Table 7. NADPH production from glucose in the udder at different stages of lactation of crossbred Holsteins during treatment with rbST.

Period of lactation	Control Group	rbST Group	Control vs rbST
Requirement of all NADPH for fatty acid synthesis (μmol/min)			
Pretreated	1317±295	1666±682	NS
Early	1677±616	2820±868	P<0.05
Mid	1838±525	2470±979	NS
Late	1725±542	2459±1024	NS
Requirement of all NADPH formation from glucose via the pentose phosphate pathway (%)			
Pretreated	30±7	24±5	NS
Early	39±8	34±19	NS
Mid	41±11	29±5	NS
Late	37±17	29±9	NS

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

P-values by unpaired t-test between control animals and rbST treated animals.

Table 8. Fatty acid composition of milk fat in the udder at different stages of lactation of crossbred Holsteins during rbST administration.

		Fatty acid chain length (μmol/ml milk)			
		Pretreatment	Early lactation	Mid lactation	Late lactaion
Control group	C6	0.86±0.47	1.69±2.02	1.13±0.49	1.27±0.76
	C8	0.63±0.24	0.80±0.28	0.95±0.39	0.94±0.35
	C10	1.25±0.28	1.57±0.56	1.82±0.96	1.79±0.66
	C12	1.19±0.36	1.44±0.43	1.87±0.89	1.76±0.57
	C14	3.98±1.31	4.68±0.84	6.15±2.04	5.94±1.44
	C16:0	16.83±1.81	20.74±7.85	20.37±2.09	23.73±7.28
	C16:1	1.00± 0.50	1.01±0.49	1.00±0.49	1.53±0.60
	C18:0	8.06±0.70	7.71±1.37	8.73±2.54	8.10±2.10
	C18:1	11.63±2.38	11.19±2.04	13.63±1.13	14.28±2.11
	C18:2	0.80±0.20	0.83±0.48	0.70±0.34	0.90±0.40
	Total	46.06±5.87	51.65±13.34	56.35±7.40	60.24±14.15
rbST treated	C6	0.98±0.10	1.56±0.48	1.40±0.69	1.48±0.50
	C8	0.59±0.24	1.03±0.31	1.01±0.23	1.03±0.36
	C10	1.31±0.58	2.10±0.63	2.05±0.45	2.16±1.00
	C12	1.44±0.71	2.04±0.61	2.11±0.61	2.40±1.05
	C14	5.17±2.38	7.43±2.14	7.65±1.85	8.78±4.12
	C16:0	20.49±7.54	26.88±2.75	24.01±12.41	25.86±6.10
	C16:1	1.06±0.60	1.02±0.32	1.20±0.51	1.60±0.60
	C18:0	7.53±1.82	9.40±0.70*	8.05±1.96	9.53±1.70
	C18:1	13.65±3.24	20.50±2.55***	18.49±4.49*	21.21±4.43**
	C18:2	0.81±0.30	1.01±0.37	1.44±0.43	1.10±0.66
	Total	52.81±15.84	73.01±8.22*	67.41±21.33	75.14±18.28

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

P-values by unpaired t-test between control animals and rbST treated animals.

Table 9. $^{3}H/^{14}C$ ratios in plasma glucose and related producted at different stages of lactation of crossbred Holstein cattle during treatment with rbST.

	Period of lactation	Control Group	rbST Group	Control vs rbST
Plasma glucose	Pretreated	0.92±0.11	0.92±0.06	NS
	Early	0.99±0.12	0.83±0.18	NS
	Mid	0.89±0.08	0.99±0.04	NS
	Late	0.91±0.18	0.88±0.19	NS
Milk lactose	Pretreated	0.85±0.03	0.89±0.21	NS
	Early	0.84±0.04	0.83±0.19	NS
	Mid	0.91±0.08	0.88±0.04	NS
	Late	0.89±0.04	0.79±0.12	NS
Milk triacylglycerol	Pretreated	3.17±1.72	3.15±1.24	NS
	Early	3.59±1.67	3.63±1.97	NS
	Mid	4.09±1.21	3.85±1.23	NS
	Late	3.58±0.98	2.81±0.97	NS
Milk citrate	Pretreated	0.42±0.07	0.59±0.16	NS
	Early	0.58±0.16	0.46±0.13	NS
	Mid	0.44±0.05	0.46±0.11	NS
	Late	0.42±0.11	0.36±0.06	NS

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

P-values by unpaired t-test between control animals and rbST treated animals.

RESULTS

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

The glucose turnover rate in both the controls and rbST-treated animals 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 stages of lactation for both groups are expressed as absolute values. In pretreatment period, there were no significant differences of the total glucose entry rate and glucose carbon recycling between the controls and rbST-treated animals. However, in early lactation, the utilization glucose turnover rate of rbST-treated animals was decreased as compared with the pretreatment period, whereas there was no change in the control animals. Comparing for the mid-lactating period, rbST-treated animals showed an elevation of plasma glucose clearance and significant increases in the glucose turnover rate ($P<0.05$) in comparison with control animals. 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 decreased during rbST administration in the early period of lactation. The percentages and values of non-mammary glucose utilization showed significantly increases during lactation advances to mid and late lactation in as compared with pretreated period in rbST-treated animals. During the course of lactation there were significant increases of body weight in both groups. Elevations of body weights were not different between groups at each period of lactation.

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

In animals treated with rbST, mammary plasma flow and milk yield initially showed significantly higher levels ($P<0.05$) in early lactation than that of control animals. The trends for persistency were observed as for udder plasma flow in rbST treated-animals throughout lactation. The values of milk lactose concentration showed no differences between groups of animals or among periods of lactation in the same group. In rbST treated-animals, the milk lactose secretion significantly increased in early lactation as

compared with pretreatment period. In rbST-treated animals, mean values of milk citrate concentration during early lactation were significantly decreased ($P<0.05$) as compared with pretreatment period. During lactation advances, the milk citrate concentration decreased in both groups. Milk triacylglycerol concentration and triacylglycerol secretion of rbST-treated animals were markedly higher in early lactation than that of pretreatment period and it was still in a high level throughout lactation.

Utilization of glucose carbon in the udder (Table 5)

A high milk lactose secretion and citrate secretion during early lactation were apparent in rbST treated-animals when compared to those of control animals. 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 controls and rbST-treated animals. The utilization of glucose carbon for synthesis of milk citrate for rbST-treated animals was significantly higher than that of control animals during mid and late lactation. The utilization of glucose for synthesis of milk triacylglycerol was significantly higher ($P<0.01$) during rbST administration throughout lactation. The ^{3}H from C-3 of glucose was recovered in milk fat. The major portion of this ^{3}H was associated with the fatty acid fraction of the saponified triacylglycerol. Less than 2% of radioactive carbon was present in triacylglycerol in both groups. The amount of ^{14}C-glucose incorporated to CO_2 in the venous blood of rbST-treated animals increased in mid and late lactation.

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 ^{3}H from [3-^{3}H]glucose into fatty acids and the flux through the pentose phosphate pathway was calculated to be increased as lactation advances in both groups. Correction of the lower $^{3}H/^{14}C$ ratio likely to be present in intracellular glucose 6-phosphate gave significant flux values of 164, 121 and 132 μmol/min for early, mid and late lactation of rbST-treated animals, respectively, in comparison with pretreatment period. 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 , as lactation advances, the intracellular glucose phosphorylated by the mammary gland were calculated to be completely metabolized via the pentose phosphate pathway in terms of absolute values and the percentages were higher when compared with pretreatment period of both groups. The percentages of metabolism of glucose 6-phosphate to the galactose moiety of lactose were slightly higher during early lactation in rbST treated-animals when compared to control animals and during lactation advance, these values decreased in both groups. Metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway was calculated in term of the proportion of glucose metabolized, which there was considerable variation with advanced lactation of both groups.

NADPH production from glucose (Table 7)

It can be calculated from the milk fat compositions and output in the present experiment that the requirements for NADPH for fatty acid synthesis increased during administration of rbST in different stages of lactation. During early lactation, the NADPH formation from glucose accounted for 2820 μmol/min of that required for fatty acid synthesis *de novo* in the mammary gland of rbST treated-animals, which was sinificantly higher than that of the value of 1677 μmol/min for the control animals.

Milk fatty acid concentrations (Table 8)

During pretreatment period, the milk fatty acid concentrations with a chain length of C_6 to C_{18} for both groups of animals were not different. During rbST administration in differnt stages lactation, the milk fatty acid concentration, particularly with a chain length of C_{16} to C_{18}, significantly increased as compared with those of control 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 between the control and rbSt treated-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, whereas the $^{3}H/^{14}C$ ratio of milk triacylglycerol was slightly higher in both groups. 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 low in both groups as lactation advances.

DISCUSSION

The supply of glucose is a principal determinant of the milk yield, since glucose requirement is used for lactose production. The administration of rbST elicited a marked increase in the milk production of crossbred dairy cattle in the present study. The absolute milk yield response to rbST administration started before the peak of lactation in early lactation and it was significantly higher than those of the control animals throughout periods of lactation. Elevated responses did not maintain for the duration of the treatment period in rbST treated animals. These results confirm the finding that an increase in milk yield in response to rbST administration will not be sustained indefinitely (Bauman, 1992), and that it is influenced by the stage of lactation (Phipps et al.1991). The low potential for extended persistency of lactation in rbST treated animals appears similar to that which occurs in higher yielding cows (Chase, 1993). However, it has been reported that the whole lactational response to somatotropin might be reduced if treatment begins very early in lactation (Bauman and Vernon, 1993; Burton et al., 1994). A marked increase in milk yield without an alteration in lactose content during early lactation in rbST treated animals indicates that this requires a substantial increase in supply of glucose to the mammary gland (Bauman and McCutcheon, 1986). Glucose is essential for milk secretion and glucose moiety of lactose arises directly from plasma glucose (Ebner & Schanbacher, 1974). In the present results, the milk secretion of animals in both controls and rbST treated animals was not dependent on the blood glucose level, since the plasma glucose concentrations remained constant over a wide range at different stages of lactation. The marked increase in the udder blood flow of rbST treated animals in the present results will support the previous conclusion from a

study in cows or goats by Linzell (1973) that glucose uptake is determined mainly by mammary blood flow.

Gluconeogenesis in ruminants has been known to be the main source of glucose production (Lindsay, 1970). In the present studies, animals were maintained on a similar concentrate intake. Relatively constant plasma glucose concentrations in both groups 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 present experiment showed that rbST treatment did not significantly affect the reversible turnover of [3-^{3}H]glucose throughout stages of lactation, while the irreversible turnover of [U-^{14}C]glucose was reduced during the early lactation but not for mid-and late lactation. Our previous experiments showed that the insulin level increased during rbST treatment in different stages of lactation in crossbred HF animals (Chaiyabutr et al., 2005). It indicates that rbST administration during early lactation antagonizes whole body turnover of glucose stimulated by insulin. Growth hormone is thought to be antagonistic to the action of insulin in tissues that are sensitive to insulin (Rose and Obara,1995), preventing the uptake of glucose by pheripheral tissue and thus sparing glucose to mammary gland , which are insensitive to insulin (McGuire et al., 1995). It also noted that glucose clearance which stimulated by insulin was also reduced during early lactation. This speculative sparing glucose utilization in tissues sensitive to insulin would partially allow for increase in lactose synthesis and milk yield. As lactation advances, the irreversible turnover of [U-^{14}C]glucose of rbST treated animals was increased in mid lactation, which was significantly higher than that of control animals. 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. There were no differences for an increased recycling of glucose-C between the controls and rbST treated animals during advanced lactation suggests that a constant level of tricarbon units originally derived from glucose being again reincorporated into glucose was not affected by rbST treatment .

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). In general an increasee in milk yield can be attributed to an increase in the rate of lactose synthesis (Linzell & Peaker, 1971). However, an increase in the lactose

yield during rbST administration was not related to the lactose concentration in milk, which largely unchanged. These results can be attributed to a difference in the activity of the mammary epithelial cells between controls and rbST treated animals. The synthesis of lactose involves a combination of glucose and UDP-galactose. The UDP-galactose originates from glucose 6-phosphate (Ebner and Schanbacher,1974). An administration rbST showed increases in both milk yield and glucose uptake by mammary gland, which were accompanied by an increase in milk glucose secretion (Chaiyabutr et al., unpublished data, Charpter VIII). These results would coincide with the calculated of metabolism of glucose 6-phosphate to the galactose moiety of lactose in rbST treated animals which was higher than that of control animals in early lactation. The availability of cytosolic glucose 6-phosphate in the cells of rbST treated animals in early lactation would be sufficient to account for the cytosolic lactose synthesis. Decreases in the metabolism of glucose 6-phosphate to the galactose moiety of lactose as lactation advances in both groups would affect the lactose synthesis and milk production.

A low enzymatic activity for lactose synthesis might be expected to appear as lactation advances in the crossbred animal. 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. 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 cell to produce lactose, citrate and triacylglycerol for milk secretion. The data obtained for the utilization of glucose carbon for the synthesis of lactose, triacylglycerol and citrate during mid and late lactation were higher in rbST treated animals as comparison with the control animals. The differences in these results between the controls and rbST treated animals without a reduction in feed intake may be explained by the difference of nutrient partition or utilization in the mammary gland. 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 9-11% throughout lactation in control animals while it increasd from 7 % in pretreatment to 18 % in early lactation after rbST administration. 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 different stages of lactation in rbST treated animals was higher than those of control animals. 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 24% to 34% of the NADPH was required during early lactation for fatty acid synthesis *de novo* from glucose metabolism in the udder of rbST treated animals, while 30% to 39 % was required in the control animals. 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 it 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 rbST treated animals was metabolized via the pentose phosphate pathway which was higher than those of control animals. In rbST treated animals, a high proportion of the glucose taken up by the udder which was oxidized in the tricarboxylic acid cycle would be apparent in mid- and late lactations. High values of both the proportion and absolute amount of glucose carbon incorporation to milk citrate and milk triacylglycerol of rbST treated animals during mid- and late lactation are evidences supporting an increased proportion of glucose 6-phosphate metabolized via the Embden-Meyerhof 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. During advanced lactation, whether an increased disequilibrium of the triose phosphate isomerase reaction occurs in the udder of rbST treated animals as compared with the control animals and causes a higher level of $^{3}H/^{14}C$ ratio in milk triacyglycerol needs to be further investigated. The high metabolism of glucose 6-phosphate in early lactation of rbST treated animals appeared to be due primarily to a high flux through the lactose synthesis and to pentose phosphate pathway, probably reflecting the high milk production during rbST treatment. Tritium and carbon-14 were also shown to be incorporated into milk citrate which provided 17 μmol/min in rbST-treated animals and 11 μmol/min in control animals for the carbon skeleton of citrate in the early lactating period as compared with pretreatment 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. 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 control animals and rbST-treated animals may have different mechanisms with a common pool of cytosolic NADPH. A significant increase in the concentration of FFA in milk was apparent in rbST-treated animals as compared with the control animals in early lactation. A similar result for an increase in milk fat content due to rbST injection has also been observed previously (West et al., 1991). It has

been known that milk fat is synthesized in the mammary epithelial cells. The fatty acids used to synthesize the milk fat arise from both blood lipids and from de novo synthesis within the mammary epithelial cells. However, an increase in milk fat after rbST injection was associated with the increased yield of long-chain fatty acids characteristic of plasma free fatty acids and body fat. Thus, the lipolytic activity would be a function of bST treatment per se in stead of the associated changes in energy balance.

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 during rbST administration in 87.5% HF animals. Of the glucose taken up by the udder of rbST treated animals during early lactation, an average 18% and 21% were metabolized in the pentose phosphate pathway and contributed to NADPH production, respectively. The sufficient pool of intracellular glucose concentration during rbST administration, has effect on an increase in glucose 6-phosphate which increased flux through the lactose synthesis and pentose cycle pathway. Although we know a great deal of differences that occur between the control animals and rbST treated animals, we do not know the different enzymatic activities during rbST administration in different stages of lactation which affect the rate of metabolic pathways. There is still a need for more information, for example,on whether the high enzymatic activity of fructose 1-6 diphosphatase or the lower enzymatic activity of pyruvate dehydrogenase occurs in rbST treated animals throughout the stages of lactation or occurs during early lactation which causes an increase in the metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway and tricarboxylic acid cycle.

Acknowledgements

This work was supported by the Thai Research Fund, Grant No. BRG 2/02/2545.

REFERENCES

Annison, E.F. and J.L. Linzell. 1964. The oxidation and utilization of glucose and acetate by the mammary gland of the goat in relation to their over all metabolism and milk formation. J. Physiol. 175 : 372-385.

Bauman, D.E. 1992. Bovine somatotropin: review of an emerging animal technology. J. Dairy Sci. 75:3432-3451.

Bauman, D.E. and R.G. Vernon. 1993. Effects of bovine somatotropin on lactation. Ann. Rev. Nutr. 13:437-461.

Bauman, D.E., C.J.Peel, W.D. Steinhour, P.J. Reynolds, H.F. Tyrell, A.C.G. Brown, and G.L.Haaland.1988. Effect of bovine somatotropin on metabolism of lactating dairy cows: Influence on rates of irreversible loss and oxidation of glucose and nonesterified fatty acids. J. Nutr. 118:1031-1040.

Bauman, D.E. and C.L. Davis. 1975. Regulation of lipid metabolism. In I.W. McDonald and A.C.I. Warner (eds.) Digestion and Metabolism in the Ruminant. The University of New England Publishing Unit, Armidale, N.S.W. pp. 496-509.

Breier,B. H., P. D. Gluckman, S.N. McCutcheon, and S.R. Davis. 1991. Physiological responses to somatotropin in the ruminant. J. Dairy Sci. 74:20.

Bickerstaffe, R., E.F. Annison and J.L. Linzell. 1974. The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. J. Agric. Sci. 82 : 71-85.

Burton, J. L., B. W. McBride, E. Block, D. R. Glimm, and J.J. Kennelly. 1994. A review of bovine growth hormone. Can.J.Anim.Sci. 74:167.

Chaiyabutr, N. and C. Buranakarl. 1989. Effects of exogenous urea infusion on glucose metabolism in acute heat stressed swamp buffaloes (Bubalus Bubalis). Br. Vet. J. 145 : 538-545.

Chaiyabutr, N., A. Faulkner and M. Peaker. 1980. The Utilization of glucose for the synthesis of milk components in the fed and starved lactating goat *in vivo*. Biochem. J. 186 : 301-308.

Chaiyabutr, N., S. Komolvanich, S. Sawangkoon, S. Preuksagorn, and S. Chanpongsang. 1997. The regulation of body fluids and mammary circulation during late pregnancy and early lactation of crossbred Holstein cattle feedingg on different types of roughage. J. Anim. Physiol. and Anim. Nutri. 77: 167-179.

Chaiyabutr N, Komolvanich S, Preuksagorn S & Chanpongsang S (2000a) Plasma levels of hormones and metabolites as affected by the forages type in two different types of crossbred Holstein cattle. Asian-Aust J Anim Sci 13(10), 1359-1366.

Chaiyabutr N, Preuksagorn S, Komolvanich S & Chanpongsang S (2000b) Comparative study on the regulation of body fluids and mammary circulation at different states

of lactation in crossbred Holstein cattle feeding on different types of roughage. J Anim Physiol and Anim Nutri 83, 74-84.

Chaiyabutr, N., S. Komolvanich, S. Sawangkoon, S. Preuksagorn and S. Chanpongsang. 1998. Glucose metabolism in vivo in crossbred Holstein cattle feedingg on different types of roughage during late pregnancy and early lactation. Com. Biochem. and Physiol. Part A. 119: 905-913.

Chase, L.E. 1993. Developing nutrition programs for high producing dairy herds. J. Dairy Sci. 76:3287-3293.

Christopherson, S.W. and R.L. Glass. 1969. Cardiovascular methyl esters by alcoholysis in an essentially non alcoholic solution. J. Dairy Sci. 52 : 1289-1290.

Davis, C.L. and D.E. Bauman. 1974. General metabolism associated with the synthesis of milk. In B.L. Larson and V.R. Smith (eds.) Lactation Vol.II. New York and London : Academic Press, pp.3-30.

Ebner, K.E. and F.L. Schanbacher. 1974. Biochemistry of lactose and related carbohydrates. In B.L. Larson and V.R. Smith (eds.) Lactation Vol.II. New York and London : Academic Press, pp.77-113.

Hardwick, D.C. 1965. The incorporation of carbondioxide into milk citrate in the isolated perfused goat udder. Biochem. J. 95 : 233-237.

Hardwick, D.C., J.L. Linzell and T.M. Mepham. 1963. The metabolism of acetate and glucose by the isolated perfused udder. 2.The contribution of acetate and glucose to carbon dioxide and milk constituents. Biochem. J. 88 : 213-220.

Katz, J., B.R. Landau and G.E. Bartsch. 1966. The pentose cycle, triosephosphate isomerization, and lipogenesis in rat adipose tissue. J. Biol. Chem. 241 : 727-740.

Katz, J. and R. Rognstad. 1976. Futile cycles in the metabolism of glucose. Curr. Top. Cell. Regul. 10 : 237-289.

Katz, J., R. Rognstad and R.G. Kemp. 1965. Isotope discrimination effects in the metabolism of tritiated glucose. J. Biol. Chem. 240 : 1484-1486.

Katz, J. and P.A. Wals. 1970. Effect of pheazine methosulfate on lipogenesis. J. Biol. Chem. 245 : 2546-2548.

Katz, J. and P.A. Wals. 1972. Pentose cycle and reducing equivalents in rat mammary gland slices. Biochem.J. 128 : 879-899.

Katz, J., P.A. Wals and R.L. Van De Velde. 1974. Lipogenesis by Acini from mammary gland of lactating rats. J. Biol.Chem. 249 : 7348-7357.

Katz, J. and H.G. Wood. 1963. The use of $C^{14}O_2$ yields from glucose-1-and 6-C^{14} for the evaluation of the pathways of glucose metabolism. J. Biol. Chem. 238 : 517-523.

Kuhn, N.J., D.T. Carrick and C.J. Wilde. 1980. Milk synthesis. J. Dairy Sci. 63 : 328-336.

Lindsay, D.B. 1970. In A.T. Phillipson (ed.) Physiology of digestion and metaboliam in the ruminant. Oriel Press. Newcastle upon Tyne, pp. 438-451.

Linzell, J.L. 1973. The demands of the udder and adaptation to lactation.. In J.M. Payne, K.G. Hibbitt and B.F. Sansom (eds.) Production disease in farm animals. London : Bailliere, Tindal, pp. 89-106.

Linzell, J.L. and M. Peaker. 1971. Mechanisms of milk secretion. Physiol. Rev. 51 : 564-597.

McGuire, M.A., J. M. Griinari, D.A. Dwyer and D. E. Bauman.1995. Role of insulin in the regulation of mammary synthesis of fat and protein. J. Dairy Sci. 78:816.

Phipps, R., C. Madakadze, T. Mutsvangwa, D.L. Hard, and G.D. Kerchove. 1991. Use of bovine somatotropin in the tropics: the effect of sometribove on milk production of *Bos indicus,* dairy crossbred and *Bos Taurus* cows in Zimbabwe. J. Agri. Sci., Cambridge. 117:257-263.

Rose, M. T. and Y. Obara. 1995. Effect of growth hormone on the response to insulin and glucose turnover in sheep. J. Agric. Sci. 126: 107.

Teles, F.F.F., C.K. Young and J.W. Stull. 1978. A method for rapid determination of lactose. J.Dairy Sci. 61 : 506-508.

Thompson, G.E. and E.M. Thomson. 1977. Effect of cold exposure on mammary circulation, oxygen consumption and milk secretion in the goat. J. Physiol. 272 : 187-196.

Thomson, E.M., A.M. Snoswell, P.I. Clarke and G.E. Thompson. 1979. Effect of cold exposure on mammary gland uptake of fat precursors and secretion of milk fat and carnitine in the goat. Q.J. Exp. Physiol. 64 : 7-16.

West, J.W., B.G. Mullinix, and T.G. Sandifer. 1991. Effects of bovine somatotropin

on physiologic responses of lactating Holstein and Jersey cows during hot, humid weather. J. Dairy Sci. 74(3):840-851.

White, J.C.D. and D.T. Davis. 1963. The determination of citric, acid in milk and milk sera. J. Dairy Res. 30 : 171-189.

Wood, H.G., G.J. Peeters, R. Verbeke, M. Lauryssens and B. Jacobson. 1965. Estimation of the pentose cycle in the perfused cow's udder. Biochem. J. 96 : 607-615.

CHAPTER X

General discussion

Chapter X

General Discussion

The present study was designed to clarify whether a shorter lactation persistency of crossbred cattle containing 87.5%Holstein genes during lactation advance was due to the reduction of the growth hormone level (Chaiyabutr et al., 2000b) or associated with some other mechanisms. The results presented in this report indicate that administration of rbST affects bodily functions both intra-mammary and extra-mammary functions. The rbST-treated animals increased body fluid compartments throughout all periods of study i.e. TBW, ECW and blood volume, while the control animals decreased TBW in comparison to pretreatment values in the early period of lactation(Charpter III and IV). The treatment of rbST being initiated at the earlier stage of lactation exerts it effect on an increase in empty body water (EBW). An increase in the EBW in rbST-treated animals would be due to an increase in ECF compartment, while ICF compartment did not change through the period of study. Increased ECF in rbST-treated animals might be partly resulted from the decrease in fat mass during early lactation. These results are agreed with the report in human that an expansion of both ECW and TBW was apparent after growth hormone administration in growth hormone deficient patients (Janssen et al., 1997). There are a number of possible explanations for this apparent finding. An increase in TBW and ECW would be influenced by an increase in voluntary intake (MacFarlane et al., 1959), which has been reported to occur after a few weeks of rbST administration (Coghlan et al., 1977). A greater percentage increase in live weight of rbST-treated animals could be considered, at least in part, to be the direct effect of somatotropin on the increased body cell mass and fat free mass. This would be attributable to an accumulation of body water. The sodium retention effect of somatotropin on the renal tubular reabsorption of sodium (Wyse et al.,1993), would be another explanation for water retention in the ECW compartment.

The higher TBW and ECW of animals receiving rbST would not only provide a higher reservoir of soluble metabolites for biosynthesis of milk but also slow down any elevation of

body temperature during lactation in hot conditions. An increase in both metabolic activity and heat production has been reported in bST-treated cows (West et al., 1991). It was suggested that even though bST increases heat production, it also increases heat dissipation (Johnson et al., 1991, West et al., 1994). However, the rbST-treated animals showed no significant changes in the water turnover rate per fat free, wet, body weight ($kg^{0.82}$) and the biological half-life of tritiated water, in any periods measured in the experiment, in comparison to the control animals. This indicates that water loss with the increase in milk yield of the rbST-treated animals might be compensated by a larger body water pool, which restores their body fluids to equilibrium, with no significant changes of body water turnover rate and water half-life. In contrast to the rbST-treated animals, the biological half-life of tritiated water in the control animals was significantly shorter, while the water turnover rate was significantly higher as lactation advanced to mid and late lactation. These changes would be due to the process of lactation requiring more water and more loss of water secretion in milk, which is generally known to be about 87% and would account for these phenomena. The control animals being 87.5%HF were genetically similar to the exotic *bos taurus* breed which might lead to poor adjustment in a tropical environment (Chaiyabutr et al., 2000a; Nakamura et al., 1993). The TBW and ICW of the control animals showed to be decreased during advanced lactation; it should be assumed that these changes are the factors influencing lactation persistency. Animals could not maintain their body fluids which resulted in the rapid approach of the end of their normal short lactation.

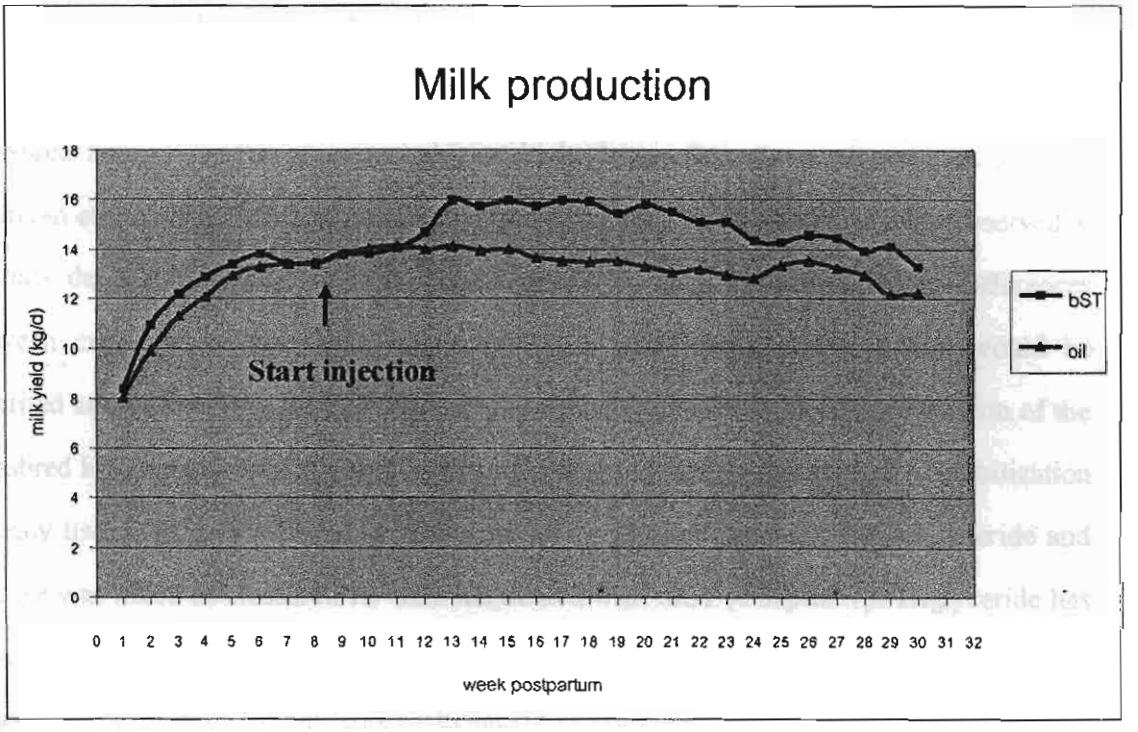
In the present study, increases in mammary blood flow to the udder of rbST-treated animals agree with several reports in both cows and goat (Davis et al., 1988; Gulay et al., 2004; Mepham et al; 1984). A marked increase in mammary blood flow of rbST-treated animals could not be attributed to a change in blood volume and plasma volume, which remained nearly constant as a percent of body weight. In lactating dairy cows, increase in blood flow to the mammary gland may allow plasma volume to remain nearly constant despite loss of body weight (Woodford et al., 1984). Several investigations show the effect

of rbST on mammary circulation was indirect, mediated via IGF-I (Capuco et al., 2001), whereas other works have demonstrated the direct effect of IGF-I on an increase in the mammary blood flow and increase in milk production (Etherton and Bauman, 1998). An elevation of both plasma IGF-I concentration and udder blood flow was also noted in late lactating crossbred cows treated with rbST (Tanwattana et al., 2003). The present study confirms that mammary blood flow is a major determining factor for supply of nutrients for milk synthesis and follows the pattern of changes of milk yield.

The treatment of rbST was initiated at the earlier stage of lactation, milk yield increased in early lactation (+19.8 %) and in mid-lactation (+9.5%), but it decreased by 2.7% during late lactation in comparison with the pretreatment period (Figure 1). Low responses in milk yield during rbST treatment in the later stage of lactation are similar as previously reported in dairy crossbred cattle (Phipps et al., 1991). A rapid decline of yield resulting the shorter persistency of lactation of rbST-treated animals seems to be similar to those which occur in higher yielding cows (Chase, 1993). These results indicated that an increase in milk yield of dairy crossbred cattle, in response to rbST administration, will not be sustained for long, being influenced by stage of lactation.

The mechanism by which rbST directly or indirectly affects mammary gland function likely involves the DMI. The ratio of DMI to milk yield of rbST-treated animals, was lower in early lactation when compared with the pretreatment period but animals still gained weight throughout the experiment in both groups. It has been known that the support of milk secretion would come through provision of substrate and stimulation of mammary cell activity. Unfortunately, the present studies on the mammary cell activity were not available. The rbST increased milk yield relating to mammary cell activity appears contradictory. Whereas some studies show no mammogenic effect of bST (Binelli et al., 1995), other studies show a possible mammogenic effect when cattle are administered bST (Knight et al., 1992). It indicates that the increased milk yield with rbST treatment in the present study is

Figure 1. Milk yield in the controls and rbST-treated animals during prolonged treatment with placebo or rbST, respectively.



rather dependent upon the adequacy of the nutritional provision than the mobilization of body stores. A marked increase in milk yield with rbST treatment without loss of body weight, especially during early lactation, may be due to the fact that the animals were well fed to allow an adequate replacement of body reserves. Milk yield in the first lactation of crossbred animals in the present study would be lesser than those of multiparous cows (Sullivan et al. 1992), which is possibly related to the continued weight increase observed in animals during their first lactation. These results provide the physiological differences between crossbred animals and exotic breeds in partitioning ability, which would be inherited and capacity for milk production. Thus, the metabolic demands of lactation of the crossbred HF animals would be met by dietary intake during early lactation. No mobilization of body tissues as indicated by no alteration of the plasma levels of both triglyceride and glucose was noted in crossbred HF animals treated with rbST (Chapter V). Triglyceride has been known to restore during period of excess energy availability and are mobilized during periods of energy deprivation. No significant change in the plasma triglyceride concentration supports the interpretation that the extra energy to support increased milk yield arose from surplus nutrient of DMI rather than from greater mobilization of body reserves. In the present study, milk fat content of rbST-treated animals was increased, while milk protein and milk lactose were not changed by rbST treatment. Peel and Bauman (1987) reported that administration of rbST did not change milk protein percentage when cows were in positive nitrogen balance, but the milk protein percentage of cows in negative nitrogen balance tended to decline. A significant increase in the concentration of FFAin milk was apparent in rbST-treated animals as compared with the control animals in early lactation. A similar result for an increase in milk fat content due to rbST injection has also been observed previously (West et al., 1990). It has been known that milk fat is synthesized in the mammary epithelial cells. The fatty acids used to synthesize the milk fat arise from both blood lipids and from de novo synthesis within the mammary epithelial cells. Milk fat content of cows in positive energy balance is not influenced by rbST treatment, and milk fat yield follows the trend of milk production (West et al., 1990). However, an increase in milk

fat after rbST injection was associated with the increased yield of long-chain fatty acids characteristic of plasma free fatty acids and body fat (Chapter VII and IX). Thus, the lipolytic activity would be a function of bST treatment per se in stead of the associated changes in energy balance.

During early lactation, an elevation of body fluid particularly blood volume (+15 %) despised large increases in mammary blood flow (+50 %) during rbST treatment. These observations could suggest that a marked increase in blood flow through the mammary glands resulting from rbST administration would be achieved in part by local vasodilatation (Linzell, 1974), causing in distribution of milk precursors to the gland. An increase in MBF has been shown to be the effect of an increase in cardiac output perfusing to the udder without any alteration in heart rate during growth hormone treatment (Davis et al., 1988). In the present results, an increase in both blood volume and plasma volume in rbST-treated animals would provide a greater venous return and stroke volume for increase in cardiac output, resulting in increased the blood supply to the mammary gland. Thus, the rate at which the milk yield elevated after the peak period when compared with the controls, could have been due primarily to an increased availability of substrates for the mammary gland. The progressive decline in milk yield of rbST-treated animals with still a higher level of either MBF or ECW, could be accounted for by changes in intra-mammary factors. Since it has been reported that the effect of somatotropin on MBF occurs by a mechanism which did not involve the direct action of somatotropin on the udder (Collier et al., 1984). In addition, study in vitro suggests that bST does not directly stimulate mammary secretory function (Gertler et al., 1983). The indirect action of rbST on mammary function may occur through some other agent e.g. insulin like growth factor-I, as administration of rbST in late, lactating, crossbred cows elevated milk yield, which coincided with increased plasma IGF-I concentration and udder blood flow (Tanwattana et al., 2003).

The experiment in Chapter V showed an increase in milk yields and circulating levels of IGF-I throughout lactation in animals treated with rbST. These findings were similar to those of previous studies on lactating cows showing that the injection of somatotropin, elevated

plasma IGF-I concentrations (Davis et al.1987; Tunwattana et al. 2003). Somatotropin increased milk yield by a mechanism which did not involve the direct action on the mammary gland (Collier et al.1984). The indirect effects of somatotropin on milk production are thought to be mediated either via IGF-I or nutrient partitioning effects (Bauman,1992). In the present study, during long-term administrations of rbST, milk yield rose to a peak in early lactation and then gradually declined over 32 weeks of the experiment, whilst the plasma concentration of IGF-I and the mammary blood flow did not decrease in the rbST treated animals. These findings suggest that the stimulatory effect of recombinant bovine somatotropin on milk production is not mediated solely by IGF-I. Changes in milk production during the progress of lactation in rbST treated animals might not be controlled systemically but also locally within the mammary gland. There are a number of possible explanations for this apparent finding. It probably involves greater synthesis of plasma IGF-I binding proteins as lactation advances which combines with IGF-I in the blood and so modulates the level of free IGF-I before it reached the mammary gland. It has been reported that approximately 95% of the infused IGF-I is bound by IGF binding proteins (Davis et al. 1989). Mammary tissue is itself capable of synthesizing an IGF-binding protein (e.g.IGFBP-5) during mammary gland involution in late lactation and this could inhibit IGF-mediated cell survival (Tonner et al. 1997; Flint & Knight, 1997) and initiate involution and a decrease in milk yield.

The expriment in Chapter VI for the plasminogen and plasmin activities indicate that the plasminogen-plasmin system involved in the tissue remodeling associated with the declining phase of lactation and mammary gland involution. Milk plasminogen concentrations are important in determining milk production by affecting the state of involution within the mammary gland. Increasing plasmin concentration in milk as lactation advances has been reported previously by Politis et al.(1989). Long-term administration of bST in dairy cows has been shown to prevent an increase in milk plasmin activity during late lactation, suggesting that bST acts to delay mammary gland involution (Politis et al.,1990). However, in the present results, the effect of rbST on prevention of an increase in milk plasmin

activities was not apparent. A different pattern of this enzymatic system in crossbred dairy cattle would be suspected. In both the controls and rbST-treated animals showed gradual increase in milk plasmin concentrations as lactation advances. Milk plasmin is known to be influenced by the availability of plasminogen and the plasminogen activators. As plasminogen is ubiquitous in the body, thus, the plasminogen concentration in milk in animals treated with rbST would not be expected to be limiting in the present study. Milk plasminogen concentrations were not significantly different between rbST treated animals and control animals given placebo as lactation advances which was similar to that of findings in cows by Politis et al., (1990). However, the plasminogen: plasmin ratio fell in the control animals while it increased in rbST-treated animals as lactation advances. The plasminogen : plasmin ratio is a useful index of plasminogen activation. This measurement is independent of milk volume. It indicates that massive activation of plasminogen and production of plasmin occured in the control animals than rbST-treated animals. Therefore, it do not exclude the possibility that rbST is involved in maintenance of the tissue function in the present results.

The effect of long-term rbST administration for the fate of nutrients uptake by the mammary gland is mentioned (Chapter VII). The supply of glucose is a principal determinant of the milk yield, since glucose requirement is used for lactose production. A marked increase in milk yield without an alteration in lactose content during early lactation in rbST treated animals indicates that this requires a substantial increase in supply of glucose to the mammary gland (Bauman and McCutcheon, 1986). Glucose is essential for milk secretion and glucose moiety of lactose arises directly from plasma glucose (Ebner & Schanbacher, 1974). In the present study, the milk secretion of animals in both groups was not dependent on the blood glucose level, since the plasma glucose concentrations remained constant over a wide range at different stages of lactation. The marked increase in the udder blood flow of rbST treated animals in the present results will support the previous conclusion from a study in cows or goats by Linzell (1973) that glucose uptake is determined mainly by mammary blood flow.

The remainder of the discussion is concerned with metabolic fate of nutrient particularly glucose metabolism, the biosynthetic pathway for lactose synthesis (Chapter VIII), the utilization of glucose in the whole body related to the utilization in the mammary gland in both control animals and rbST-treated animals (Chapter IX).

. It is clear that changes in milk yield during rbST administration were in part accounted for changes in intra-mammary factors (ChapterVIII). An increase in milk yield during bST administration is thought to be determined primarily by lactose secretion (Linzell and Peaker, 1971). Lactose is synthesized in the mammary secretory cell from glucose derived from the blood. The concentration of milk glucose significantly increased which coincided with an increase in milk yield during rbST administration in both early and mid-lactation (Chapter VIII). This would reflect to the intracellular glucose concentration (Kuhn and White,1975; Faulkner et al,1981), since glucose freely permeates across Golgi vesicles and apical membranes of the mammary secretory cells (Faulkner & Peaker, 1987). Mammary cell cannot synthesize free glucose because they lack glucose-6-phosphatase activity (Threadgold & Kuhn, 1979). It is likely that the high concentrations of milk glucose in rbST-treated animals are related to a high rate of glucose uptake by the mammary gland, consistent with the higher mammary blood flow to the mammary gland during rbST administration (Chaiyabutr et al., 2005). During early lactation, a large portion of the conversion of intracellular glucose to intermediary metabolites of rbST-treated animals, was mainly used in the lactose biosynthetic pathway, when compared with controls. Our results in Chapter VIII clearly indicate that rbST administration exerts its galactopoietic action, in part, through both intra-mammary and extra-mammary effects.

The experiment in Chapter IX showed that rbST treatment did not significantly affect the reversible turnover of [3-^{3}H]glucose throughout stages of lactation, while the irreversible turnover of [U-^{14}C]glucose was reduced during the early lactation but not for mid-and late lactation. Experiments in Chapter V showed that the insulin level increased during rbST treatment in different stages of lactation in crossbred HF animals (Chaiyabutr et al., 2005). It indicates that rbST administration during early lactation antagonizes whole body turnover of glucose stimulated by insulin. Growth hormone is thought to be antagonistic to the action of insulin in tissues that are sensitive to insulin (Rose and Obara,1995), preventing the uptake of glucose by peripheral tissue and thus sparing glucose to mammary gland , which are insensitive to insulin (McGuire et al., 1995). It also noted that glucose clearance which

stimulated by insulin was also reduced during early lactation. This speculative sparing glucose utilization in tissues sensitive to insulin would partially allow for increase in lactose synthesis and milk yield. As lactation advances, the irreversible turnover of [U-^{14}C]glucose of rbST treated animals was increased in mid lactation, which was significantly higher than that of control animals. 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). There were no differences for an increased recycling of glucose-C between the controls and rbST treated animals during advanced lactation suggests that a constant level of tricarbon units originally derived from glucose being again reincorporated into glucose was not affected by rbST treatment .

In general an increasee in milk yield can be attributed to an increase in the rate of lactose synthesis (Linzell & Peaker, 1971). However, an increase in the lactose yield during rbST administration was not related to the lactose concentration in milk, which largely unchanged. These results can be attributed to a difference in the activity of the mammary epithelial cells between controls and rbST treated animals. The synthesis of lactose involves a combination of glucose and UDP-galactose. The UDP-galactose originates from glucose 6-phosphate(Ebner and Schanbacher,1974). In contrast to the control animals, an administration rbST showed increases in both milk yield and glucose uptake by mammary gland, which were accompanied by increases in the secretion of both milk glucose and milk glucose 6-phosphate (Chapter VIII). These results would coincide with the calculated of metabolism of glucose 6-phosphate to the galactose moiety of lactose in rbST treated animals which was higher than that of control animals in early lactation. The availability of cytosolic glucose 6-phosphate in the cells of rbST-treated animals in early lactation would be sufficient to account for the cytosolic lactose synthesis. Decreases in the metabolism of glucose 6-phosphate to the galactose moiety of lactose in mid and late lactation in both groups (Chapter VIII and IX), would affect the lactose synthesis and milk production. A low enzymatic activity for lactose synthesis might be expected to appear as lactation advances in the crossbred animal. 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. 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 cell to produce lactose, citrate and triacylglycerol for milk secretion. The data obtained for the utilization of glucose carbon for the synthesis of lactose, triacylglycerol and citrate during mid and late lactation were higher in rbST treated animals as comparison with the control animals. The differences in these results between the controls and rbST treated animals without a reduction in feed intake may be explained by the difference of nutrient partition or utilization in the mammary gland. 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 7% to18% for both groups. 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 ismetabolized 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 different stages of lactation in rbST treated animals was higher than those of control animals. 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 24% to 34% of the NADPH was required during early lactation for fatty acid synthesis *de novo* from glucose metabolism in the udder of rbST treated animals, while 30% to 39 % was required in the control animals. 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 ^{3}H from [3-^{3}H]glucose in fatty acids assuming that the NADPH formed is used exclusively for biosynthesis of fatty acids (Katz et al.,1974). This technique has been used to study the *in vitro* metabolism of rat mammary and adipose tissue (Katz and Wals 1970,1972; Katz et al., 1966) and it 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 rbST treated animals was metabolized via the pentose phosphate pathway which was higher than those of control animals. In rbST treated animals, a high proportion of the glucose taken up by the udder which was oxidized in the tricarboxylic acid cycle would be apparent in mid- and late lactations. High values of both the proportion and absolute amount of glucose carbon incorporation to milk citrate and milk triacylglycerol of rbST treated animals during mid- and late lactation are evidences supporting an increased proportion of glucose 6-phosphate metabolized via the Embden-Meyerhof 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. The high metabolism of glucose 6-phosphate in early lactation of rbST treated animals appeared to be due primarily to a high flux through the lactose synthesis and to pentose phosphate pathway, probably reflecting the high milk production during rbST treatment. Tritium and carbon-14 were also shown to be incorporated into milk citrate which showed increases during lactation advance in rbST treated animals whereas it remained the same levels as compared with pretreatment period for the carbon skeleton of citrate in control animals. 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. It is possible that the incorporation of ^{3}H into milk citrate may occur in different manners in the exchange

Figure 2 The metabolic pathway involved in the metabolism of the precursor of milk in the pretreatment period of initial lactation of control animals and rbST treated animals (The value shown are in micromole/min.)

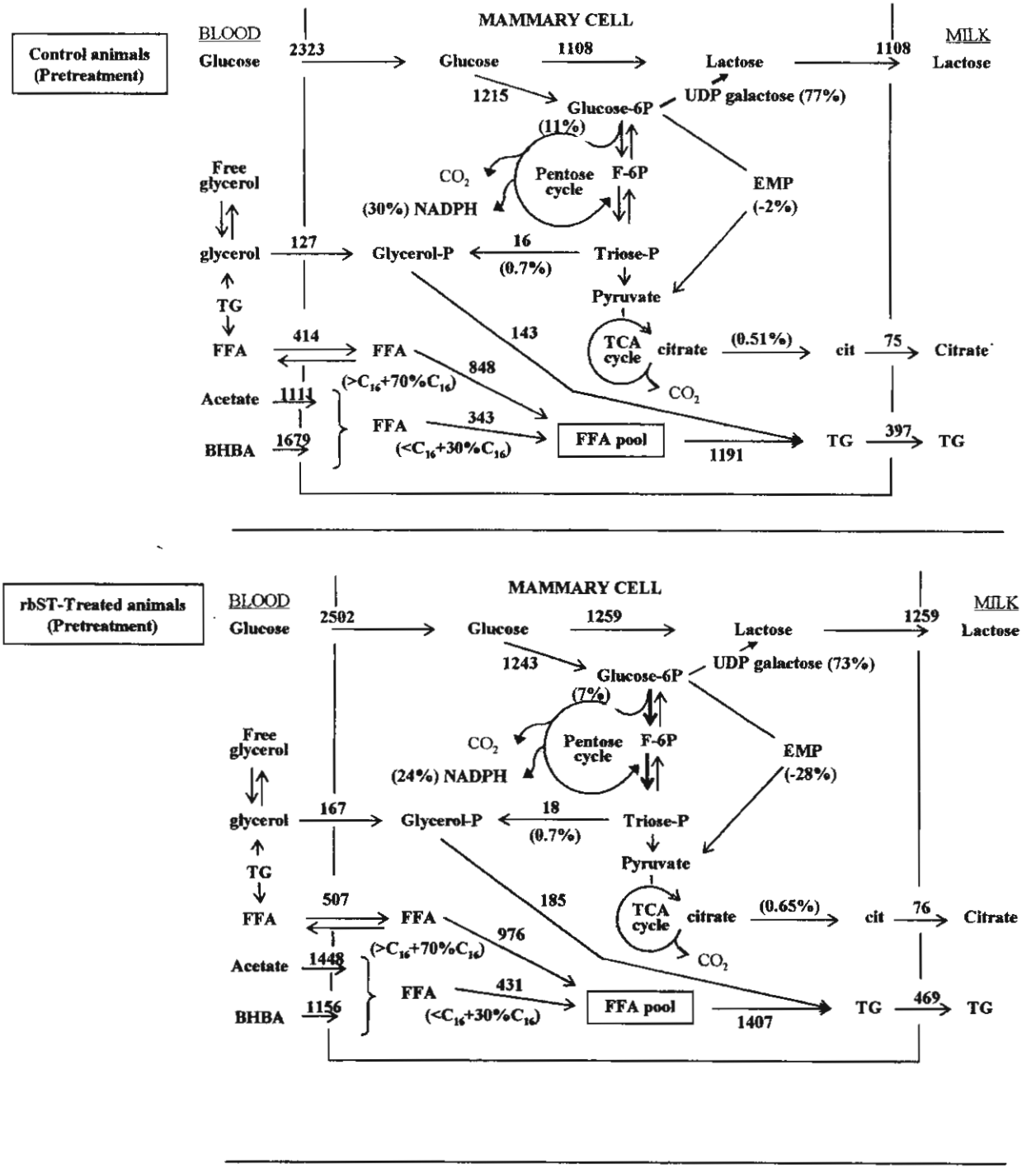
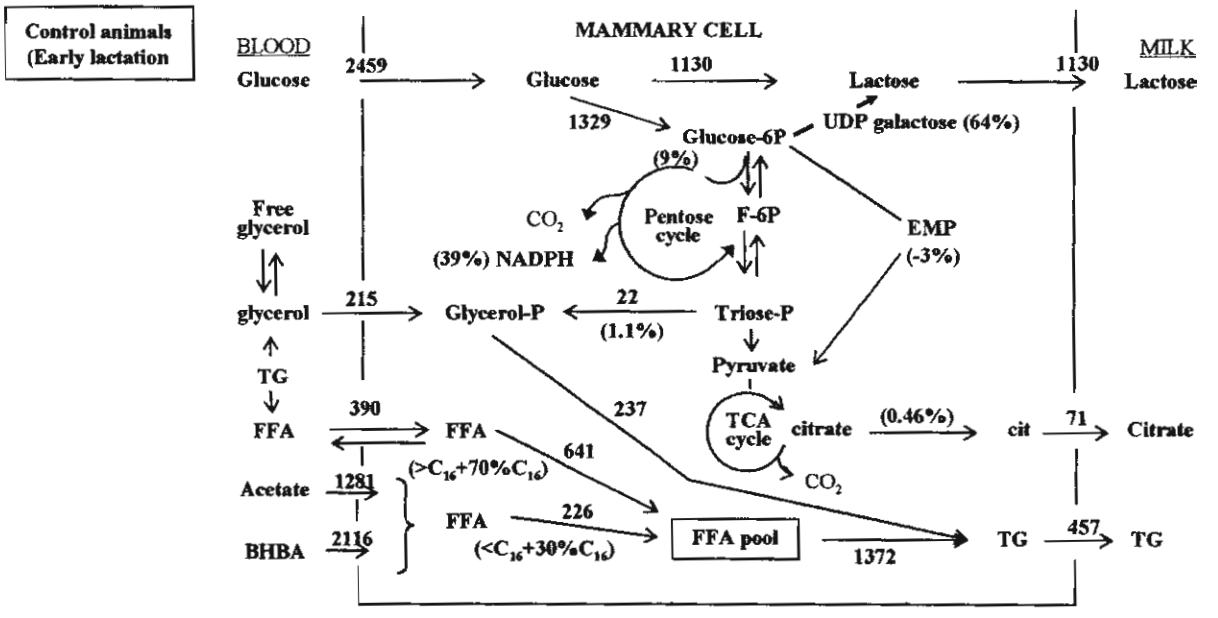


Figure 3. The metabolic pathway involved in the metabolism of the precursor of milk in the early lactation of control animals and rbST treated animals (The value shown are in micromole/min.)



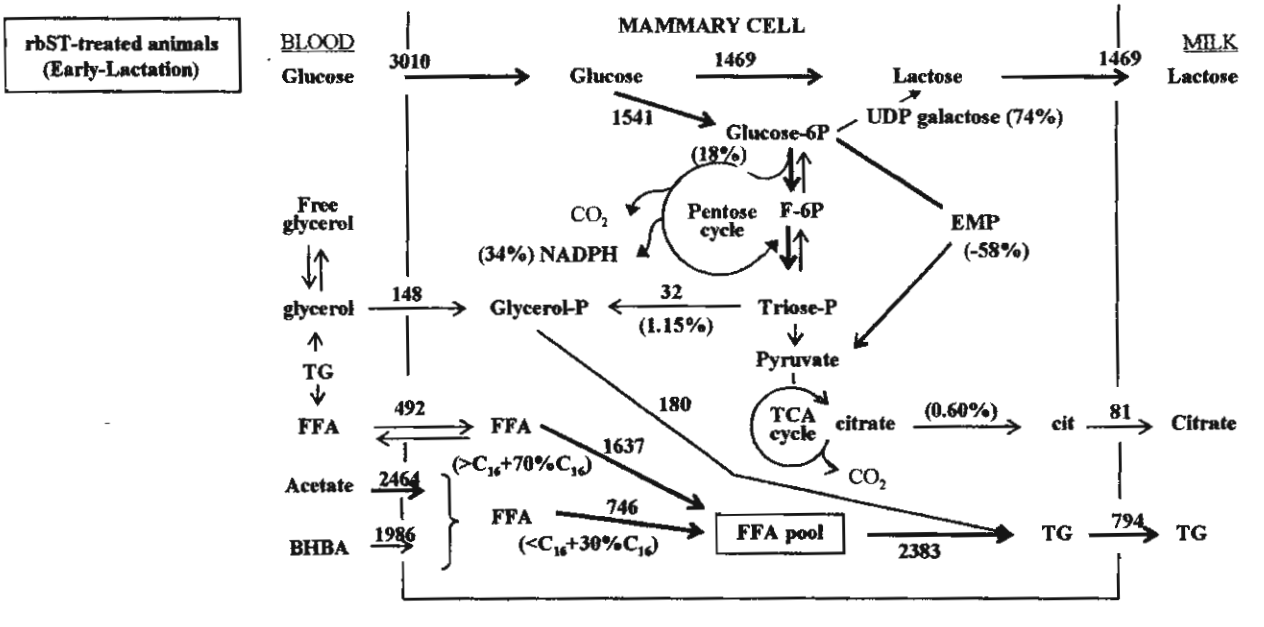
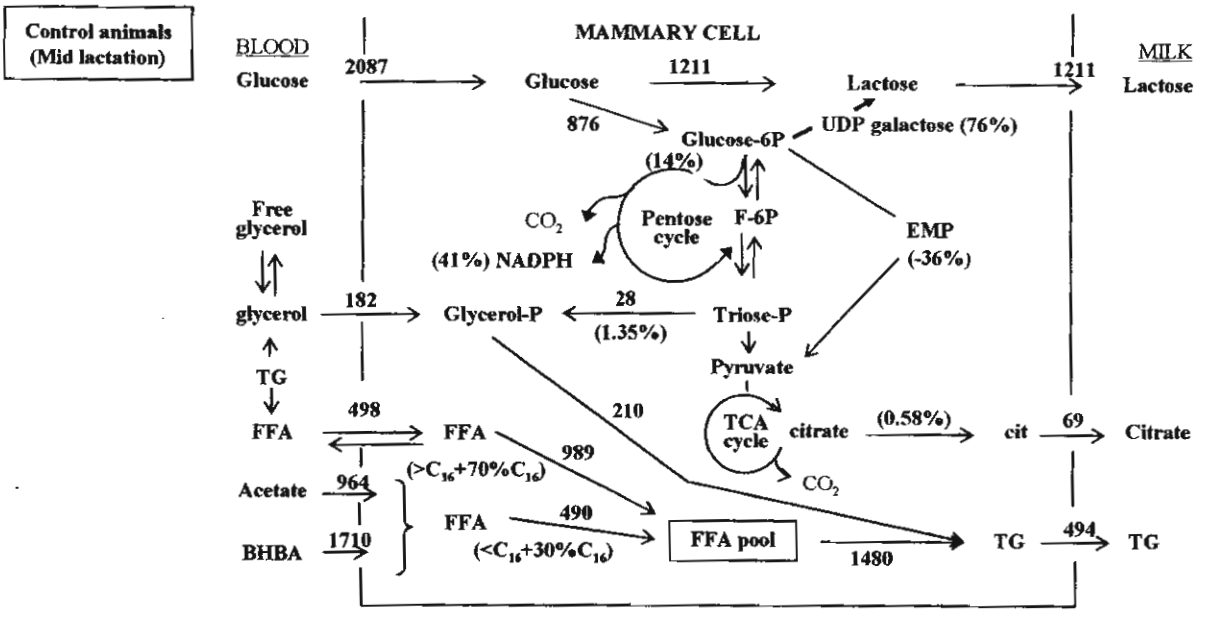


Figure 4. The metabolic pathway involved in the metabolism of the precursor of milk in the mid lactation of control animals and rbST treated animals (The value shown are in micromole/min.)



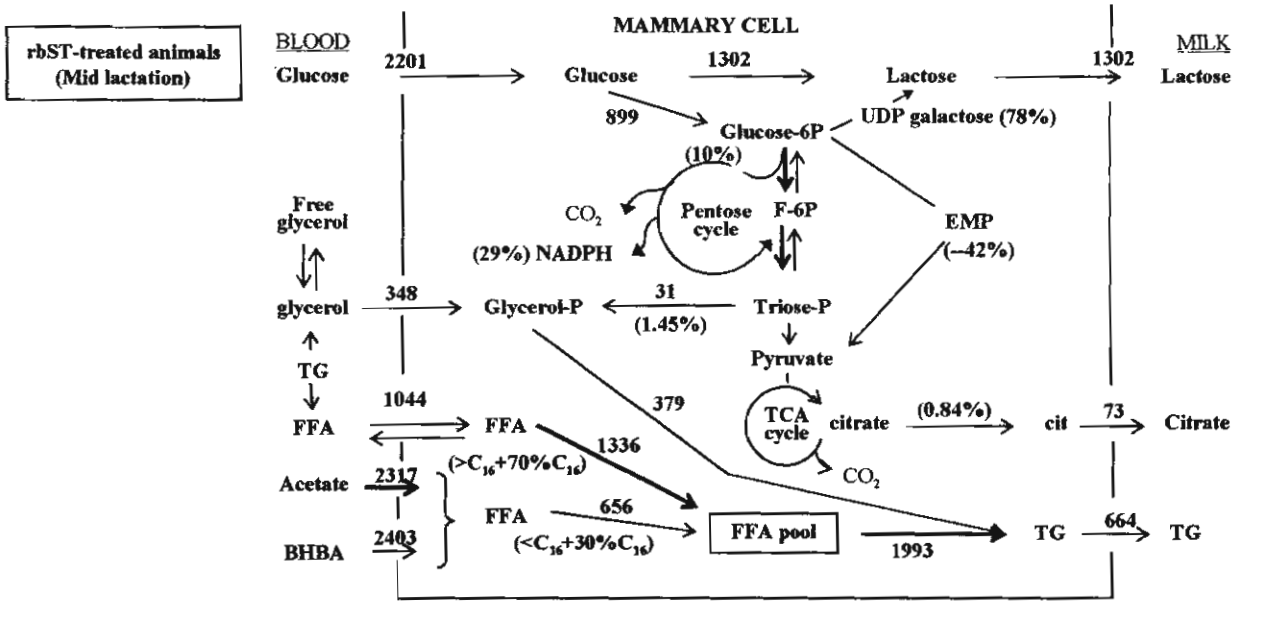
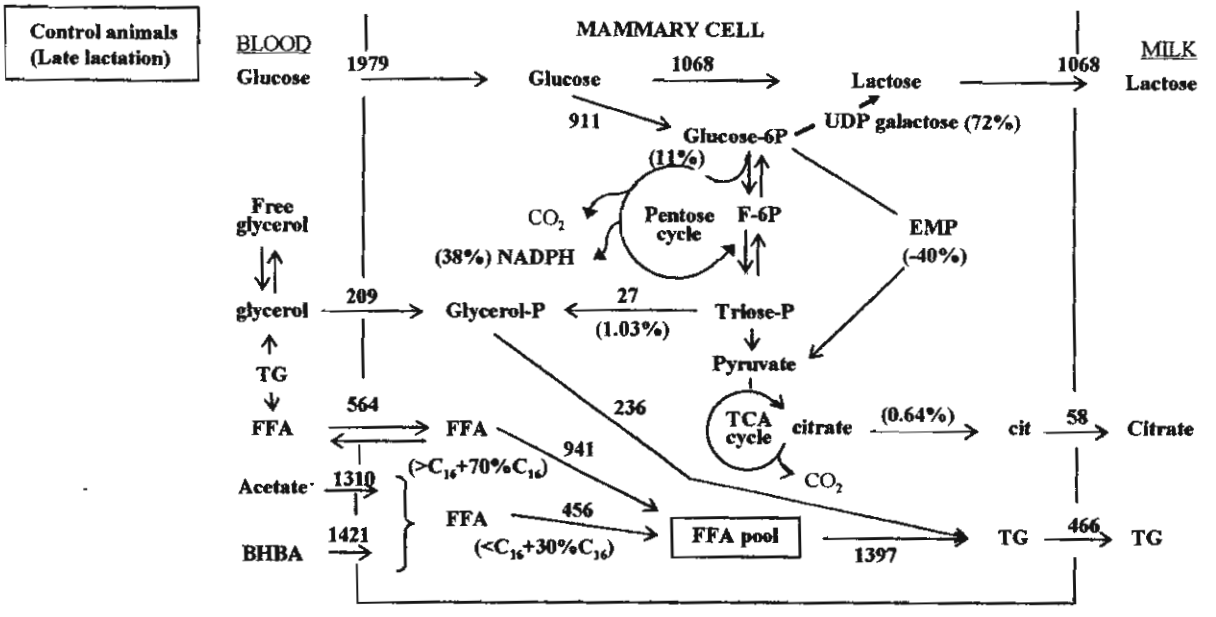
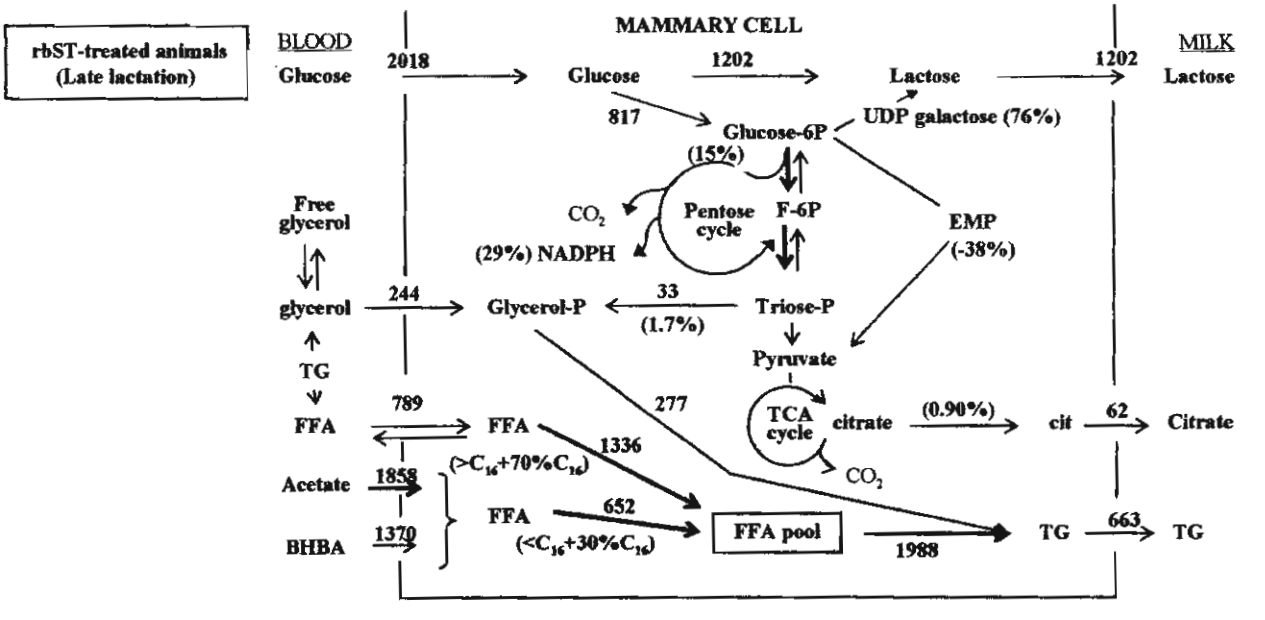


Figure 5 The metabolic pathway involved in the metabolism of the precursor of milk in the late lactation of control animals and rbST treated animals (The value shown are in micromole/min.)





reaction of the cytosolic NADP-dependent isocitrate dehydrogenase. Both fatty acid synthesis and the NADP-dependent isocitrate dehydrogenase reaction between control animals and rbST-treated 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 during rbST administration in 87.5% HF animals. As shown in Fig 2, 3, 4 & 5 (summarized of Chapter X), the glucose taken up by the udder of rbST treated animals during early lactation, an average 18% and 34% were metabolized in the pentose phosphate pathway and contributed to NADPH production, respectively. The sufficient pool of intracellular glucose concentration during rbST administration, has effect on an increase in glucose 6-phosphate which increased flux through the lactose synthesis and pentose cycle pathway. Although we know a great deal of differences in regulating glucose metabolism that occur between the control animals and rbST treated animals, we do not know the different enzymatic activities during rbST administration in different stages of lactation which affect the rate of metabolic pathways. There is still a need for more information, for example,on whether the high enzymatic activity of fructose 1-6 diphosphatase or the lower enzymatic activity of pyruvate dehydrogenase occurs in rbST treated animals throughout the stages of lactation or occurs during early lactation which causes an increase in the metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway and tricarboxylic acid cycle.

Reference

Bauman, D.E. 1992. Bovine somatotropin: review of an emerging animal technology. J. Dairy Sci. 75:3432-3451.

Bauman, D.E. and S. N. McCutcheon.1986. The effects of growth hormone and prolactin on metabolism . In; Proceeding of 6th International Symposium on Ruminant Physiology: Control of Digestion and Metabolism in Ruminants (Eds. L.P. Milligan, W. L. Grovum, and A. Dobson)Prentice Hall, Englewood Cliffs, NJ. Pp436-455.

Binelli, M., M. K. Vanderkooi, L.T. Chapin, M. J. Vanderhaar, J. D. Turner, W. M. Mosely, and H. A. Tucker. 1995. Comparison of growth hormone- releasing factor and somatotropin : Body growth and lactation of primiparous cows. J. Dairy Sci. 78: 2129-2139.

Capuco, A. V., D. L. Wood, R. Baldwin, K. Mcleod and M. J. Paape. 2001. Mammary cell number, proliferation, and apoptosis during a bovine lactation: Relation to milk production and effect of bST. J. Dairy Sci. 84: 2177-2187.

Chaiyabutr, N., S. Komolvanich, S. Thammacharoen and S. Chanpongsang. 2004.The plasma level of insulin-like growth factor-1 (IGF-1) in relation to mammary circulation and milk yield in two different types of crossbred Holstein cattle. Asian-Aus. J. Anim. Sci. 17 (3): 343-348.

Chaiyabutr, N., S. Komolvanich, S. Sawangkoon, S. Preuksagorn, and S. Chanpongsang. 1997. The regulation of body fluids and mammary circulation during late pregnancy and early lactation of crossbred Holstein cattle feeding on different types of roughage. J. Anim. Physiol. and Anim. Nutri. 77:167-179.

Chaiyabutr, N., S. Preuksagorn, S. Komolvanich, and S. Chanpongsang. 2000a. Comparative study on the regulation of body fluids and mammary circulation at different states of lactation in crossbred Holstein cattle feeding on different types of roughage. J. Anim. Physiol. and Anim. Nutri. 83:74-84.

Chaiyabutr, N., S. Komolvanich, S. Preuksagorn, and S. Chanpongsang. 2000b. Plasma levels of hormones and metabolites as affected by the forages type in two different types of crossbred Holstein cattle. Asian-Aus. J. Anim. Sci. 13(10):1359-1366.

Chaiyabutr, N., S. Thammacharoen, S. Komolvanich and S.Chanpongsang. 2005. Effects of long-term administration of recombinant bovine somatotropin on milk production and

plasma insulin-like growth factor and insulin in crossbred Holstein cows. J. Agri. Sci. Camb. (in press).

Chaiyabutr, N., A. Faulkner, and M. Peaker. 1980. Effects of starvation on the cardiovascular system, water balance and milk secretion in lactating goats. Res. Vet. Sci. 28:291-295.

Chase, L.E. 1993. Developing nutrition programs for high producing dairy herds. J. Dairy Sci. 76:3287-3293.

Coghlan, J.P., J.S.K. Fan, B.A. Scoggins, and A.A. Shulkes. 1977. Measurement of extracellular fluid volume and blood volume in sheep. Aust. J. Biol. Sci. 30:71-84.

Collier, R.J., J.P. Mcnamara, C.R. Wallace, and M.H. Dehoff. 1984. A review of endocrine regulation of metabolism during lactation. J. Anim. Sci. 59: 498-510.

Davis, C.L. and Bauman, D.E. (1974). General metabolism associated with the synthesis of milk. In Lactation Vol. II, (Ed. Larson, B.L. and V.R.Smith) New York and London : Academic Press. pp. 3-30.

Davis, S.R. and R.J. Collier. 1985. Mammary blood flow and regulation of substrate supply for milk synthesis. J. Dairy Sci. 68:1041-1058.

Davis, S. R., R. J. Collier, J. P. McNamara, H. H. Head and W.Sussman. 1988. Effects of thyroxine and growth hormone treatment of dairy cows on milk yield, cardiac output and mammary blood flow. J. Anim. Sci. 66:70-79.

Davis, S.R., P.D.Gluckman, I.C. Hart and H.V. Henderson.1987. Effects of injecting growth hormone or thyroxine on milk production and blood plasma concentrations of insulin-like growth factors I and II in dairy cows. Journal of Endocrinology. 114, 17-24.

Ebner K.E. and F.L. Schanbacher 1974. Biochemistry of lactose and related carbohydrates. In Lctation, vol.II, (BL Larson and VR Smith). New York &London : Academic Press. pp77-113.

Etherton, T. D. and D. E. Bauman. 1998. Biology of somatotropin in growth and lactation of domestic animals. Phys. Rev. 78: 745-761.

Faulkner A, N. Chaiyabutr, M. Peaker, D.T.Carrick and N. J. Kuhn. 1981. Metaobolic significance of milk glucose. J Dairy Res 48: 51-56.

Flint, D.J. and C.H.Knight 1997. Interactions of prolactin and growth hormone(GH) in the regulation of mammary gland function and epithelial cell survival. J. Mammary Gland Biology and Neoplasia **2** (1),41-48.

Gertler, A., N. Cohen and A. Maoz. 1983. Human growth hormone but not ovine or bovine growth hormones exhibits a galactopoietic prolactin-like activity in organ culture from bovine lactating mammary gland. Mol. Cell. Endrocrinol. 35:51

Gulay,M.S., A. N. Garcia, M. J. Hayen, C. J. Wilcox and H.H. Head. 2004. Responses of Holstein cows to different bovine somatotropin (bST) treatments during the transition period and early lactation. Asian-Aus. J. Anim. Sci. 17 (6): 784-793.

Hardwick, D.C., J.L. Linzell and T.M. Mepham. 1963. The metabolism of acetate and glucose by the isolated perfused udder. 2.The contribution of acetate and glucose to carbon dioxide and milk constituents. Biochem. J. 88 : 213-220.

Janssen, Y.J.H., P. Deurenberg, and F. Roelfsema.1997. Using dilution techniques and multifrequency bioelectrical impedance to assess both total body water and extracellular water at baseline and during recombinant human growth hormone (GH) treatment in GH-deficient adults. J. Clin. Endocrinol. & Metab. 82(10): 3349-3355.

Johnson, H.D., R. Li, W. Manulu, K.J. Spencer-Johnson, B.A. Becker, R.J. Collier, and C.A. Baile. 1991. Effects of somatotropin on milk yield and physiological responses during summer farm and hot laboratory conditions. J. Dairy Sci. 74(4):1250-1262.

Katz, J., B.R. Landau and G.E. Bartsch. 1966. The pentose cycle, triosephosphate isomerization, and lipogenesis in rat adipose tissue. J. Biol. Chem. 241 : 727-740.

Katz, J., R. Rognstad and R.G. Kemp. 1965. Isotope discrimination effects in the metabolism of tritiated glucose. J. Biol. Chem. 240 : 1484-1486.

Katz, J. and P.A. Wals. 1970. Effect of pheazine methosulfate on lipogenesis. J. Biol. Chem. 245 : 2546-2548.

Katz, J. and P.A. Wals. 1972. Pentose cycle and reducing equivalents in rat mammary gland slices. Biochem.J. 128 : 879-899.

Katz, J., P.A. Wals and R.L. Van De Velde. 1974. Lipogenesis by Acini from mammary gland of lactating rats. J. Biol.Chem. 249 : 7348-7357.

Kuhn, N.J., D.T. Carrick and C.J. Wilde. 1980. Milk synthesis. J. Dairy Sci. 63 : 328-336.

Kuhn, N.J., D.T. Carrick and C.J. Wilde. 1980. Lactose synthesis: the possibilities of regulation. J Dairy Sci 63, 328-336.

Kuhn N.J. and White, A. 1975 Milk glucose as an index of the intracellular glucose concentration of rat mammary gland. Biochemical J 152, 153-155.

Knight, C. J., J. E. Hillerton, M. A. Kerr, R. M. Teverson, A. Turvey, and C, J. Wilde. 1992. Separate and additive stimulation of bovine milk yield by the local and systemic galactopoietic stimuli of frequent milking and growth hormone. J. Dairy Res. 59: 243-252

. Linzell, J.L. 1973. The demands of the udder and adaptation to lactation.. In Production disease in farm animals (Eds. J.M. Payne, K.G. Hibbitt and B.F. Sansom). London : Bailliere, Tindal, pp. 89-106.

Linzell, J.L. 1974. Mammary blood flow and methods of identifying and measuring precursors of milk. In: Lactation 1., (Eds B.L Larson,. and V.R. Smith).N.Y.and London: Academic Press. pp.143-225.

Linzell,J.L. and Peaker, M.,1971. Mechanism of milk secretion. Physiological Review, 51, 564-597

Macfarlane, W.V., R.J.H. Morris, B. Howard, and O.G. Budtz-Olsen.1959. Extracellular fluid distribution in tropical Merino sheep. Aust. J. Agric. Res. 10:269- 286.

McGuire, M. A., D. E. Bauman, D.A.Dwyer and W.S. Cohick.1995. Nutritional modulation of the somatotropin/ insulin-like growth factor system : Response to feed deprivation in lactating cows. Journal of Nutrition 125 (3): 493-502

McGuire, M.A., J. M. Griinari, D.A. Dwyer and D. E. Bauman.1995. Role of insulin in the regulation of mammary synthesis of fat and protein. J. Dairy Sci. 78:816.

Mepham,T.B., S.E. Lawrence, A.R. Peters and I.C. Hart. 1984. Effects of exogenous growth hormone on mammary function in lactating goats. Horm. Metab. Res. 16:248.

Peel, C. J. and D. E. Bauman. 1987. Somatotropin and lactation. J. Dairy Sci. 70: 474- 486.

Politis, I., E.Block, and J.D Turner. 1990. Effect of somatotropin on the plasminogen and plasmin system in the mammary gland: Proposed mechanism of action for somatotropin on the mammary gland. Journal of Dairy Science, 73,1494-1499.

Politis, I., E. Lachance, E. Block, and J.D.Turner. 1989. Plasmin/plasminogen in bovine milk: a relationship with involution. Journal of Dairy Science, 72, 900-906

Phipps, R., C. Madakadze, T. Mutsvangwa, D.L. Hard, and G.D. Kerchove. 1991. Use of bovine somatotropin in the tropics: the effect of sometribove on milk production of *Bos indicus*, dairy crossbred and *Bos Taurus* cows in Zimbabwe. J. Agri. Sci., Cambridge. 117:257-263.

Rose, M. T. and Y. Obara. 1995. Effect of growth hormone on the response to insulin and glucose turnover in sheep. J. Agric. Sci. 126: 107.

Sullivan, J.L., Huber, J.T., Denise, K., Hoffman, R.G., Kung, L.,Franson, S.E. and K.S. MADSEN. 1992. Factors affecting response of cows to biweekly injections of sometribove. J. Dairy Sci.756-763.

Tanwattana, P., S. Chanpongsang, and N. Chaiyabutr. 2003. Effects of Exogenous Bovine Somatotropin on Mammary Function of Late Lactating Crossbred Holstein Cows. Asian-Aus. J. Anim. Sci. 16(1):85-96.

Threadgold L. C. and Kuhn, N.J. 1979. Glucose-6-phosphate hydrolysis by lactating rat mammary gland. Int. J. Biochem. 10, 683-685.

Tonner, E., M. C. Barber, M.T. Travers, A. Logan and D. J. Flint. 1997. Hormonal control of insulin-like growth factor-binding protein-5 production in the involuting mammary gland of the rat. Endocrinology.138: 5101-5107.

West, J.W. 1994. Interactions of energy and bovine somatotropin with heat stress. J. Dairy Sci. 77:2091-2102.

West, J. W., K. Bondari and J. C. Jhonson.1990. Effects of bovine somatotropin on milk yield and composition, body weight, and condition score of Holstein and Jersey cows. J. Dairy Sci. 73: 1062-1068.

West, J.W., B.G. Mullinix, and T.G. Sandifer. 1991. Effects of bovine somatotropin

on physiologic responses of lactating Holstein and Jersey cows during hot, humid weather. J. Dairy Sci. 74(3):840-851.

Wood, H.G., G.J. Peeters, R. Verbeke, M. Lauryssens and B. Jacobson. 1965. Estimation of the pentose cycle in the perfused cow's udder. Biochem. J. 96 : 607-615.

Wyse, B., M. Waters, and C. Sernia. 1993. Stimulation of the rennin-angiotensin system by growth hormone in Lewis dwarf rats. Am. J. Physiol. 265:E332-E339.

X