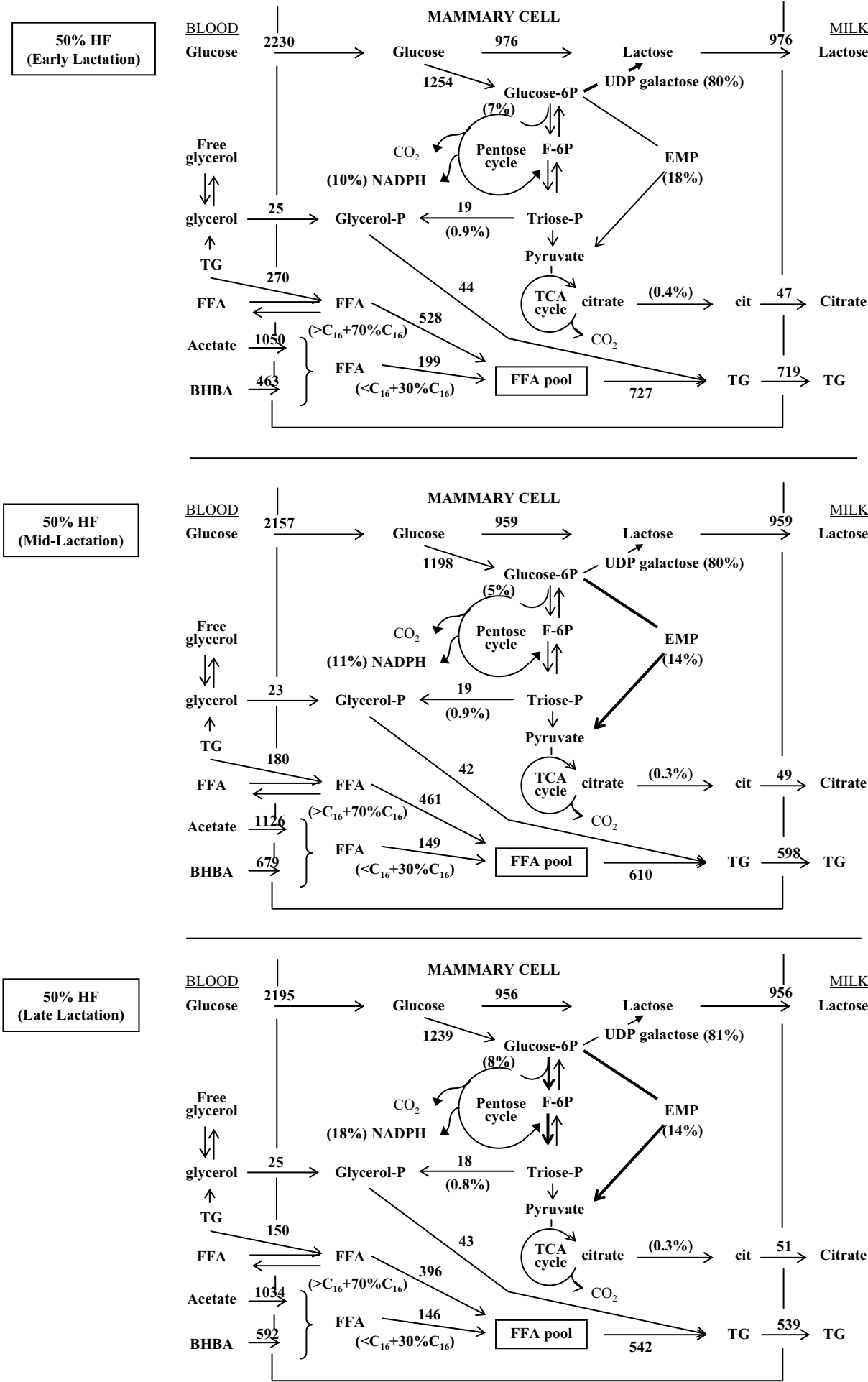
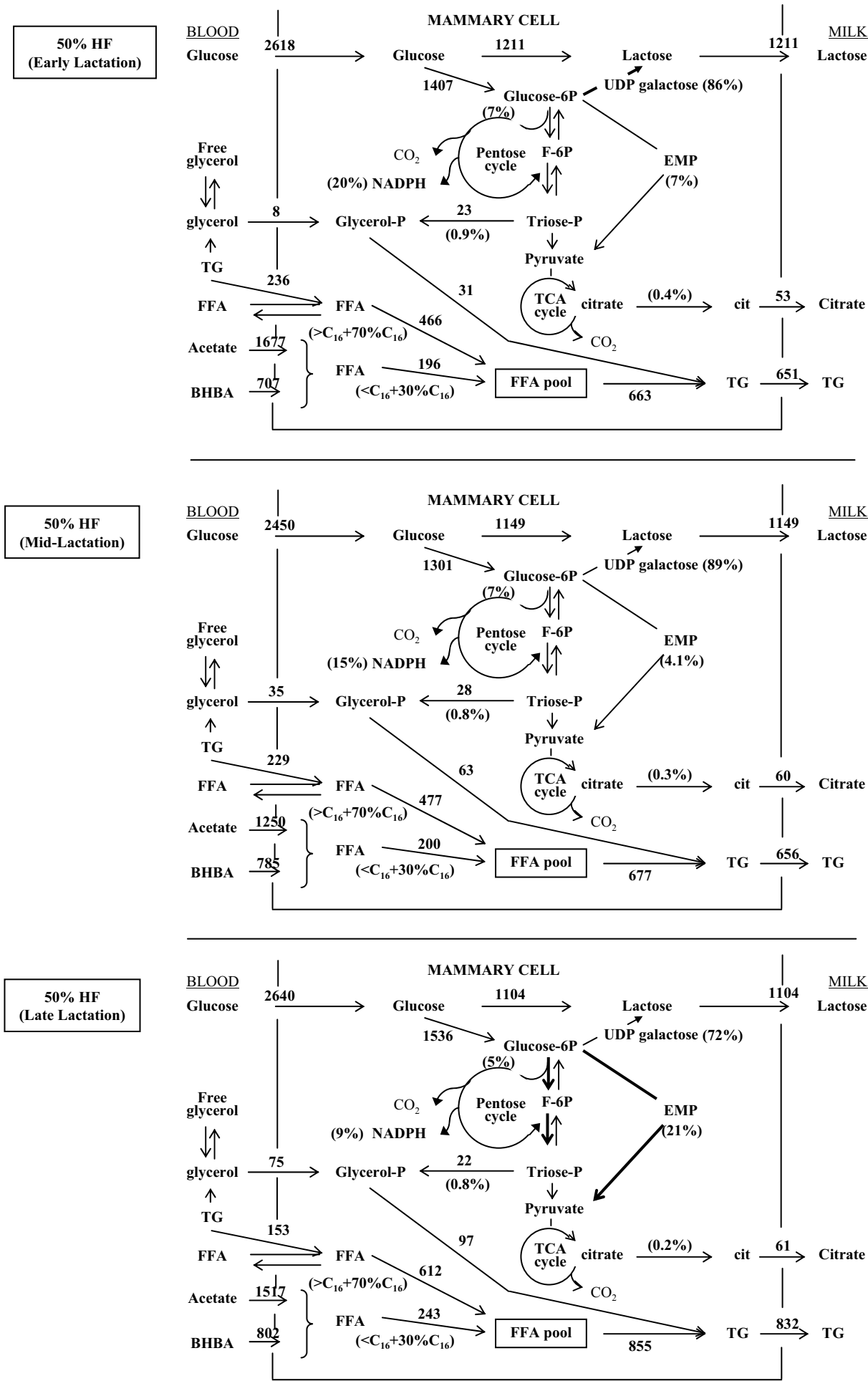


Figure 4 The metabolic pathway involved in the metabolism of the precursor of milk in different state of lactation of 50% HF feeding on ureatreated rice straw (The value shown are in micromole/min.).



Chapter XI

General Discussion

The results presented in this report indicate that bodily functions vary during pregnancy and lactation in both 50%HF and 87.5%HF animals feeding on either hay or urea treated rice straw. Changes in water balance during late pregnancy and lactation have been observed in both types of crossbred animals. It is well known that during lactation blood volume, plasma volume and water turnover are increased (Hanwell and Peaker, 1977; MacFarlane and Howard, 1970). In agreement with the present results, plasma volume, blood volume and water turnover rates were significantly higher in lactating animals than in pregnant animals in all groups (Chapter III).

The packed cell volume significantly decreased after parturition in both groups of 87.5%HF animals while it did not significantly change in 50%HF animals. The packed cell volume of 50%HF animals was significantly higher than that of 87.5%HF animals fed either hay or treated rice straw. In the early lactating period the significant reductions of total body water were noted in both groups of 87.5%HF fed either hay or treated rice straw, while this was not apparent for 50%HF animals. Plasma osmolality remained unchanged between late pregnancy and lactating period, indicating that osmotic homeostasis was being maintained throughout the experiment. During lactation udder blood flow was nearly three times higher than in late pregnant animals in all groups. The udder blood flow of 87.5%HF was significantly higher while mammary resistance was significantly lower than in 50%HF animals. Milk secretion in early lactation of 87.5%HF animals fed either hay or urea treated rice straw was significantly higher than that of 50%HF animals in early lactation. The ratio of udder blood flow to milk secretion ratio was in the similar range for all groups of experiment animals. The ratio of DM intake to milk production for 87.5%HF animals fed either hay or urea treated rice straw was significantly lower than that of 50%HF animals. The difference between breeds indicates that 87.5%HF animals which has a higher milk yield but a lower adjustment for the regulation of body fluids during pregnancy and lactation

in comparison to 50%HF animals. Mammary growth during pregnancy has been known to be a prerequisite for satisfactory lactation. In the present study, the pattern of mammary growth varies during pregnancy and early lactation for both 87.5%HF and 50%HF animals. There were no significant differences in heart rate and arterial blood pressure between the periods of late pregnancy and early stage of lactation in all groups of animals.

It is clear that the degree of local vasoconstriction was greater during pregnancy and less in lactation for both types of crossbred cattle. In late pregnancy, the mammary circulation of 50%HF appeared to be less than that of 87.5%HF animals which could be due to variations in the developments of mammary blood vessels and mammary cells. The high genetic blood level closing to the exotic *bos taurus* breed of 87.5%HF animals may cause a rapid rate in the development of the secretory activity of the mammary cell in the late pregnancy. Local vasodilations produced by the active cells (Hanwell & Peaker, 1977; Lacasse et al., 1996) would decrease in the resistance of the vascular bed and a higher mammary blood flow of 87.5%HF in comparison with 50%HF animals (Chapter III).

During lactation advance (Chapter V), there were no significant differences in body weight, heart rate, mean arterial blood pressure, plasma osmolality, plasma volume and blood volume among groups of 87.5%HF animals and 50%HF animals fed either hay or urea treated rice straw. Water turnover rate, total body water space and total body water as a percentage of body weight of 50%HF animals were significantly higher than those of 87.5%HF animals fed either hay or urea treated rice straw. The udder blood flow and milk secretion of 87.5%HF were significantly higher in early lactation and markedly declined when lactation advanced in comparison with those of 50%HF animals fed either hay or urea treated rice straw. The ratio of mammary blood flow to milk yield for all groups was in a similar range during early lactation while it significantly increased in mid- and late lactation for both groups of 87.5%HF animals. In 50%HF animals feeding on either hay or urea treated rice straw, mammary blood flow, mammary resistance and milk secretion continued in an unchanged manner during lactation advance. During the course of lactation, a decline of 10% per month of previous milk yield has been observed during mid-lactation in both

groups of 87.5%HF while declining of 2% per month was apparent in 50%HF animals. Dietary inadequacies would not suspect to occur in the present study. Since, the pattern of altering lactation persistency in 87.5%HF animals was apparent in both groups of 87.5%HF animals feeding on either hay or urea treated rice straw. These results indicate that genetic makeup closer to the exotic *bos taurus* breed and a high milk yield of 87.5%HF animals, show a poor adjustment to the tropical environment and poorer lactation persistency in comparison with 50%HF animals.

The remainder of the discursion is concerned with metabolic fate of nutrient particularly glucose metabolism, the utilization in the whole body is related to the utilization in the mammary gland in either pregnancy or lactation. The effect of hormonal control that determines the fate of nutrients should be mentioned. The results in Chapter IV show that total glucose entry and utilization rates increased significantly during early lactation in comparison to late pregnancy for all groups. Recycling of glucose-C was approximately 20% in both crossbred cattle fed either hay or urea treated rice straw and was unaffected by the stage of late pregnancy or early lactation. Comparing 50%HF and 87.5%HF animals, arterial plasma glucose concentrations were slightly higher during pregnant period but significantly higher in lactating periods in 50%HF animals. The ratio of specific radioactivity of arterial blood bicarbonate relative to that of arterial blood glucose-¹⁴C in the lactating period significantly decreased in 50%HF animals fed either urea treated rice straw or hay. An increase in udder blood flow during early lactation was significantly higher in 87.5%HF animals than in 50%HF animals. The uptake, arteriovenous differences and extraction ratio for glucose across the udder significantly increased in the lactating period for all crossbred animals. Glucose uptake by the udder of 87.5%HF animals accounted for 65% of the total glucose turnover rate compared to a value of 46% in the lactating 50%HF animals. These results indicate that both crossbred cattle fed either urea treated rice straw or hay exhibit the same body glucose turnover rate. The 87.5%HF animal has a high milk yield and has high body and udder glucose metabolisms compared with 50%HF animals in early lactation.

In early lactating 87.5%HF animals feeding on either hay or urea treated rice straw, the high milk yields and lactose secretion were related to glucose uptake by the udder and udder blood flow as compared with those of 50%HF animals. The marked decreases in udder blood flow, glucose uptake, lactose secretion and milk yield were apparent in mid- and late lactation of both groups of 87.5%HF animals. In contrast to both groups of 50%HF animals, no significant changes in udder blood flow, udder glucose uptake, lactose secretion and milk yields were apparent throughout the course of lactation. Total glucose entry rate using 3- ^3H glucose infusion, recycling of carbon glucose and plasma glucose clearance significantly increased during late lactation for 50%HF and 87.5%HF animals feeding on urea treated rice straw. The utilization rates of glucose using $[\text{U}-^{14}\text{C}]$ glucose infusion were not significantly different for different groups of animals and periods of lactation. The present results indicate that 87.5%HF animals have a higher milk yield but a shorter peak yield and poorer persistency in comparison with 50%HF animals (Chapter VI). Changes in the utilization of glucose by the mammary gland for milk production in both groups of crossbred animals during feeding on either hay or urea treated rice straw would be dependent on changes in intramammary factors.

There are many factors have been reported to capable of influencing lactation persistency in ruminant. The hormonal control of substrates uptake of the mammary gland and milk yield that might be expected to occur in both groups of crossbred animals. However, the way in which hormones act is complex (Chapter VIII). It is impossible to speculate on how hormones act in intramammary metabolism in the present study. There were substantial increases in the mean levels of plasma total triiodothyronine (T_3), insulin and glucagon at the onset of lactation, and there were maintained in high levels during lactation advance for all groups of experiments. The mean levels of prolactin and thyroxine (T_4) were not significantly different for different 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. These results indicate that thyroid hormones, prolactin and cortisol would not influence on poorer lactation persistency in 87.5%HF animals. The present

results also confirm that the plasma prolactin concentration is less significant in ruminant lactation (Hart, 1973). The major negative influence on declining of milk yield is concurrent pregnancy during lactation (Coulon et al., 1995). However, in the present experiment all lactating animals were not recurrent pregnancy. In the present results, the high levels of both plasma progesterone and estradiol concentration significantly declined after parturition and remained low through lactating period. Thus, a consequence of increasing plasma oestrogens during pregnancy that were directly inhibitory of milk secretion would be omitted. Growth hormone is one of the anterior pituitary hormones shown to play an essential role in the establishment and maintenance of lactation (Tucker, 1974). The mean levels of plasma 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. Altering lactation persistency in 87.5%HF is regulated mainly by growth hormones chronically acting through the period of lactation.

In general the rate of milk secretion is thought to be determined primarily by lactose secretion (Rook & Wheelock, 1967; Linzell & Peaker, 1971a), an increase in milk yield can be attributed to an increase in the rate of lactose synthesis. Glucose is essential for milk secretion (Hardwick, Linzell & Price, 1961) and glucose moiety of lactose arises directly from plasma glucose (Ebner & Schanbacher, 1974). No significant differences of mammary arteriovenous differences within the same group of both 87.5%HF and 50%HF animals at different periods of lactation (Chapter IX) suggest that glucose uptake is determined mainly by mammary blood flow (Linzell, 1973). The differences of plasma glucose concentration between 87.5%HF and 50%HF animals can not be used to interpret conclusively changes in the utilization of glucose by the mammary gland in the different periods of lactation. In Chapter VI, mammary blood flow, milk yield and lactose yield were not affected by an alteration of plasma glucose concentrations in both groups of 87.5%HF and 50%HF animals

feeding on either hay or urea treated rice straw. The present results may indicate that the plasma glucose concentration is not a major factor in determining lactose output. This evidence suggests that the major change occurs in the metabolism of the mammary cell in different periods of lactation. It might be reasonable to believe that the reduction of glucose uptake by the mammary gland during mid- and late lactation of both groups of 87.5%HF animals may not be a result from the decrease in the rate of phosphorylation of glucose by hexokinase. The decrease in mammary glucose uptake has been shown to be insulin-independent. However, whether insulin acts on the phosphorylation step continues to be debatable. Since, the plasma insulin concentrations of both 87.5%HF and 50%HF animals showed similar ranges in different groups of animals and in different periods of lactation (Chapter VIII). The results in Chapters X indicate that the calculated percentage of metabolism of glucose 6-phosphate to galactose moiety of lactose during early lactation was about 95% and 83% in 87.5%HF animals and 50%HF animals respectively (see Fig.1,2,3 & 4) which was consistent with the results reported in normally fed goats and cows (Wood et al., 1965; Linzell, 1968). For both groups of 87.5%HF animals, the percentages of these values decreased during lactation advance. The synthesis of lactose involves combination of glucose and UDP-galactose; the UDP-galactose originates from glucose-6-phosphate (Ebner & Schanbacher, 1974). The present experiment indicates that the availability of cytosolic glucose 6-phosphate in the cell is sufficient to account for the cytosolic lactose synthesis in all periods of lactation. In mid- and late lactation of both groups of 87.5%HF animals a decrease in enzymatic activity for lactose synthetase might be expected to occur, possibly amino acids for the synthesis of α -lactalbumin which regulates lactose synthesis (Linzell & Peaker, 1971a), could be affected by a fall in mammary blood flow. The activity of glucose 6-phosphatase is low or absent in the mammary tissue (Davis & Bauman, 1974), reducing the chance for reversibility of the glucose 6-phosphate to glucose reaction. Thus, it is possible that a decrease in the process of lactose synthesis might occur at the last step catalyzed by lactose synthetase (Jones, 1978), and at the same time there is an increase in cytosolic concentration of glucose 6-phosphate. The other possibility for the reduction of

glucose uptake during lactation advance in 87.5%HF animals may be due to the inhibition of the enzyme hexokinase by high cytosolic concentration of glucose 6-phosphate (Gumaa et al., 1971). However, lactose synthesis is a complex process (Kuhn et al., 1980), there is still a need for more information to elucidate the changes in enzymatic activity in this particular system in different stages of lactation and in different breeds of animals.

The present experiments show that at the same time as the decrease in the rate of lactose synthesis in mid- and late lactation in both groups of 87.5%HF animals, there were no significant changes in the rate of fatty acid synthesis *de novo* in the mammary gland during lactation advance (Chapter X). In contrast to 87.5%HF animals, the present results *in vivo* have shown that there were a low in the flux through the pentose phosphate pathway in 50%HF animals. As shown in Fig 1,2,3 & 4 (summarized of Chapter X), of the glucose taken up by the gland, 21% and 6% were metabolized completely in the pentose phosphate pathway in both groups of 87.5%HF animals and 50%HF animals respectively. The present studies indicate that glucose metabolism accounted for 37% and 13% of the NADPH from pentose phosphate pathway required for fatty acid synthesis *de novo* in the udder in the 87.5%HF and 50%HF animals respectively, although the similarity of values of mammary glucose uptake was observed during mid- lactation. Some of the difference in glucose and lipid metabolism between 87.5%HF and 50%HF animals may be accounted for by the fact of the different breeds of animals with different rates of udder metabolism. The consistency of the data obtained is indicative that the high rate of glucose metabolism accounted for the NADPH production in the pentose phosphate pathway during mid- lactation which was also associated with the high transfer of ^{14}C -glucose to glycerol for their esterification in milk fat. It has been shown in the present study that calculated values for triacylglycerol production, expressed as percentages of glycerol synthesized from U- ^{14}C glucose (Fig.1,2,3 &4), are lower than those reported in studies *in vitro* by Hardwick and co-worker (1963), in which 23% of triacylglycerol glycerol was synthesized from glucose in the perfused goat udder. This difference could be attributed to the fact that the U- ^{14}C glucose taken up by the mammary gland in intact conscious animals can be accounted for as lactose, citrate, CO_2 and

lost as venous-plasma lactate. Some glucose may also be converted into amino acids for milk protein synthesis (Linzell & Mephram, 1968). However, in the 87.5%HF animals the rate of triacylglycerol synthesized from U- ^{14}C glucose was lower in early lactation and conversely, the values expressed as percentages were higher during mid- and late lactation. An increase in the proportion of ^{14}C -glucose converted to triacylglycerol was consistent with the calculated data in mid- and late lactation in 87.5%HF animals in which a large proportion of glucose 6-phosphate was metabolized via the Embden-Meyerhof pathway (Chapter X).

An uptake of milk fat precursors confirmed the theory that acetate, β -hydroxybutyrate, long chain fatty acids of triacylglycerol and free fatty acid fractions of plasma are major precursor of milk fatty acids. In the present study, the balance data for the utilization of both short chain and long chain fatty acid were performed by calculating their likely contribution to milk free fatty acid knowing its composition and subtracting these values from the measured uptake of the substrates (Fig.1,2,3 &4). Acetate and β -hydroxybutyrate were grouped together because it is known that they both contribute to the synthesis of milk fatty acids up to and including C_{16} (Annison et al., 1968; Palmquist et al., 1969). It clearly indicates that in normally fed animals acetate and are the main substrates for milk fat synthesis and β -hydroxybutyrate also make a more significant contribution to oxidative metabolism in the udder. The present studies confirmed the previous reports that there was a negligible oxidation of free fatty acid by the udder in normally fed animals (Bickerstaffe, Annison & Linzell, 1974). The insignificant net uptake of free fatty acids by the mammary gland has been shown to be due to the simultaneous uptake of free fatty acids and release into mammary venous blood of fatty acid derived from plasma triacylglycerol which are hydrolysed during their uptake by the mammary gland (West et al., 1972). During mid- and late lactation for both groups of 87.5%HF animals there were marked decreases in utilization of both acetate and β -hydroxybutyrate which were due to the decrease in their supplies from the blood stream (Chapters IX).

In the present experiment, the high correlation between the milk urea concentration and plasma urea concentration was apparent in all periods of lactation of both 50%HF and 87.5%HF animals feeding on different types of roughage. Concentrations of both plasma and milk urea of animals feeding on urea treated rice straw were higher than those animals fed with hay. In all groups of HF crossbred animals, the type of roughage and the level of feeding have been shown to have no effects on the concentration of milk allantoin in all periods of lactation. Breed differences were in general small and non-significant for both plasma and milk urea concentrations but were significant for the plasma glucose concentration. The values of the plasma protein and milk protein concentrations in all groups were not affected by feeding on either hay or urea treated rice straw among all periods of lactation. No significant difference was apparent for either urea-N or allantoin-N excreted in milk by 50%HF animals throughout all periods of lactation. Milk yield rather than the concentration of either urea or allantoin in milk appeared to be an important factor affecting their excretion in milk. The present result seems reasonable to assume that the relatively stable levels of both milk urea and milk allantoin concentrations indicate a constant level of feeding and synthesis of microbial protein in the rumen during feeding on either hay or urea treated rice straw as a source of roughage (Chapter VII).

The composition of the aqueous phase of milk was not affected by different stages of lactation. The movement of ions via paracellular route caused in concentration of sodium and chloride together and in concentration of potassium and lactose together. Normally the concentration of the major ions between extracellular, intracellular and milk is regulated by the activity of ion pumps located on both basal and apical membrane of the secretory cell (Linzell & Peaker, 1971a,b). The movement of these ions has been proposed to occur by a transcellular route (Linzell & Peaker, 1971a,b). The movement of the ions by a transcellular route can apply for the present experiment, since no changes of the relationship between lactose and potassium, sodium and chloride concentrations were noted during different stages of lactation in both types of crossbred animal. Thus, these results clearly suggest the

mechanism of movement of ions in 87.5%HF and 50%HF animals feeding on either hay or urea treated rice straw to be by the paracellular route (Chapter IX).

The present experiment has demonstrated that major changes occur in the metabolism of the mammary gland of the lactating 87.5%HF. A variety of physiological changes, which occur in the whole animal, have been shown to be the result of different breeds. Although we now know a great deal of the changes that occur, we do not know the physiological signal for a poorer lactation persistency which decreases the rate of milk secretion in 87.5%HF animals during lactation advance. There is still a need for more information, for example about hormones, which regulate the secretory activity of mammary cells directly in the late lactation animals and many of the intracellular metabolites concerning the process of milk secretion in the ruminant mammary gland both *in vivo* and *in vitro*.

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การเปรียบเทียบผลตอบแทนที่ได้รับในโคนมลูกผสมที่เลี้ยงด้วยฟางข้าวปรุงแต่งด้วยยูเรีย หรือหญ้าแห้งร่วมกับการให้อาหารข้นชนิดเดียวกัน

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บทนำ

ปัญหาที่สำคัญปัญหาหนึ่งที่พบในการเลี้ยงโคนมในประเทศไทยคือ การขาดแคลนอาหารหยาบคุณภาพดีไว้ใช้เลี้ยงโคนมตลอดทั้งปี อันเป็นผลมาจากการที่สภาพภูมิอากาศร้อนและแล้งในประเทศไทยมีช่วงระยะเวลานานติดต่อกันหลายเดือน บางแห่งมีช่วงระยะเวลานานถึง 6 เดือน (สมชาย จันทรพ่องแสง, 2540) การที่เกษตรกรขาดแคลนอาหารหยาบคุณภาพดี จึงมีการจัดเตรียมอาหารหยาบชนิดอื่นทดแทน โดยเฉพาะอาหารหยาบที่ได้จากของเหลือใช้ทางการเกษตรที่มีอยู่จำนวนมาก สามารถหาได้ง่าย ราคาถูก เช่น ต้นข้าวโพด เปลือกข้าวโพดฝักอ่อน (เพ็ญศรีและคณะ, 2532) เปลือกสับปะรด ฯลฯ นอกจากวัตถุดิบที่ได้กล่าวมาแล้ว ประเทศไทยยังมีของเหลือใช้อีกชนิดหนึ่งคือฟางข้าว ในแต่ละปีจะมีฟางข้าวที่เหลือจากการเก็บเกี่ยวข้าวเปลือกนับล้านตันมีรายงาน เช่น ในปี พ.ศ. 2538-2539 มีฟางข้าวที่ผลิตได้แต่ละปีเฉลี่ยมากกว่า 19 ล้านตัน (คิดจากอัตราส่วนฟางข้าวต่อข้าวเปลือก เท่ากับ 1:1 โดยน้ำหนัก สำนักงานเศรษฐกิจการเกษตร 2539)

ลักษณะที่สำคัญของฟางข้าวคือ มีคุณค่าทางอาหารต่ำ เช่น มีเยื่อใยสูง โปรตีนต่ำ ซิลิกาสูง ซึ่งทำให้สัตว์ไม่ชอบกินและมีความน่ากินน้อยรวมทั้งเปอร์เซ็นต์การย่อยได้ต่ำเฉลี่ย 45-50% (Doyle et al, 1986) ดังนั้นเพื่อให้คุณค่าทางอาหารของฟางข้าวสูงขึ้นจึงมีการพยายามหาวิธีปรับปรุงคุณภาพของฟางข้าวโดยใช้สารเคมี เช่น การใช้ด่าง (Sundstol, 1985) รวมทั้งยูเรียซึ่งเป็นสารเคมีอีกชนิดหนึ่งที่ถูกนำมาใช้อย่างแพร่หลายในการปรับปรุงฟางข้าวโดยการทำเป็นฟางปรุงแต่ง (Promma, 1988) ซึ่งพบว่าสามารถทำให้สัตว์กินฟางข้าวมากขึ้นและมีเปอร์เซ็นต์การย่อยได้สูงขึ้น

อย่างไรก็ตามผลของการให้ฟางข้าวปรุงแต่งแก่โคนมเป็นระยะเวลานานเทียบกับการให้หญ้าแห้งในโคนมพันธุ์ผสมยังมีการศึกษาน้อย ดังนั้นจุดประสงค์ของการศึกษานี้เพื่อต้องการทราบถึงผลตอบแทนที่เกิดขึ้นในโคนมทดลองลูกผสมที่เลี้ยงด้วยฟางข้าวปรุงแต่งด้วยยูเรียเทียบกับการเลี้ยงด้วยหญ้าแห้งโดยมีการให้อาหารข้นเสริมชนิดเดียวกันในระดับที่พอเพียงต่อการสร้างนมตลอดระยะเวลาของการให้นม

วัสดุอุปกรณ์และวิธีการ

สัตว์ทดลอง

ใช้โคสาวลูกผสม 2 สายพันธุ์ คือ โคสาวลูกผสม 87.5%Holstein Friesian (87.5%HF) และ โคสาวลูกผสม 50%Holstein Friesian (50%HF) ที่ทราบประวัติและสายเลือดแน่นอน มีการตั้งท้อง ประมาณ 6 เดือน อายุประมาณ 18-24 เดือน จำนวน 16 ตัว โดยแบ่งสัตว์ทดลองออกเป็น 4 กลุ่ม ๆ ละ 4 ตัว ดังนี้

กลุ่มที่ 1 โคนมลูกผสม 87.5%HF จำนวน 4 ตัว ได้รับอาหารหยาบเป็นหญ้าแห้งพันธุ์แพงโกลา (pangola hay, *Digitaria decumbens*) กับอาหารข้นชนิดเดียวกันตลอดการทดลอง

กลุ่มที่ 2 โคนมลูกผสม 87.5%HF จำนวน 4 ตัว ได้รับอาหารหยาบเป็นฟางข้าวปรุ่งแต่งด้วยยูเรีย 5% กับอาหารข้นชนิดเดียวกันตลอดการทดลอง

กลุ่มที่ 3 โคนมลูกผสม 50%HF จำนวน 4 ตัว ได้รับอาหารหยาบเป็นหญ้าแห้งพันธุ์แพงโกลา (pangola hay, *Digitaria decumbens*) กับอาหารข้นชนิดเดียวกันตลอดการทดลอง

กลุ่มที่ 4 โคนมลูกผสม 50%HF จำนวน 4 ตัว ได้รับอาหารหยาบเป็นฟางข้าวปรุ่งแต่งด้วยยูเรีย 5% กับอาหารข้นชนิดเดียวกันตลอดการทดลอง

สัตว์ทดลองทุกตัวเลี้ยงอยู่ในโรงเรือนที่เปิดด้านข้าง มีอุณหภูมิแวดล้อมได้รุ่มช่วงกลางวัน สูงสุด $34 \pm 1^{\circ}\text{C}$ และอุณหภูมิในช่วงกลางคืนต่ำสุด $26 \pm 1^{\circ}\text{C}$ ในระยะท้องก่อนคลอดสัตว์ทุกตัวได้รับอาหารข้นและอาหารหยาบตามกลุ่มสัตว์ทดลองจนกระทั่งคลอด

ในระยะให้นมสัตว์ทุกตัวได้รับอาหารหยาบทั้งฟางปรุ่งแต่งและหญ้าแห้งกินเต็มที่ร่วมกับอาหารข้นชนิดเดียวกันเฉลี่ย 7-10 กก./วัน โดยปริมาณอาหารข้นปรับตามสภาพโคนมแต่ละตัวโดยใช้เกณฑ์ body score เฉลี่ยเท่ากับ 2.5 บันทึกปริมาณอาหารที่กินของสัตว์แต่ละตัวในแต่ละวันตลอดการทดลอง บันทึกปริมาณน้ำนมที่ผลิตได้จากการรีดนมในแต่ละวันในช่วงเช้าเวลา 6.00-7.00 น. และในช่วงบ่าย 16.00-17.00 น. โดยใช้เครื่องรีดและทำการบันทึกปริมาณน้ำนมในแต่ละวันตลอดระยะเวลาทดลอง 300 วันภายหลังคลอด อาหารข้นถูกแบ่งออกเป็น 2 ส่วนและเลี้ยงในช่วงเช้าและช่วงบ่ายก่อนรีดนม โคนมทดลองทุกตัวที่ใช้ตลอดระยะเวลาการให้นม 300 วันเป็นโคนมที่ไม่มีการตั้งท้องตลอดระยะเวลาทดลอง

ชนิดอาหาร

ส่วนประกอบของอาหารชั้นและอาหารหยาบ แสดงตามตารางที่ 1

ตารางที่ 1 แสดงส่วนประกอบของอาหารชั้นและอาหารหยาบ (% น้ำหนักวัตถุดิบแห้ง)

	หญ้าแห้งแพงโกลา	ฟางข้าวปรุ่งแต่ง	อาหารชั้น
วัตถุดิบแห้ง	92.1	58.0	89.4
โปรตีนหยาบ	4.3	8.9	17.8
ADF	48.9	61.2	21.5
NDF	81.0	67.2	28.8
ลิกนิน	6.6	8.8	7.0
เถ้า	10.2	16.8	5.6

ส่วนประกอบของอาหารชั้น 100 กก. ประกอบด้วย กากถั่วเหลือง 30 กก. เมล็ดฝ้าย 25 กก. มันสำปะหลังเม็ด 25 กก. รำข้าว 15 กก. ไคแคลเซียมฟอสเฟต 2 กก. โซเดียมไบคาร์บอเนต 1.7 กก. โพแทสเซียมคลอไรด์ 0.7 กก. และฟริมิกซ์ 0.6 กก.

การเตรียมฟางปรุ่งแต่งเตรียมจากการใช้ยูเรีย (46%N) จำนวน 5 กก. ละลายในน้ำ 100 ลิตร ราดลงบนฟางข้าวแห้งจำนวน 100 กก. ปิดด้วยผ้าพลาสติกทิ้งไว้เป็นเวลา 21 วันแล้วจึงนำออกมาใช้เลี้ยงโค ส่วนหญ้าแห้งพันธุ์แพงโกลา ซื้อจากบริษัทเอกชน

การคิดผลตอบแทน : ในการคิดผลตอบแทนของการศึกษาในครั้งนี้ไม่ได้นำค่าราคาตัวสัตว์ ค่าสาธารณูปโภค และค่าตอบแทนคนงานดูแลสัตว์มาคิดในต้นทุนด้วย ดังนั้นต้นทุนการผลิตคิดจากราคาเฉลี่ยของอาหารวัตถุดิบที่ใช้เลี้ยงสัตว์ตลอดการทดลอง ส่วนรายได้คิดจากผลผลิตน้ำนมตลอดระยะเวลา 300 วัน คำนวณจากราคาเฉลี่ยของนมที่ขายในราคาตลาดคูณกับปริมาณนมที่ผลิตได้จากโคแต่ละกลุ่ม

ราคาหญ้าแห้งพันธุ์แพงโกลา	ก.ก. 4.15 บาท
ราคาฟางปรุ่งแต่งยูเรีย 5%	ก.ก. 1.98 บาท
ราคาอาหารชั้น	ก.ก. 7.02 บาท
ราคาน้ำนม	ก.ก. 9.00 บาท

ผลการทดลองและวิจารณ์

ปริมาณอาหารหยาบและขี้ (น้ำหนักสด) ที่โคนมทุกกลุ่มกิน ได้แสดงไว้ในตารางที่ 2 เมื่อเทียบในกลุ่มโคนมสายพันธุ์เดียวกัน กลุ่มที่ได้รับฟางปรงแต่งมีแนวโน้มว่าจะกินอาหารในรูปของน้ำหนักสดสูงกว่ากลุ่มที่ได้รับหญ้าแห้ง โดยโคนมที่กินฟางปรงแต่งเฉลี่ยตลอด 300 วันของการให้นมในกลุ่ม 50%HF จะกินอาหารเท่ากับ 14.4 กก./วัน และ 87.5%HF กินอาหารเท่ากับ 15.0 กก./วัน เทียบกับกลุ่มโคนมที่กินหญ้าเฉลี่ย 12.4 กก./วัน ในโคนม 50%HF และ 12.4 กก./วัน ในโคนม 87.5%HF เมื่อเปรียบเทียบกันระหว่างโคนมสายพันธุ์ต่างกันที่ได้รับอาหารหยาบชนิดเดียวกันพบว่าปริมาณการกินอาหารไม่แตกต่างกัน อย่างไรก็ตามเมื่อเปรียบเทียบปริมาณชนิดของอาหารหยาบที่โคนมกินได้พบว่าไม่มีความแตกต่างกันในปริมาณที่โคนมกินได้ในกลุ่มสัตว์ทดลองทุกกลุ่ม ส่วนปริมาณอาหารขี้มีแนวโน้มว่ากลุ่มที่ได้รับอาหารหยาบที่เป็นฟางปรงแต่งจะกินอาหารขี้ในปริมาณมากกว่ากลุ่มโคที่กินหญ้าแห้ง ในโคนมลูกผสม 50%HF ที่ได้รับฟางปรงแต่งจะกินอาหารขี้ 9.8 กก./วัน เทียบกับ 8.2 กก./วันในกลุ่มโคชนิดเดียวกันที่กินหญ้าแห้ง ($P<0.05$) ในทำนองเดียวกันโคนม 87.5%HF ที่กินฟางปรงแต่งจะกินอาหารขี้เท่ากับ 10.6 กก./วัน ส่วนกลุ่มโคที่กินหญ้าแห้งกินอาหารขี้เท่ากับ 8.4 กก./วัน จากผลของการศึกษาเมื่อคิดปริมาณการกินทั้งอาหารขี้และอาหารหยาบที่โคนมได้รับจะแตกต่างกันระหว่างกลุ่มโคนมที่กินหญ้าแห้งและฟางปรงแต่ง ($P<0.05$) ทั้งนี้เป็นผลจากความชื้นในอาหารหยาบที่แตกต่างกัน (ตารางที่ 1) ซึ่งจะมีผลกระทบต่อปริมาณการกินอาหารขี้แตกต่างไปด้วย แต่เมื่อคิดคำนวณปริมาณการได้รับอาหารเป็นวัตถุแห้งต่อวัน (dry matter intake) ไม่พบข้อแตกต่างระหว่างโคนมลูกผสมทุกกลุ่ม (Chaiyabutr et al., 1999) จึงอาจเป็นไปได้ว่าความต้องการอาหารของร่างกายโคนมแสดงในรูปของวัตถุแห้ง (dry matter) น่าจะเป็นค่าบ่งชี้ความต้องการอาหารต่อวันที่แน่นอนกว่าการคิดจากอาหารในรูปน้ำหนักสด แต่อย่างไรก็ตามจากการคิดผลตอบแทนจากราคาดันทุนซึ่งจำเป็นต้องใช้ในรูปแบบน้ำหนักสด

ต้นทุนค่าอาหาร ปริมาณน้ำนม รายได้ และผลตอบแทนที่ได้รับแสดงไว้ในตารางที่ 3 ต้นทุนค่าอาหารคิดจากราคาของหญ้าแห้ง 4.15 บาท/กก. ฟางปรงแต่ง 1.98 บาท/กก. และอาหารขี้ 7.02 บาท/กก. ส่วนราคามูลคี่ค่าเฉลี่ย 9 บาท/กก. จะเห็นได้ว่าต้นทุนค่าอาหารค่อนข้างสูง โดยเฉพาะหญ้าแห้งและอาหารขี้ เนื่องจากหญ้าแห้งพันธุ์แพงโกลาซื้อมาจากบริษัทเอกชน ซึ่งผลิตอยู่เพียงรายเดียวในประเทศไทย ส่วนอาหารขี้จะมีราคาสูงกว่าราคาอาหารในท้องตลาดที่มีราคาเฉลี่ย 5-5.50 บาท/กก. การที่อาหารขี้ในการทดลองนี้มีราคาสูง เนื่องจากผู้วิจัยได้พยายามคัดเลือกใช้วัตถุดิบที่ใช้ในการผสมที่มีคุณภาพดีและไม่มีความแปรปรวนในองค์ประกอบทางเคมี ซึ่งจะแตกต่างจากวัตถุดิบที่ใช้กันอยู่ทั่วไป ในการผสมอาหารสำหรับโคนมและอาหารขี้ที่ใช้ในครั้งนี้มีระดับพลังงานและโปรตีนเท่ากันตลอดการทดลอง ดังนั้นจึงอาจจะมีคุณค่าทางอาหารสูงเกิน

กว่าความต้องการของโคนม โดยเฉพาะอย่างยิ่งในช่วง 201-300 วันของการให้นม จึงทำให้ต้นทุนค่าอาหารในการทดลองครั้งนี้ค่อนข้างสูง

ปริมาณนมที่ผลิตได้ในโคแต่ละกลุ่มโดยคิดตลอดระยะเวลาการให้นม 300 วัน (ตารางที่ 4) ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P>0.05$) ส่วนรายได้ในโคแต่ละกลุ่มจะมีความแตกต่างกัน รายได้ที่เกิดขึ้นจากกลุ่มโคนม 50%HF ที่ได้รับฟางปรุงแต่งจะสูงสุดเท่ากับ 29 บาท/วัน และกลุ่ม 87.5%HF ที่ได้รับฟางปรุงแต่งจะมีรายได้ต่ำสุดเท่ากับ 5.2 บาท/วัน กลุ่มที่กินหญ้าแห้งในกลุ่ม 50%HF จะมีรายได้เกิดขึ้นเท่ากับ 10.3 บาท/วัน เทียบกับกลุ่ม 87.5%HF ที่มีรายได้เท่ากับ 12.9 บาท/วัน การที่กลุ่ม 87.5%HF ที่กินฟางปรุงแต่งมีรายได้ต่ำกว่ากลุ่มอื่นมากเป็นผลมาจากการได้รับอาหารชั้นในปริมาณที่สูง โดยมีค่าใช้จ่ายในส่วนนี้สูงกว่าโคนม 50%HF ที่กินฟางปรุงแต่งเช่นเดียวกัน 5.8 บาท/วัน และสูงกว่าโคนมอีก 2 กลุ่มที่กินหญ้าแห้งโดยในโคนม 50%HF เท่ากับ 16.5 บาท/วัน และ 15.3 บาท/วัน สำหรับโคนม 87.5%HF

เมื่อพิจารณาในแต่ละช่วงของการให้นมจะพบว่าในช่วงระยะ 100 วันแรก โคนม 87.5%HF ที่กินหญ้าแห้งจะมีผลให้เกิดรายได้มากที่สุดเท่ากับ 47.7 บาท/วัน และกลุ่มโคนม 87.5%HF ที่กินฟางปรุงแต่งจะทำให้เกิดรายได้รองลงมาเท่ากับ 43.5 บาท/วันเมื่อเทียบกับโคนม 50%HF ซึ่งให้เห็นว่าในช่วงต้นของการให้นม โคนม 87.5%HF จะมีศักยภาพในการผลิตสูงกว่าโคนม 50%HF ส่วนในช่วง 101-200 วัน โคนมกลุ่ม 50%HF ที่กินหญ้าแห้งหรือฟางปรุงแต่งจะทำให้เกิดรายได้ 21.4 บาท/วัน และ 31.6 บาท/วัน ตามลำดับ รวมทั้งในช่วงระยะ 201-300 วัน มีรายได้เท่ากับ 4.81 บาท/วัน และ 20.3 บาท/วัน ตามลำดับ ซึ่งมีค่าสูงกว่ากลุ่ม 87.5%HF ที่กินทั้งหญ้าแห้งหรือฟางปรุงแต่ง ผลดังกล่าวบ่งชี้ให้เห็นว่าในช่วงกลางและท้ายของการให้นม โคนม 50%HF มีแนวโน้มว่าจะมีศักยภาพในการผลิตสูงกว่าโคนม 87.5%HF

จากข้อมูลทั้งหมดชี้ให้เห็นว่าในฟาร์มทั่วไปที่มีการจัดการให้อาหารในระดับปานกลาง การเลี้ยงโคนมลูกผสม 50%HF อาจจะเหมาะสมกว่าโคนม 87.5%HF เพราะโคนมที่ใช้ในการศึกษาในครั้งนี้ได้รับอาหารในปริมาณที่ใกล้เคียงกันและมีปริมาณที่เหมาะสมกับความต้องการของโคนม แต่โคนม 87.5%HF จะผลิตนมได้ดีกว่าในช่วงแรกของการให้นม ส่วนในช่วงกลางและท้ายมีแนวโน้มว่าจะลดลงในระดับที่เร็วกว่าโคนม 50%HF ซึ่งอาจเป็นผลจากปัจจัยทางพันธุกรรมหรือปัจจัยทางสรีรวิทยาบางอย่างที่เป็นขีดจำกัดของโคนม 87.5%HF โดยไม่เกี่ยวกับการตั้งท้องของโคนมในช่วงที่มีการศึกษา เพราะโคนมทุกตัวไม่ได้รับการผสมพันธุ์ในช่วงดังกล่าว ดังนั้นรายได้ที่เกิดจากผลิตผลน้ำนมของโคนม 50%HF จะมีมากกว่า นอกจากนี้การใช้ฟางข้าวปรุงแต่งสามารถใช้เป็นอีกทางเลือกหนึ่งที่เหมาะสมจะใช้เป็นอาหารหยาบทดแทนอาหารหยาบชนิดอื่นที่มักจะขาดแคลนในช่วงหน้าแล้ง เพราะมีราคาที่ไม่สูงเกินไป การจัดเตรียมก็ไม่ลำบากและถ้ามีการจัดการให้ร่วมกับการให้อาหารชั้นที่เหมาะสมก็สามารถทำให้การเลี้ยงโคนมก่อให้เกิดรายได้แก่เกษตรกรได้

ตารางที่ 2 ปริมาณอาหารหยาบและอาหารชั้นที่โคนมลูกผสมกินในแต่ละช่วงของการให้นม

พันธุ์โคนม	ช่วงวันการให้นม	น้ำหนักสดของอาหารที่โคนมกิน (กก./วัน)		
		อาหารหยาบ	อาหารชั้น	อาหารหยาบ+ชั้น
50%HF (หญ้าแห้ง)	1-100 วัน	3.76±0.54	9.41±0.34	13.16±0.66
	101-200 วัน	3.95±0.46	7.33±1.87	11.29±1.71
	201-300 วัน	4.84±0.29	7.88±1.51	12.73±1.79
	ค่าเฉลี่ย	4.19±0.20 ^a	8.21±1.04 ^a	12.39±1.53 ^a
50%HF (ฟาง)	1-100 วัน	4.74±0.37	9.23±0.51	13.97±0.72
	101-200 วัน	3.94±0.55	10.19±0.08	14.13±0.54
	201-300 วัน	5.08±0.29	9.96±0.11	15.04±0.31
	ค่าเฉลี่ย	4.59±0.10 ^b	9.79±0.22 ^b	14.38±0.24 ^b
87.5%HF (หญ้าแห้ง)	1-100 วัน	4.34±1.39	7.89±1.94	12.23±0.56
	101-200 วัน	3.72±0.78	9.53±1.69	13.25±1.67
	201-300 วัน	4.12±0.47	7.72±3.09	11.84±2.71
	ค่าเฉลี่ย	4.06±0.64 ^{ab}	8.38±2.08 ^{abc}	12.44±1.36 ^a
87.5%HF (ฟาง)	1-100 วัน	4.26±0.41	9.04±0.64	13.30±0.74
	101-200 วัน	4.48±0.47	11.25±0.97	15.72±0.81
	201-300 วัน	4.54±0.31	11.39±0.91	15.93±0.68
	ค่าเฉลี่ย	4.43±0.14 ^{ab}	10.55±0.78 ^{bc}	14.98±1.50 ^b

ค่าเฉลี่ยที่มีอักษร a,b,c ไม่เหมือนกันในแต่ละกลุ่มแสดงระดับนัยสำคัญทางสถิติ ($P<0.05$) โดยใช้ unpaired t-test

ตารางที่ 3 แสดงต้นทุนค่าอาหาร รายได้ และผลกำไรที่ได้รับในแต่ละช่วงของการให้นม

พันธุ์โคนม	ช่วงวัน การให้นม (วัน)	อาหารหยาบ (บาท/วัน)	อาหารข้น (บาท/วัน)	อาหาร ทั้งหมด (บาท/วัน)	ปริมาณน้ำนม (กก./วัน)	รายได้ (บาท/วัน)	ผลกำไร
50%HF (หญ้าแห้ง)	1-100	15.60±2.24	66.04±2.36	81.64±3.40	9.60±0.69	86.40±6.28	4.75±6.53 [*]
	101-200	16.38±1.90	51.49±1.31	67.88±12.40	9.92±1.21 [*]	89.30±10.89 [*]	21.42±15.07 [*]
	201-300	20.11±1.19	53.33±1.06	75.43±11.75	8.92±1.03 [*]	80.24±9.29 [*]	4.81±15.39
	ค่าเฉลี่ย	17.37±2.41 ^a	57.62±7.29 ^a	74.98±10.18 ^a	9.47±0.97 ^a	85.31±8.79 ^a	10.33±10.67 ^a
50%HF (ฟาง)	1-100	9.39±0.74	64.79±3.55	74.19±3.90	12.15±1.98	109.43±17.85	35.25±16.39
	101-200	7.80±1.08	71.51±0.55	79.32±1.10	12.32±1.92 [*]	110.89±17.30 [*]	31.56±16.32 [*]
	201-300	10.01±0.57 ^b	69.92±0.79 ^a	79.99±1.00 ^a	11.14±2.54 ^a	100.27±22.90 ^a	20.28±23.13 ^a
	ค่าเฉลี่ย	9.08±0.19	68.74±1.53	77.83±3.30	11.87±2.12	106.86±19.10	29.03±18.31
87.5%HF (หญ้าแห้ง)	1-100	18.02±5.76	55.38±13.65	73.40±7.90	13.45±1.38 [*]	121.07±12.48 [*]	47.67±83.93 [*]
	101-200	15.44±3.24	66.89±11.86	82.34±11.50	10.04±1.13 ^{**}	90.36±10.25 ^{**}	8.05±11.63 ^{**}
	201-300	17.09±1.97	54.21±21.69	71.31±20.10	6.01±0.60	54.17±5.42	-17.14±22.40 ^{**}
	ค่าเฉลี่ย	16.85±2.65 ^a	58.83±14.63 ^a	75.68±9.40 ^a	9.83±0.60 ^a	88.53±5.41 ^a	12.85±10.67 ^a
87.5%HF (ฟาง)	1-100	8.44±0.81	63.43±4.52	71.87±4.50 ^{**}	12.81±2.06 ^{**}	115.40±18.60 ^{**}	43.53±14.50 ^{**}
	101-200	8.86±0.92	78.95±6.77	87.81±6.30 ^{**}	9.61±1.13 ^{**}	86.52±10.20 ^{**}	-1.30±5.85 ^{**}
	201-300	9.00±0.60 ^b	79.94±6.40 ^a	88.45±5.90 ^a	6.91±0.97 ^a	62.27±8.80 ^a	-26.67±10.72 ^a
	ค่าเฉลี่ย	8.77±0.28	74.11±5.50	82.88±9.80	9.78±1.26	88.06±11.42	5.19±8.11

- ค่าเฉลี่ยที่มีอักษร a,b ที่ไม่เหมือนกันในแต่ละพารามิเตอร์ของโคนมกลุ่มต่าง ๆ แสดงความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) โดยใช้ unpaired t-test
- เปรียบเทียบข้อแตกต่างอย่างมีนัยสำคัญทางสถิติโดยใช้ paired t-test * $P<0.05$, ** $P<0.01$ ระหว่างช่วงการให้นมเปรียบเทียบกับช่วง 1-100 วันแรกของโคนมกลุ่มเดียวกัน

ตารางที่ 4 แสดงต้นทุนค่าอาหาร รายได้ และผลกำไรที่ได้รับในแต่ละช่วงของการให้นม (บาท/ตัว/300 วัน)

พันธุ์โคนม	จำนวนวัน ให้นม	อาหารหยาบ (บาท)	อาหารข้น (บาท)	รวมค่าอาหาร ทั้งหมด(บาท)	ปริมาณน้ำนม ทั้งหมด (กก.)	รายได้จาก น้ำนม(บาท)	ผลกำไร (บาท)
50%HF (หญ้าแห้ง)	300 วัน	5210±247 ^a	17286±2185 ^a	22495±2065 ^a	2844±293 ^a	25595±2638 ^a	3099±3200 ^a
50%HF (ฟาง)	300 วัน	2726±60 ^b	20623±495 ^b	23349±465 ^a	3562±636 ^a	32055±5727 ^a	8709±5493 ^a
87.5%HF (หญ้าแห้ง)	300 วัน	5054±794 ^a	17649±4389 ^{ab}	22704±3628 ^a	2951±181 ^a	26560±1624 ^a	3856±3201 ^a
87.5%HF (ฟาง)	300 วัน	2629±85 ^b	22233±1651 ^b	24863±1568 ^a	2936±381 ^a	26420±3428 ^a	1557±2433 ^a

ค่าเฉลี่ยที่มีอักษร a,b ไม่เหมือนกันในแต่ละกลุ่มแสดงระดับนัยสำคัญทางสถิติ ($P<0.05$) โดยใช้ unpaired t-test

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