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]

N. Chaiyabutr*, S. Komolvanich, S. Preuksagorn and S. Chanpongsang[1]
Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand

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×Red Sindhi (50×50=50% HF) and Holstein Friesian×Red Sindhi (87.5×12.5=87.5% HF). They were divided into four groups of 4 animals each 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. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 3 : 334-347*)

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 torelance 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

* Address reprint request to N. Chaiyabutr, Tel: +66-2-2520737, Fax: +66-2-2553910, E-mail: Narongsak.c@chula.ac.th.
[1] Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.
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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×Red Sindhi (50:50=50% HF) and Holstein Friesian×Red Sindhi (87.5:12.5=87.5% HF). They were divided into four groups of 4 animals each. Each group of animals consisted of four animals from the same breed. Animals from the same breed type in each group were fed with either rice straw treated with 5% urea or pangola hay (*Digitaria decumbens*) as the source of roughage throughout the experiments. All the animals were housed in sheds. The maximum temperature in the shed at noon was 34±1℃ and the minimum temperature at night was 26±1℃. Before parturition, animals were individually fed a concentrate of an average of 4.0 kg/day (DM basis) and roughage to maintain 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 06:00-07:00 h and the other half between 16:00-17:00 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.

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).

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

Experimental procedures

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, UK.) 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-^{3}H)-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 1100 h a priming dose of radioactive glucose in 20 ml of sterile NSS containing 60 μCi (3-^{3}H) 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-^{3}H) glucose for 4 h (Peristaltic pump; EYLA Model 3). During the final 1 hour (1400-1500 h) 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, C_{16}-C_{18}) and plasma free fatty acids (FFA, C_{16}-C_{18}) 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-^{14}C]glucose and [3-^{3}H]glucose were obtained from the Radiochemical Center, Amersham Bucks, UK. 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. $^{14}CO_2$ was liberated and trapped as $K^{14}CO_3$ 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 (C_6 to C_{18}) 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℃. 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 mol/min, was calculated from the equation

$$T = I/G_A$$

where I=rate of infusion of U-^{14}C glucose or 3-^{3}H glucose (μCi/min) and G_A= specific activity of ^{14}C- or ^{3}H-glucose in arterial plasma at equilibrium (μCi/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-^{3}H 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-^{3}H glucose (μmol/min) and P_{AG}=arterial plasma glucose concentration (μmol/ml).

Uptake of substrates by the udder (U_M), expressed as μmol/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 (μmol/ml) and P_V=concentration of substrate in mammary venous plasma (μmol/ml).

Milk substrate output (OS), expressed as μmol/min, was calculated from the equation:

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

where M=milk secretion rate (ml/min) and Cm= concentration of substrate in milk (μmol/l).

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

$$R_{CO_2} = Q_B \times (^{14}CO_{2V} - {}^{14}CO_{2A})/G_A$$

where Q_B=udder blood flow (ml/min), $^{14}CO_{2A}$= arterial blood $^{14}CO_2$ (μCi/ml), $^{14}CO_{2V}$=mammary venous blood $^{14}CO_2$ (μCi/ml) and G_A=specific activity of ^{14}C-glucose in arterial plasma at equilibrium (μCi/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 (μmol/min), M_A=total activity of ^{3}H or ^{14}C in the milk components (μCi), G_A=specific activity of ^{14}C- or ^{3}H-glucose in arterial plasma at equilibrium (μCi/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 μmol/min, was calculated from the equation:

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

where n=chain length of the fatty acid (6 to 16) and FFA_n=output in milk of fatty acid chain length n (μmol/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 μmol/min, was calculated from the equation:

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

where U_G=mammary glucose uptake (μmol/min) and L=output of lactose in milk (μmol/min).

Net metabolism of glucose (B) to the galactose or glucose moiety of lactose, expressed as μmol/min, was calculated from the equation:

$$B = L$$

where L=output of lactose in milk (μmol/min).

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

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

where Y=specific yield of $^{14}CO_2$ from (1-^{14}C) glucose via the pentose phosphate pathway (Katz and Wood, 1963).

If the NADPH formed via PC were used

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 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)
Glucose turnover rate					
(3-^{3}H)glucose	Early	5,662.6 ± 695.5^{a}	4,965.6 ± 564.2^{a}	4,713.5 ± 804.5^{a}	5,115.1 ± 567.6^{a}
(μmol/min)	Mid	4,587.5 ± 1,198.8^{b}	5,514.8 ± 803.6^{ab}	5,228.0 ± 1,081.7^{ab}	6,481.2 ± 988.6^{a}
	Late	4,602.8 ± 900.9^{c}*	5,766.1 ± 669.0^{bc}	6,657.5 ± 1,313.1^{ab}**	7,453.9 ± 862.4^{a}*
(U-^{14}C)glucose	Early	4,712.3 ± 747.5^{a}	3,980.4 ± 399.1^{a}	4,471.2 ± 751.9^{a}	3,911.8 ± 726.5^{a}
(μmol/min)	Mid	3,874.3 ± 757.4^{a}	3,696.1 ± 270.8^{a}	3,755.4 ± 540.9^{a}	4,301.4 ± 390.8^{a}
	Late	3,793.0 ± 475.6^{ab}	3,669.8 ± 331.8^{b}	4,186.3 ± 691.3^{ab}	4,451.3 ± 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	Early	1,702.1 ± 409.9^{a}	1,127.5 ± 197.8^{b}	1,333.4 ± 211.9^{ab}	1,288.6 ± 70.3^{b}
clearance (ml/min)	Mid	1,471.2 ± 417.8^{a}	1,486.8 ± 358.6^{a}	1,534.7 ± 130.5^{a}	1,618.3 ± 59.0^{a}**
	Late	1,296.2 ± 177.1^{c}	1,488.7 ± 106.2^{bc}	1,749.1 ± 262.2^{b}**	2,122.8 ± 244.1^{a}**
Non mammary	Early	1,784.7 ± 986.0^{a}	2,537.2 ± 820.5^{a}	1,600.3 ± 957.1^{a}	2,669.3 ± 698.2^{a}
Glucose utilization	Mid	2,106.5 ± 891.9^{a}	3,356.7 ± 666.9^{ab}	3,000.5 ± 1,199.9^{ab}	4,093.7 ± 1,127.4^{b}
(μmol/min)	Late	2,151.7 ± 569.1^{a}	3,570.7 ± 354.5^{b}	4,499.3 ± 1,317.3^{bc}	4,849.0 ± 938.7^{c}
Non mammary	Early	31.4 ± 16.6^{a}	50.2 ± 11.6^{a}	33.4 ± 16.6^{a}	51.7 ± 9.6^{a}
Glucose utilization	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 ($p<0.05$).

exclusively for reductive biosynthesis of fatty acids, the ^{3}H-incorporation from (3-^{3}H) glucose into fatty acids would equal the $^{14}CO_2$ 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:

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

where Z=(Total ^{3}H in milk fatty acid)/t × G_A × (U_G-L)

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

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

The $^3H/^{14}C$ ratio in the plasma and related products was calculated from the equations:

$^3H/^{14}C$ glucose=$^3H/^{14}C$ in plasma glucose relative to a $^3H/^{14}C$ ratio of 1 in the infusion,

$^3H/^{14}C$ lactose = $^3H/^{14}C$ in milk lactose relative to a $^3H/^{14}C$ ratio of 1 in the infusion,

$^3H/^{14}C$ citrate = $^3H/^{14}C$ in milk citrate relative to a $^3H/^{14}C$ ratio of 1 in the infusion, and

$^3H/^{14}C$ triacyglycerol=$^3H/^{14}C$ in milk triacyglycerol relative to a $^3H/^{14}C$ 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 Duncans 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 ± 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-[^{3}H] glucose infusion and the 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

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 (87.5:12.5)	HF:RS (50:50)	HF:RS (87.5:12.5)	HF:RS (50:50)
Udder blood flow (ml/min)	Early	7,160 ± 1,807^{a}	3,887 ± 543^{a}	4,619 ± 1,149^{b}	4,314 ± 575^{b}
	Mid	4,745 ± 836^{a}	4,090 ± 398^{a}	3,843 ± 872^{a}	5,068 ± 1,054^{a}
	Late	5,026 ± 724^{ab}	3,942 ± 500^{b}	3,995 ± 883^{b}	5,371 ± 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,c} Mean values within a row indicated with different superscripts are significantly different ($p<0.05$).

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 clearance ($p<0.01$) 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.

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 (87.5:12.5)	HF:RS (50:50)	HF:RS (87.5:12.5)	HF:RS (50:50)
Milk lactose secretion (μmol/min)	Early	1,845.7 ±438.8^{a}	976.3 ±122^{b}	1,543.8 ±544.6^{ab}	1,210.6 ±156.8^{b}
	Mid	1,025.7 ±165.4^{a}*	958.9 ±110^{a}	1,096.5 ± 59.5^{a}	1,149.4 ±225.4^{a}
	Late	921.9 ± 77.6^{ab}*	955.7 ± 72^{ab}	846.9 ±101.4^{b}*	1,104.4 ±211.9^{a}
Milk citrate secretion (μmol/min)	Early	113.0 ± 15.7^{a}	47.2 ± 7.7^{b}	93.3 ± 35.7^{a}	52.6 ± 6.7^{b}
	Mid	51.8 ± 15.7^{a}	49.2 ± 10^{a}	50.3 ± 11.2^{a}	59.5 ± 12.2^{a}
	Late	40.6 ± 1.6^{a}	50.5 ± 5.9^{a}	43.4 ± 14.6^{a}	60.7 ± 21.9^{a}
Milk triacylglycerol secretion (μmol/min)	Early	700.1 ±148.7^{a}	719.7 ±174.1^{a}	584.4 ±206.2^{a}	650.9 ±191.3^{a}
	Mid	415.9 ± 37.4^{c}	598.3 ± 83.8^{ab}	495.7 ± 93.1^{bc}	655.8 ± 34.9^{a}
	Late	507.5 ±133.9^{b}	538.8 ±149.4^{b}	440.4 ±100.4^{b}	831.8 ± 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 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)
[^{14}C] Glucose incorporation (μmol/min) into:					
milk lactose	Early	2,189.3 ±530.2^{a}	1,339.4 ±217^{b}	2,000.5 ±743.8^{ab}	1,334.0 ±300.8^{b}
	Mid	1,594.5 ±279.4^{a}*	1,219.4 ±356^{a}	1,227.3 ±161.2^{a}	1,291.0 ±354.5^{a}
	Late	1,158.8 ±347.8^{a}*	1,228.2 ±358^{a}	873.1 ±119.8^{a}*	1,084.0 ±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 CO_2	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 CO_2	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).

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}H]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 μmol/min for 87.5% HF and 50% HF animals

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 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)
Flux through the pentose phosphate pathway calculated as ^3H incorporation into milk fatty acid (equivalent μ mol 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 ^3H incorporation into milk fatty acid (equivalent μ mol 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 ($p<0.05$).

feeding on hay, respectively, and 253 and 149 μmol/min for 87.5% HF and 50% HF animals feeding on urea treated rice straw, respectively. Correction of the lower $^3H/^{14}C$ ratio likely to be present in intracellular glucose 6-phosphate gave flux values of 299 and 118 μmol/min for 87.5% HF and 50% HF animals feeding on hay, respectively, and 314 and 220 μmol/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: glucose 6-phosphate → glyceraldehyde 3-phosphate+$3CO_2$ (Katz and Wood, 1963).

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 μmol/min for 87.5% HF and 50% HF animals feeding on hay respectively and 255 and 90 μmol/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 C6 to C18 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 C16 to C18, increased to the same level as that in 50% HF animals.

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

The $^3H/^{14}C$ ratio in arterial plasma glucose was lower than that of the infusion in both groups of crossbred HF cattle. These values were not different

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 (87.5:12.5)	HF:RS (50:50)	HF:RS (87.5:12.5)	HF:RS (50:50)
Requirement of all NADPH for fatty acid synthesis (μmol/min)				
Early	1,773.06 ± 296.44^a	2,053.24 ± 652.89^a	1,452.99 ± 742.15^a	2,063.16 ± 808.55^a
Mid	1,181.16 ± 124.74^b	1,565.51 ± 219.09^{ab}	1,578.33 ± 516.27^{ab}	1,996.28 ± 177.20^a
Late	1,425.87 ± 253.95^b	1,474.31 ± 364.64^a	1,147.57 ± 404.55^b	2,474.08 ± 317.72^a
Requirement of all NADPH formation from glucose via the pentose phosphate pathway (%)				
Early	25.8 ± 15.2^a	10.0 ± 3.3^a	16.1 ± 3.9^a	19.5 ± 16.8^a
Mid	42.5 ± 21.8^a	11.1 ± 6.8^b	31.9 ± 20.5^{ab}	14.8 ± 9.6^b
Late	32.1 ± 26.6^a	17.8 ± 13.4^a	14.5 ± 5.8^a	8.9 ± 5.1^a

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

among groups of animals, indicating some recycling of glucose-C in the whole animal. A slight decrease in the $^{3}H/^{14}C$ ratio was seen in milk lactose in both groups of 50% HF animals,whereas the $^{3}H/^{14}C$ ratio of milk triacylglycerol was slightly higher in both groups of 87.5% HF animals. The ^{3}H and ^{14}C from glucose were also shown to be incorporated into milk citrate. The $^{3}H/^{14}C$ ratio of milk citrate was slightly higher in 87.5% HF during early lactation.

DISCUSSION

The present results and those of Chaiyabutr 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 (Chaiyabutr et al., 1998). The udder blood flow showed significant differences between 50% HF and 87.5% HF animals in early lactation while the

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 (μmol/ml milk)	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)
Early lactation	C6	1.25± 0.52^{a}	2.78± 1.01^{b}	1.03± 0.57^{a}	2.52± 1.70^{b}
	C8	0.57± 0.14^{a}	1.25± 0.49^{bc}	0.44± 0.28^{ac}	1.10± 0.86^{ab}
	C10	0.95± 0.16^{a}	2.23± 0.91^{b}	0.72± 0.47^{ac}	1.93± 1.58^{ab}
	C12	0.98± 0.17^{a}	2.32± 0.79^{b}	0.80± 0.51^{ac}	1.80± 1.39^{ab}
	C14	3.59± 0.83^{a}	8.08± 2.80^{b}	3.20± 1.73^{ac}	6.61± 3.56^{ab}
	C16:0	16.40± 5.82^{a}	29.70± 8.56^{b}	16.72± 3.59^{ac}	24.69± 8.00^{ab}
	C16:1	1.06± 0.87^{a}	1.69± 0.53^{a}	1.02± 0.23^{a}	1.71± 0.36^{a}
	C18:0	13.20± 5.34^{a}	23.05± 5.54^{b}	12.35± 3.55^{a}	14.45± 4.46^{a}
	C18:1	14.88± 8.97^{ac}	23.05± 6.16^{a}	14.50± 2.16^{c}	17.53± 5.01^{ac}
	C18:2	0.82± 0.38^{a}	1.20± 0.27^{a}	0.96± 0.42^{a}	1.64± 0.47^{a}
	Total	53.71±21.34^{a}	95.34±24.40^{b}	51.74±10.25^{ac}	73.97±26.33^{ab}
Mid lactation	C6	1.38± 0.51^{a}	1.87± 0.53^{a}	1.64± 0.65^{a}	2.57± 0.52^{a}
	C8	0.65± 0.21^{a}	0.80± 0.25^{a}	0.77± 0.33^{a}	1.21± 0.26^{a}
	C10	1.10± 0.38^{a}	1.43± 0.49^{a}	1.43± 0.60^{a}	2.23± 0.50^{a}
	C12	1.16± 0.34^{a}	1.51± 0.58^{a}	1.59± 0.63^{a}	2.16± 0.53^{a}
	C14	4.42± 1.33^{a}	6.40± 1.80^{ab}	5.85± 2.12^{ab}	7.25± 1.37^{b}
	C16:0	19.21± 4.04^{a}	26.28± 6.63^{a}	22.08± 6.04^{a}	25.26± 5.98^{a}
	C16:1	1.00± 0.49^{a}	1.69± 0.41^{a}	1.02± 0.34^{a}	1.15± 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± 5.29^{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. $^{3}H/^{14}C$ 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 (87.5:12.5)	HF:RS (50:50)	HF:RS (87.5:12.5)	HF:RS (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 ($p<0.05$).

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 (Chaiyabutr 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 [U-^{14}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-^{3}H]glucose may represent the total glucose turnover rate as the ^{3}H is not recycled from products of partial glucose degradation (Katz et al., 1965). Thus one way of estimating ^{14}C-recycling is by simultaneously injecting [3-^{3}H] glucose and [U-^{14}C]glucose as in the present experiments. The incresed 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 concent- rations 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 CO_2 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-^3H]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 ^{3}H 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 ^{3}H from [3-^{3}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 $^{3}H/^{14}C$ ratio in milk triacyglycerol 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 ^{3}H is lost to NADPH or water in metabolism via the pentose phosphate pathway or glycolytic pathway, so it is likely that ^{3}H incorporation into milk citrate was also via NADP^{3}H. The ratio of tritium in milk citrate to that of plasma glucose in both types of crosssbred HF animals in the present experiments would appear to support this hypothesis. However, the different values of the specific radioactivity of the ^{3}H in milk citrate were apparent in 50% HF and 87.5% HF animals. It is possible that the incorporation of ^{3}H 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 was 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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