Studies on the Mode of Uptake of Plasma Glucose, Acetate, β-hydroxybutyrate Triglyceride Fatty Acids and Glycerol by the Mammary Gland of Crossbred Holstein Cattle Feeding on Different Types of Roughage

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ABSTRACT: The present experiment was carried out to study the utilization of substrates in the mammary gland of crossbred Holstein Friesian during feeding on different types of roughage. Sixteen pregnant crossbred Holstein heifers consisted of two breed types of eight animals each; Holstein Friesian×Red Sindhi (50:50=50%HF) and Holstein Friesian×Red Sindhi (87.5:12.5=87.5%HF). Animals were divided into four groups of the same breed type in each group which were fed with either rice straw treated with 5% urea or pangola hay (Digitaria decumbens) as the source of roughage throughout the experiments. Four consecutive experimental periods were carried out in late pregnancy (20-23 days before parturition), early lactation (30 days postpartum), mid-lactation (120 days postpartum) and late lactation (210 days postpartum). Measurement of mammary blood flow in combining with measurement of AV difference was performed for the mammary uptake of substrates. In the period of lactation, udder blood flow was nearly three times higher than that of late pregnant period (p<0.05) in both 50%HF and 87.5%HF feeding on either hay or urea treated rice straw. During mid- and late lactation of both groups of 87.5%HF animals, mammary blood flow and milk yield showed decrease when compared to those during the early lactating period while the trends for persistency were apparent in both groups of 50%HF animals throughout experimental periods. The mean arterial plasma concentrations of glucose, acetate, β-hydroxybutyrate and free glycerol in each group remained constant throughout experimental periods. During late pregnancy in all groups, the AV difference and extraction ratio of glucose, β-hydroxybutyrate and triacylglycerol across the mammary gland markedly lowered (p<0.05), which coincided with a lower net uptake by the mammary gland in comparison to the early lactating period. The mean arterial plasma concentration, AV difference and extraction ratio for acetate showed no significant differences between late pregnancy and the early lactating period. The AV difference of free glycerol showed apparent release from mammary tissue during late pregnancy in all groups. In mid- and late lactation, the mammary uptake for glucose, acetate and β-hydroxybutyrate in both groups of 87.5%HF animals showed apparent decrease as compared to that in the early lactating period, whereas no appearances were observed in 50%HF animals feeding either hay or urea treated rice straw. The mean arterial plasma concentrations for free fatty acid (FFA) and triacylglycerol (C16 to C18) were higher in late pregnancy than in early lactation in both types of crossbred animals. The values of AV difference and the net uptake by the mammary gland for FFA were variable during late pregnancy and lactating periods in all groups. There were no significant differences for AV difference, extraction ratio and net uptake of triacylglycerol during lactation advance in both groups of 50%HF and 87.5%HF animals feeding either hay or urea treated rice straw. These results suggest that the adaptations to either hay or urea treated rice straw by the mammary gland of crossbred HF animals allow for an adequate nutrient supply during pregnancy and lactation. There is no difference in the mode of mammary uptake of substrates in the same crossbred animals in response to feeding hay or urea treated rice straw. The differences in utilizing nutrients by the mammary gland for milk production between 87.5%HF and 50%HF animals would be dependent on changes in both intra-mammary factors and extra-mammary factors. (Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 10 : 1445-1452)

Key Words: Mammary Gland Uptake, Crossbred Holstein Cattle, Substrates, Roughage

INTRODUCTION

In tropical countries, selecting the types of suitable cross breeding of indigenous and exotic cattle for milk production is practiced. However, low milk production of dairy herd of both exotic and crossbred cattle in the tropics is still the main problem in dairy farming. In adequate for foraging during the dry summer months is one of the problems which may limit milk production of dairy cattle in the topic. Animals are fed mainly on crop residues such as rice straw which has a low nutritive value. Urea has been used to improve the feeding value of low quality roughage (Klopfenstein, 1978). There have been very few studies how the urea treated rice straw is efficiently utilized by crossbred dairy cattle.

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 substrate supplying to the mammary gland is determined by the substrate concentration in the plasma and mammary blood flow. It is evident that substrates supply to the mammary...
gland is often inadequate to maintain the maximum rate of milk synthesis (Linzell and Mepham, 1974). The mammary gland may produce 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. During pregnancy, mammary growth has been known to be a prerequisite for lactation. Prolonged undernutrition in early lactation has been shown to be the cause of an impairment of lactational ability throughout the course of lactation (Broster et al., 1969). Little is known about the utilization of substrates in the mammary gland in different types of crossbred cattle during feeding on low quality roughage, although the genetic potential for a high milk yield of 87.5%HF animal showed homeorhetic adaptation and hormonal regulation for mammary function which differed from 50%HF animal during different periods of lactation (Chaiyabutr et al., 2000b). Therefore, the present experiment was conducted to obtained more information on the controlling mechanism for milk synthesis in regarding to the utilization of substrate by the mammary gland in different types of crossbred Holstein cattle feeding on either hay or urea treated rice straw. The present experiment was performed by using techniques for measuring mammary blood flow and combining with the measurement of AV difference of a substrate across the mammary gland for the mammary uptake during late pregnancy and lactation.

MATERIALS AND METHODS

Animals and management

Sixteen pregnant, crossbred Holstein heifers (23-25 months old and no later than 150 days in gestation) were utilized for the experiments. These animals consisted of two breed types of eight animals each: 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 from the same breed type in each group which 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 the shed. The maximum temperature in the shed at noon was 34±1°C and the minimum temperature at night was 26±1°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 (2.5, scale=1 to 5) until calving. In lactating period, animals were maintained similar moderate body condition score (2.5) by 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 06:00-07:00 h and the other half between 16:00-17:00 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×21 day system of urea treatment. After 21 days, the treated rice straw with 5% urea was offered to the 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 | 48.9        | 61.2                    | 21.5        |
| 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). (1 kg of premix contains vitamin A 1,000,000 IU., vitamin D3 250,000 IU., vitamin E 3.5 g., selenium 0.2 g, ferrous 4.0 g, cobalt 60 mg, manganese 4.0 g, zinc 8.0 g, iodine 0.1 g, potassium 0.03 g and phosphorus 21 g).

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 entire experimental period. During lactating periods of experiments, animals were milked twice daily at around 06:00 h and 17:00 h. On the day of the experiment at around 11:00 h, around 7 ml of blood sample was taken from either the milk vein or the coccygeal artery by venipuncture with a #21 needle into heparinized tubes before udder blood flow measurement. Blood sample tubes were kept in crushed ice and then centrifuged at 3,000 rpm for 30 min at 4°C. Plasma samples were kept in aliquots at -40°C for chemical studies. Milk yield was measured and recorded daily.

Udder blood flow measurements

Udder blood flow measurements were performed twice in each experimental period. 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 (0.9%NaCl) and diluted to a concentration of 100 mg/L. The mixture solution was infused using 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, around 7 ml of 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 2 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 (Kit Human GmbH, Germany). Plasma free fatty acid (FFA, C16-C18) concentrations were measured by using gas chromatography (Shimazu GC-7AG Gas Chromatograph) using 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 (Kit, 310-A, Sigma-Aldrich Co., USA). Plasma acetate concentrations were determined by head-space gas chromatographic method as described by Akane et al. (1990). Plasma glycerol concentrations were determined by enzymatic method (Kit, 337-A Sigma-Aldrich, Co., USA).

Calculation

Uptake of substrates by the mammary gland was calculated by mammary plasma flow multiplied with arteriovenous concentration difference (AV difference) of each substrate. Extraction ratio or efficiency was calculated as 100 times the AV difference of a substrate divided by its arterial concentration.

Statistics

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

RESULTS

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

Udder blood flow was higher in the lactating period of than that of late pregnant period in both 50%HF and 87.5%HF animals feeding on either hay or urea treated rice straw (p<0.05). In the early lactating period, 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 both groups of 50%HF animals. In mid- and late lactation, both mammary blood flow and milk yield showed decrease when compared to those in early lactating period of both groups of 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 throughout experimental periods.

Arterial plasma concentration, AV difference and mammary uptakes of glucose and acetate (Table 3)

The mean arterial plasma glucose concentration remained constant throughout periods of study in each group. However, the plasma glucose concentration of

| Table 2. Udder blood flow and milk secretion during late pregnancy and different stages of lactation (mean±SD) |
|---|---|---|---|---|---|---|---|---|
| Period of experiment | Hay+concentration | Urea treated rice straw+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) |
| Udder blood flow (ml/min) | 2,696±265* | 1,034±354b | 1,876±363b | 1,396±406b |
| Pregnant | | 3,887 | | |
| Early | 7,160±1807†† | 3,887±543b | 4,619±1149†† | 4,314±575b††† |
| Mid | 4,745±836 | 4,090±398 | 3,843±872 | 5,068±1054 |
| Late | 5,026±724b | 3,942±500b | 3,995±883b | 5,371±932b |
| Milk secretion (ml/min) | 7.6±1.1 | 7.3±0.9 | 8.1±0.6 | 8.6±1.7 |
| Early | 7.0±0.5b | 7.3±0.6b | 6.4±0.8** | 8.5±1.7a |
| Mid | 7.6±1.1 | 7.3±0.9 | 8.1±0.6 | 8.6±1.7 |
| Late | 7.0±0.5b | 7.3±0.6b | 6.4±0.8** | 8.5±1.7a |

Values by paired t-test. * p<0.05 with respect to the early period of lactation in each group.
† p<0.05, †† p<0.01, ††† p<0.001 with respect to the late pregnant period in each group.
Mean values within a row indicated with different superscripts are significantly different (p<0.05).
50%HF animals fed either hay or urea treated rice straw was higher than those of 87.5%HF animals in all periods of experiment especially in early lactating period (p<0.05). Compared to late pregnancy, A-V difference and extraction efficiency of glucose in early lactation were increased approximately 4-5 times in 87.5%HF and 2-3 times in 50%HF, respectively. The mean arterial plasma concentration, A-V difference and extraction ratio for acetate showed no significant differences when compared to those in the early lactating period. The net mammary uptake in late pregnancy markedly lowered for glucose (p<0.001) and for acetate (p<0.05) 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 as compared to that in the early lactating period. Mammary uptake of glucose and acetate showed no differences among periods of lactation in 50%HF animals fed either hay or urea treated rice straw.

**Arterial plasma concentration, A-V difference and mammary uptakes of β-hydroxybutyrate and glycerol (Table 4)**

The mean arterial plasma concentrations for β-hydroxybutyrate and free glycerol remained constant throughout experimental periods in each group. During late pregnancy in all groups, the AV difference and extraction

### Table 3. Arterial plasma concentrations, mammary arteriovenous differences and mammary uptake for glucose and acetate during late pregnancy and different stages of lactation (mean±SD)

| Period of experiment | Hay+concentration (87.5:12.5) | Hay+concentration (50:50) | Urea treated rice straw+concentration (87.5:12.5) | Urea treated rice straw+concentration (50:50) |
|----------------------|-------------------------------|---------------------------|-----------------------------------------------|-----------------------------------------------|
| **Glucose**          |                               |                           |                                               |                                               |
| Arterial concentrate (µmol/ml) |                                |                           |                                               |                                               |
| Pregnant             | 3.67±0.32                     | 4.06±0.59                 | 3.49±1.04                                     | 3.60±0.81                                     |
| Early                | 3.40±0.53                     | 4.46±0.42                 | 3.54±0.19                                     | 4.16±0.13                                     |
| Mid                  | 3.15±0.46                     | 3.77±0.35                 | 3.39±0.56                                     | 3.99±0.45                                     |
| Late                 | 3.54±0.40                     | 3.86±0.19                 | 3.79±0.33                                     | 3.52±0.17                                     |
| A-V (µmol/ml)        | 0.16±0.06                     | 0.32±0.20                 | 0.22±0.07                                     | 0.20±0.07                                     |
| Mid                  | 0.74±0.04                     | 0.83±0.12                 | 0.90±0.15                                     | 0.78±0.05                                     |
| Late                 | 0.71±0.08                     | 0.77±0.08                 | 0.83±0.19                                     | 0.69±0.04                                     |
| Extraction (%)       | 4±2                           | 8±6                       | 6±2                                           | 6±1                                           |
| Udder uptake (µmol/min) | 301±108                     | 254±226                   | 291±91                                        | 188±65                                        |
| Mid                  | 2,482±483                     | 2,157±275                 | 2,225±169                                     | 2,450±437                                     |
| Late                 | 2,451±604                     | 2,195±454                 | 2,158±591                                     | 2,640±479                                     |
| **Acetate**          |                               |                           |                                               |                                               |
| Arterial concentrate (µmol/l) |                                |                           |                                               |                                               |
| Pregnant             | 977±121                       | 915±226                   | 1,117±428                                     | 810±261                                       |
| Early                | 713±201                       | 691±146                   | 919±452                                       | 811±189                                       |
| Mid                  | 736±203                       | 821±211                   | 963±477                                       | 714±222                                       |
| Late                 | 966±105                       | 714±144                   | 893±275                                       | 664±235                                       |
| A-V (µmol/l)         | 467±78                        | 326±259                   | 675±255                                       | 407±163                                       |
| Mid                  | 441±128                       | 403±218                   | 581±322                                       | 330±108                                       |
| Late                 | 506±97                        | 380±132                   | 483±117                                       | 378±228                                       |
| Extraction (%)       | 49±14                         | 34±24                     | 62±16                                         | 50±10                                         |
| Udder uptake (µmol/min) | 902±136.4                     | 257±273.6                 | 931.0±404.1                                   | 390.2±201.4                                   |
| Mid                  | 2,186.7±742.2                 | 1,050.3±325.1             | 2,427.7±1,797.8                               | 1,677.3±990.6                                 |
| Late                 | 1,512.7±291.6                 | 1,126.1±637.1             | 1,733.1±1,158.8                               | 1,250.3±527.9                                 |

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

**p<0.05, **p<0.01, ***p<0.001 with respect to the period of pregnancy in each group.**

**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 (mean±SD)

| Period of experiment | Hay+concentration | Urea treated rice straw+concentration |
|----------------------|-------------------|---------------------------------------|
|                      | HF:RS (87.5:12.5) | HF:RS (50:50)                         |
|                      | Mid               | Late                                  |
| Beta-hydroxybutyrate |                   |                                       |
| Arterial concentrate (µmol/l) |       |                                       |
| Pregnant             | 680.2±153.6       | 539.0±160.4                           |
|                      | 646.0±140.3       | 800.5±569.9                           |
| Early               | 648.0±151.8††   | 536.7±195.9                           |
| Mid                 | 563.0±154.2       | 517.7±102.9                           |
| Late                | 531.3±79.6†††    | 432.5±119.9                           |
|                      | 523.0±67.0        | 408.7±74.2                           |
|                      | 503.5±64.6        | 431.3±19.5                            |
| A-V (µmol/l)         |                   |                                       |
| Pregnant             | 69.5±42.5         | 80.7±25.6                             |
|                      | 61.7±51.1         |                                       |
| Early               | 235.7±65.6†††    | 217.7±103.1                           |
| Mid                 | 213.5±78.4†††    | 211.5±56.2                            |
| Late                | 199.3±53.4        | 212.7±22.5                            |
| Extraction (%)       |                   |                                       |
| Pregnant             | 10±4              | 10±11                                 |
|                      | 9±6               |                                       |
| Early               | 36±7†††           | 40±6††                               |
| Mid                 | 38±11††           | 41±5††                               |
| Late                | 38±8              | 49±4                                 |
| Udder Uptake (µmol/min) |       |                                       |
| Pregnant             | 131.6±73.0        | 77.2±33.7                             |
|                      | 48.0±51.5         |                                       |
| Early               | 1217.5±380.7†††  | 706.6±272.6                           |
| Mid                 | 749.8±330.7      | 785.3±240.6                           |
| Late                | 716.7±126.5††     | 801.7±168.8                           |
| Glycerol            |                   |                                       |
| Arterial concentrate (µmol/l) |       |                                       |
| Pregnant             | 92.7±22.9         | 144.0±22.8                            |
|                      | 89.0±48.3         |                                       |
| Early               | 61.3±20.2         | 95.2±33.0                             |
| Mid                 | 59.5±20.6         | 112.5±30.4                            |
| Late                | 54.0±22.0         | 107.2±27.7                            |
| A-V (µmol/l)         |                   |                                       |
| Pregnant             | -7.5±14.0         | -41.2±25.3                            |
|                      | -4.7±34.2         |                                       |
| Early               | 10.3±9.9          | -3.7±17.2                             |
| Mid                 | 11.0±15.4         | 9.2±4.8                               |
| Late                | 13.5±17.8         | 9.5±4.4                               |
| Extraction (%)       |                   |                                       |
| Pregnant             | -11±18            | -30±17                                |
|                      | -5±37             |                                       |
| Early               | 16±11             | 4±17                                   |
| Mid                 | 17±28             | 10±8                                  |
| Late                | 27±17             | 18±7                                  |
| Udder uptake (µmol/min) |       |                                       |
| Pregnant             | -16.5±28.6        | -40±32.6                              |
|                      | -9.1±27.1         |                                       |
| Early               | 50.1±40.8         | 7.6±54.5                              |
| Mid                 | 42.6±59.1         | 35.4±34.6                             |
| Late                | 50.1±34.0         | 74.6±35.3                             |

P-values by paired t-test. * p<0.05 with respect to the early period of lactation in each group. 
††† p<0.05, †† † p<0.01, † † † p<0.001 with respect to the period of pregnancy in each group. 
-ab Mean values within a row indicated with different superscripts are significantly different (p<0.05).

The ratio of β-hydroxybutyrate across the mammary gland markedly lowered (p<0.05) which coincided with a lower net uptake by the mammary gland in comparison to the early lactating period. The AV difference of free glycerol showed a release from mammary tissue during late pregnancy in all groups. In mid- and late lactation, the mammary uptake for β-hydroxybutyrate in both groups of 87.5% HF animals showed a decrease as compared to that in the early lactating period whereas no appearances of mammary uptake for free glycerol were observed in mid- and late lactation. The net mammary uptakes for β-hydroxybutyrate and glycerol in 50% HF animals fed either hay or urea treated rice straw remained constant throughout the course of lactation.

Arterial plasma concentration, AV difference and free fatty acid uptake of free fatty acid and triacylglycerol (Table 5)

The mean arterial plasma concentrations for free fatty acid and triacylglycerol (C16 to C18) were higher in late pregnancy compared with early lactation in 50%HF and 87.5% HF animals fed either hay or urea treated rice straw. The values of AV difference and the net uptake by the mammary gland for FFA were variable during the pregnant and lactating periods in all groups. During late pregnancy, the AV difference, the extraction ratio and net uptake of...
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 (mean±SD)

| Period of experiment | Arterial concentrate (µmol/l) | Udder uptake (µmol/min) |
|----------------------|-------------------------------|------------------------|
|                      | HF:RS (87.5:12.5)             | HF:RS (50:50)          | Urea treated rice straw+concentration (50:50) |
|                      | Pregnant                      | Early                  | Mid                      | Late                  |
|                      | 369.5±83.0a                   | 302.0±111.3b           | 260.7±191.7              | 182.5±62.4c           |
| Free fatty acid (C16-18) | 526.7±135.3bc                 | 314.4±115.8           | 375.1±191.3              | 350.4±129.3c          |
|                      | 393.7±90.3cd                 | 317.5±171.5           | 200.7±50.3               | 237.9±76.0d          |
|                      | 573.3±165.6e                 | 446.5±223.5           | 298.8±146.4              | 288.8±117.7e          |
| A-V (µmol/l)         | Pregnant                      | Early                  | Mid                      | Late                  |
|                      | -74.6±118.1                   | 90.4±204.5            | -121.4±198.8             | -5.3±56.9             |
|                      | -67.2±37.7d                  | -34.3±26.8            | 6.53±106.3               | -24.5±34.3            |
|                      | -20.7±39.5f                  | 23.6±77.4             | -45.7±39.5               | 15.7±56.7             |
| Extraction (%)       | Pregnant                      | Early                  | Mid                      | Late                  |
|                      | -25±36                       | 12±29                 | -14±14                   | 14±6                  |
|                      | -13±13                       | -16±24                | -39±23                   | 2±24                  |
|                      | -4±7                         | 1±13                  | -20±25                   |                      |
|                      | -19±8ab                      | 2±16                  | -39±23                   |                      |
| Udder uptake (µmol/min) | Pregnant                      | Early                  | Mid                      | Late                  |
|                      | -224.6±339.1                 | 56.5±134.7            | -124.9±81.9              | -174±40.6            |
|                      | -124.9±81.9                  | -158.4±179.4          | -79.0±114.9              | -143±118.1            |
|                      | -174±40.6                    | 61.5±235.2            | -143±118.1               |                      |
|                      | -176±187.9                   | 45.6±192.7            | -348±1222.7              | 61.2±175.8            |
| Triacylglycerol (C16-18) | Pregnant                      | Early                  | Mid                      | Late                  |
|                      | 229.3±145.6                  | 63.6±27.9f            | 108.6±64.1               | 82.4±34.4f           |
|                      | 180.6±41.4f                  | 69.6±15.1f            | 83.9±37.1                | 71.4±7.5b            |
|                      | 204.5±69.7f                  | 111.4±13.2f           | 80.5±16.0                | 134.7±13.1f          |
|                      | 195.6±118.4f                 | 96.7±20.0f            | 88.4±40.2                | 62.9±10.2f           |
| A-V (µmol/l)         | Pregnant                      | Early                  | Mid                      | Late                  |
|                      | 10.3±16.2                    | 52.7±26.9f            | 81.3±48.9                | 53.5±25.2f           |
|                      | 25.0±39.9                    | 36.8±20.3f            | 65.7±31.1                | 55.1±11.9f           |
|                      | 8.4±14.9                     | 68.8±16.0f            | 52.4±13.5                | 84.7±30.3f           |
|                      | -7.0±28.9                    | 71.7±15.8f            | 64.4±25.2                | 41.1±5.5f            |
| Extraction (%)       | Pregnant                      | Early                  | Mid                      | Late                  |
|                      | 3±3                          | 80±10f                | 73±9                     | 65±9                  |
|                      | 13±3                         | 50±24                 | 77±7                     | 75±11                 |
|                      | 4±6                          | 63±17f                | 65±9                     |                      |
|                      | -7±3                         |                      | 74±3                    |                      |
| Udder uptake (µmol/min) | Pregnant                      | Early                  | Mid                      | Late                  |
|                      | 28.9±47.3                    | 258.9±113f            | 298.9±203.0              | 186.8±64.6f          |
|                      | 29.0±22.8                    | 103.1±61.7f           | 179.6±81.3               | 150.2±46.0f          |
|                      | 40.7±40.5                    | 269.9±69.9f           | 147.1±54.7               | 245.4±120.8          |
|                      | -9.1±36.0                    | 236.3±47.8f           | 229.0±61.9               | 153.1±24.4           |

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.

Free fatty acid (C16-18) across the mammary gland markedly lowered in comparison to early lactating period (p<0.05). There were no significant differences for AV difference, extraction ratio and net uptake of triacylglycerol with lactation in both groups of 50%HF and 87.5%HF animals fed either hay or urea treated rice straw.

**DISCUSSION**

The mammary uptake of different substrates based on changes in AV difference and extraction ratio across the mammary gland of both 50%HF and 87.5%HF animals were varied during the transition period from pregnancy to lactation. During late pregnancy, the extent of glucose utilization by the mammary gland showed the low value of both AV difference and extraction ratio in comparison to lactating periods in both types of crossbred HF animals feeding either hay or urea treated rice straw. An increase in both AV difference and extraction ratio of glucose coincided with a marked increases in the rate of mammary blood flow around three folds during the transition period from pregnancy to lactation in all groups. This indicates that glucose utilization by the mammary gland during lactation would be dependent on both the mammary blood flow and the activity of the mammary epithelial cell. An increase in both mammary blood flow and efficiency of mammary extraction of glucose would be crucially important in providing glucose to the mammary epithelial cells for
biosynthesis to establish lactation but less for oxidation in this time. These results support the finding that the udder of ruminating animals account for 60-85% of glucose used for lactose synthesis (Annison and Linzell, 1964). However, in the late lactating period of both groups of 87.5%HF animals feeding on either hay or urea treated rice straw showed the reduction in the extraction efficiency and AV difference without change in arterial plasma concentration. Several mechanisms may be involved in controlling the utilization of glucose within the mammary gland, for example, reduction in the specific glucose transporter in the mammary epithelial cell (Burnol et al., 1990); or the decrease in biological signal especially the growth hormone level during late lactation for facilitating the transport of glucose into the mammary cell (Chaiyabutr et al., 2000b). However, these have not been fully investigated in crossbred animals. The significant decreases in the mammary blood flow and milk yield in late lactation of both 87.5%HF animals were also noted. Because of a short persistency of milk yield in 87.5%HF animals, then the question arises as to whether the rate of mammary metabolism for glucose influences mammary blood flow or mammary blood flow influences the rate of tissue metabolism of glucose in 87.5%HF animals during lactation advance, since 87.5%HF animals had a higher body glucose metabolism than 50%HF animals (Chaiyabutr et al., 2000a).

For 50%HF animals, feeding either hay or urea treated rice straw were sufficient to meet the animals’ requirements for glucose metabolism. A slight higher level in the plasma glucose concentration in lactating 50%HF animals would be an index for this adjustment.

No differences in the AV difference and extraction ratio of acetate across the mammary gland were apparent between late pregnancy and the lactating period in both types of crossbred animals feeding on either hay or urea treated rice straw. Because the efficiency of mammary extraction of acetate was unchanged, a portion of acetate taken up by the mammary gland either late pregnancy or lactation serves as a source of energy for mammary tissue via oxidation process (Jarrett and Potter, 1950). These results support the previous report that acetate rather than glucose is the main energy substrate for the mammary gland (Rook et al., 1965). However, during lactation, the mammary uptake of acetate for biosynthesis of short chain fatty acid in milk was mainly dependent upon the high rate of mammary blood flow in both types of crossbred animals. The other volatile fatty acid in the form of β-hydroxybutyrate arise mainly from butyrate in the rumen (Leng and West, 1969). A lower level of both AV difference and extraction ratio of β-hydroxybutyrate across the mammary gland without changes in the arterial plasma concentrations during late pregnancy of both types of crossbred HF animals were apparent. It indicates that the utilization of β-hydroxybutyrate by the mammary tissue was not obvious during late pregnancy in both crossbred HF animals feeding on either hay or urea treated rice straw. However, the greater energy requirement of the late pregnant animal resulting in increased hepatic ketogenesis for β-hydroxybutyrate production during greater mobilization of fat reserves has been noted (Schultz, 1974). An increase in the efficiency of the mammary gland for extraction and net uptake of β-hydroxybutyrate during lactation indicate that β-hydroxybutyrate is used as a precursor for biosynthesis of normal milk fat during this time.

In the present experiment, the high values of the arterial plasma concentration of FFA and triacylglycerol were apparent in the late pregnancy of both types of crossbred HF animals. This phenomenon has been proposed as an indication of under-nutrition (Reid and Hinks, 1962). However, previous studies by Chaiyabutr and coworkers (1997; 2000c) indicated that both 50%HF and 87.5%HF animals feeding on either urea treated rice straw or hay as roughages did not show any under-nutritional effects. Occurrence of body fat mobilization during late pregnancy should be regarded as a physiological phenomenon by altering the endocrine signals, not a consequence of under-nutrition in both 50%HF and 87.5%HF animals feeding on either hay or urea treated rice straw (Lindsay, 1973; Chaiyabutr et al., 2000b). During lactation, 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 (West et al., 1967). The net uptake of circulating triacylglycerol by the mammary gland significantly increased in the lactating period as to the late pregnancy in all groups. A higher enzymatic activity of lipoprotein lipase in the lactating mammary tissue would increase release of FFA relative to pregnant tissue (Shirley et al., 1973). However, in late pregnancy, the rate of glycerol release from mammary tissue provides an indication of the rate of lipolysis in the fat store (stroma) of the mammary gland. It may relate to a low glycerokinase activity in adipose tissue which could not metabolize free glycerol (Vaughan, 1961). In contrast to late pregnancy, there were a small net uptake of glycerol by the mammary gland paralleled by a little uptake of triacylglycerol during lactating period of both types of crossbred animals feeding either hay or urea treated rice straw. A possible explanation for this is partial hydrolysis of circulating triacylglycerol and utilization of glycerol occurring in the lactating mammary tissue which contains both high activities of lipoprotein lipase and enzyme of triglyceride esterification for supporting normal milk production.
In conclusion, the present results provide more information of the adaptations of the mammary gland of crossbred HF animals feeding on either hay or urea treated rice straw during late pregnancy and lactation which allow for an adequate nutrient supply. There is no difference in the mode of mammary uptake of substrates in the same crossbred animals in response to feeding hay or urea treated rice straw. The differences in utilizing nutrients by the mammary gland for milk production between 87.5%HF and 50%HF animals would be dependent on changes in both intra-mammary factors and extra-mammary factors.

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