ผลของโบวายน์โซมาโตโทรปินต่อผลผลิตน้ำนม การย่อยได้ของอาหาร
และระดับฮอร์โมนเลปตินในพลาสม่าของแพะนมลูกผสมในช่วงต้นของการให้นม

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THE EFFECT OF BOVINE SOMATOTROPIN ON MILK PRODUCTION, NUTRIENT DIGESTIBILITY AND PLASMA LEPTIN OF LACTATING GOAT DURING EARLY LACTATING PERIOD

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เที๊ยท เหงียน: ผลของโบวายโซมาโตโทรปินต่อปริมาณนม การย่อยได้ของอาหาร และระดับฮอร์โมนเลปติน ในพลาสม่าของแพะนมลูกผสมให้ช่วงต้นของการให้นม (THE EFFECT OF BOVINE SOMATOTROPIN ON MILK PRODUCTION, NUTRIENT DIGESTIBILITY AND PLASMA LEPTIN OF LACTATING GOAT DURING EARLY LACTATING PERIOD) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ.น.สพ. สมชาย จันทร์ผ่องแสง อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ.น.สพ.ดร. สัมพันธ์ ธรรมเจริญ, 66 หน้า

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของการให้อาร์บีเอสทีต่อปริมาณน้ำนมการย่อยได้ของสารอาหารและพลาสมาเลปตินของแพะนมลูกผสมในช่วงต้นของการให้นมและเพื่อทดสอบว่าอาร์บี-เอสทีสามารถออกฤทธิ์ต่อการกินอาหารโดยตรงหรือโดยอ้อมผ่านฮอร์โมนเลปตินการทดลอง ใช้แพะนมลูกผสมจำนวน ๑๐ ตัวเป็นสัตว์ทดลอง หนึ่งสัปดาห์หลังคลอด สัตว์ทดลองถูกแบ่งเป็น ๒ กลุ่มแบบสุ่ม กลุ่มละ ๕ ตัว ทำการฉีดอาร์บีเอสที หรือสารควบคุมในวันที่ ๗ และวันที่ ๒๒ หลังคลอด หลังการฉีดอาร์บีเอสทีระดับของกลูโคสฮอร์โมนไอจีเอฟลำดับที่หนึ่งเพิ่มขึ้นภายใน๒๔ ชั่วโมง และพบว่าระดับของฮอร์โมนเลปตินของกลุ่มที่ได้รับอาร์บีเอสทีสูงกว่ากลุ่มควบคุมภายใน ๔๘ ชั่วโมง หลังการให้อาร์บีเอสทีพบว่าการกินอาหารลดลงในขณะที่ปริมาณน้ำนมและการกินน้ำเพิ่มขึ้นโดยที่ไม่พบความแตกต่างของน้ำหนักตัว

ผลดังกล่าวทำให้ปริมาณการกินอาหารต่อน้ำหนักตัวในกลุ่มที่ได้รับอาร์บีเอสทีน้อยกว่ากลุ่ม-ควบคุม และทำให้ประสิทธิภาพการใช้อาหารสูงกว่าการวิเคราะห์ส่วนประกอบน้ำนมจากตัวอย่างน้ำนมในวันที่ ๘ หลังการให้อาร์บีเอสทีครั้งที่สองไม่พบความแตกต่างใดๆเมื่อเปรียบเทียบกับกลุ่มควบคุมและจากการ ศึกษาถึงการย่อยได้ของสารอาหารการสะสมของไนโตรเจนและระดับสารอัลลัลโตอินนั้นไม่พบความแตกต่างใดๆที่ชัดเจนจากการให้อาร์บีเอสทีผลการทดลองบ่งชี้ว่า การให้อาร์บีเอสทีมีผลต่อประสิทธิภาพการสร้างน้ำนมในแพะนมลูกผสมในช่วงต้นของการให้น้ำนมอย่างไรก็ตามการให้อาร์บี-เอสทีในช่วงดัง กล่าว ส่งผลให้การกินอาหารลดลงโดยที่ผลดังกล่าว อาจเกิดขึ้นจากการเพิ่มระดับของฮอร์โมนเลปติน

ภาควิชา : สัตวบาล ลายมือชื่อนิสิต .....

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KEYWORDS: GOAT / MILK YIELD / FEED INTAKE / LEPTIN / SOMATOTROPIN.

THIET NGUYEN: THE EFFECT OF BOVINE SOMATOTROPIN ON MILK PRODUCTION, NUTRIENT DIGESTIBILITY AND PLASMA LEPTIN OF LACTATING GOAT DURING EARLY LACTATING PERIOD. ADVISOR: PROF. SOMCHAI CHANPONGSANG. CO-ADVISOR: SUMPUN THAMMACHAROEN, Ph.D., 66 pp.

This study was designed to investigate the effect of bovine somatotropin on milk production, nutrient digestibility and plasma leptin of crossbred lactating goat during early lactating period and further that somatrotopin could influence feed intake during early lactation directly or indirectly via plasma leptin. The experiment was performed using ten crossbred lactating goats. One week after parturition, animals were randomly divided into two groups (n=5). Recombinant bovine somatotropin (rbST) or vehicle was injected at day 7^{th} and 22^{nd} after parturition to each group of animals. rbST increased plasma glucose, IGF-I concentration within 24 hours after supplementation. Within 48 hours after rbST supplementation, plasma leptin concentrations from the treatment group was higher than those from control group. In addition, rbST supplementation decreased dry matter intake while increased milk yield and water intake. Body weight of animals from each group was not different. Supplementation with rbST significantly decreased dry matter intake per body weight and then increased feed efficiency. Milk composition was not different between each treatment. Similarly, the nutrients digestibility, nitrogen retention and urinary allantoin concentration were not affected by rbST supplementation. The present experiment suggested that rbST supplementation could improve lactation performance of crossbred dairy goat during early lactating period. However, rbST supplementation during this period decreased feed intake and this behavioral effect was apparently caused by the direct mechanism of an increment of plasma leptin concentration.

Department : Animal Husbandry Student's Signature .....

Field of Study : Animal Nutrition Advisor's Signature .....

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LIST OF ABBREVIATIONS

ACTH	=	Adrenocorticotrophic hormone
ADF	=	Acid detergent fiber
ADFD	=	Acid detergent fiber digestibility
bST	=	Bovine somatotropin
BW	=	Body weight
cAMP	=	Cyclic adenosine monophosphate
CBG	=	Corticosteroid binding globulin
CNS	=	Central nervous system
CP	=	Crude protein
CPD	=	Crude protein digestibility
DM	=	Dry matter
DMD	=	Dry matter digestibility
DMI	=	Dry matter intake
DMI/BW	=	Dry matter intake/body weight
DNA	=	Deoxyribonucleic acid
GH	=	Growth hormone
GHR	=	Growth hormone receptors
GHRF	=	Growth hormone releasing factor
HCl	=	Hydrochloric acid
hST	=	Human somatotropin
IGF-I	=	Insulin-like growth factor-I
IGFBP-5	=	Insulin-like growth factor binding protein-5
IGFs	=	Insulin-like growth factors
IU	=	International unit
ME	=	Metabolized energy
mRNA	=	Messenger ribonucleic acid
MY	=	Milk yield
N	=	Nitrogen

NDF	=	Neutral detergent fiber
NDFD	=	Neutral detergent fiber digestibility
NPY	=	Neuropeptide Y
OMD	=	Organic matter digestibility
oST	=	Sheep somatotropin
PRL	=	Prolactin
pST	=	Porcine somatotropin
PTH	=	Para thyroid hormone
rbGH	=	Recombinant bovine growth hormone
rbST	=	Recombinant bovine somatotropin
SE	=	Standard error
SNF	=	Solid not-fat
SS	=	Somatostatin
T_3	=	Triiodothyronine
T_4	=	Thyroxine
TSH	=	Thyroid stimulating hormone
VFA	=	Volatile fatty acid
WI	=	Water intake

CHAPTER I
INTRODUCTION

One of the interesting biological technologies that have been introduced to the livestock production is the synthetic somatotropin (ST) or growth hormone (GH). Exogenous ST has been used to increase production in farm animals. In dairy animals, ST has been shown to increase lactation performance (Bauman and Vernon, 1993). This effect has been investigated extensively in dairy cattle after available of recombinant bovine ST (rbST). Supplementation with rbST has been shown to increase milk yield with minor change in milk composition. In addition, rbST has been demonstrated to increase milk yield effectively in dairy goat as well (Disenhaus et al., 1995; Polratana et al., 2004; Sallam et al., 2005). There are many factors that can determine the magnitude of rbST effect on milk yield which include the nutritional status, feed quality, and environmental factors. Cows injected with rbST adjust their nutritional status partly by changing voluntary food intake related to feed quality and their milk production (Bauman, 1992). In general, supplementation with rbST increased milk yield during early lactation as well as during late lactation (Etherton and Bauman, 1998). For the dairy animals in Thailand, lactation performance is attenuated partly by the inadequacy of nutritional status. However, it has been shown that rbST could increase milk yield in both dairy cows and goats (Polratana et al., 2004; Chaiyabutr et al., 2005).

During peri-parturition and early lactation, dairy animals use more energy and protein than what they can consume. More energy expenditure and less energy input lead to negative energy balance. Dairy animals in negative energy balance utilize body reserves to meet the additional requirements for milk synthesis. It was then speculated that the use of rbST in early lactation could cause a greater energy deficiency in dairy cattle (NRC, 2001), frequently associated to an insufficient feed intake. However, feed intake is a complex behavior that provides energy and necessary nutrients to the body. Some previous studies found that rbST supplement did not change feed intake in dairy sheep (Sallam et al., 2005) and dairy goat (Disenhaus et al., 1995), whereas others found that there were an increase of feed intake in dairy cattle (Boonsanit et al., 2010)

and late lactation of dairy goat (Polratana et al., 2004). According to Chanchai et al. (2010[a]) reported that the misty-fans cooling associate with rbST supplementation in dairy cattle could increase feed intake which was coincided to the reduction of plasma leptin level. It was known that exogenous leptin administration decreased feed intake and body weight (Kershaw and Flier, 2004). Moreover, fasting or underfed for three days has been shown to result in a significant reduction in plasma leptin level (Weigle et al., 1997). Thus, feeding behavior may be directly affected by rbST or indirectly via plasma leptin concentration.

There was a little information about the effects of exogenous rbST supplementation on alteration of plasma leptin concentration in related with feed intake in dairy goats. Therefore, the aim of current experiment was to investigate the effect of rbST in dairy goat during early lactation period with regard to feed intake, milk production, nutrient digestibility and plasma leptin concentration.

CHAPTER II
LITERATURE REVIEW

2.1 Endocrinology aspects on milk production

The physiology of lactation consists of mammary gland development (mammogenesis), the start of milk synthesis (lactogenesis) and maintenance of milk synthesis (galactopoiesis). The mammary gland development is stimulated mainly by oestrogen and progesterone, somatotropin, insulin-like growth factor-I (IGF-I) and prolactin and inhibited by mammary derived growth inhibitor. It is well-advocated that lactogenesis is stimulated by prolactin, oestrogen, glucocorticoids, insulin and inhibited by progesterone while galactopoiesis is influenced by some hormones such as somatotropin, thyroid hormones, and prolactin for stimulation and glucocorticoids, oestrogen for inhibition.

2.1.1 Hormonal effects on lactogenesis

Progesterone, secreted by the corpus luteum, has been suggested to work by increasing the mammary threshold to prolactin, by altering the secretion of prolactin from the pituitary or acting as a glucocorticoid receptor antagonist. The level of progesterone was high during gestation and serves to inhibit lactogenesis until just before parturition. The level decreased about 2 days before parturition to remove the inhibition of milk synthesis (Mellor et al., 1987).

There is considerable species variability in the effects of hormones on lactogenesis. Prolactin, oestrogen, insulin and glucocorticoids initiate lactation, provided well-developed lobule-alveolar system. Prolactin, secreted by anterior pituitary, is important for maintenance of lactation in most species, but it is not important during ruminant lactation. In dairy goat, inhibition of prolactin secretion had only small influences on milk yield and administration with prolactin did not increase milk yield. Oestrogen, produced by the walls of the developing ovarian follicles, stimulates the release of prolactin into blood from the anterior pituitary and increases the number of

prolactin receptors in mammary cells. There is a surge of prolactin several hours before parturition (Jacquemet and Prigge, 1991). Insulin is important in stimulating glucose uptake and the expression of milk protein genes required for lactogenesis. Insulin concentration was low during lactation in most species (Neville and Picciano, 1997). This would be important for shunting the nutrients away from body depots and had more available nutrients for milk synthesis. Glucocorticoids, produced by adrenal cortex, bind to receptors in mammary tissue, increase the development of the rough endoplasmic reticulum and increase the secretion of α-lactalbumin and β-casein (Kehrli, 1991).

2.1.2 Hormonal effects on galactopoiesis

Prolactin is required for the maintenance of milk production in rats, with decreases in milk yield of 50% or more after bromocriptine administration (bromocriptine blocks the release of prolactin hormone). However, prolactin produced less effect on milk yield in ruminant. In addition, milk yield from ruminant has been shown to influence significantly by ST deficiency. Thus, prolactin seems to play important role on galactopoiesis in rodent and primate. Somatotropin is important hormone in ruminant (Knight and Wilde, 1993).

Thyroid hormones are required for maximal milk production. During lactation there is decreased conversion of thyroxine (T_4) to the active hormone triiodothyronine (T_3) in liver and kidney, but increased conversion to T_3 in the mammary gland. This may be enhanced the priority of the mammary gland for metabolites compared to other body tissues. Administration of thyroid hormone causes a temporary increase in milk production for several weeks if nutrients were provided sufficiently for supporting the increased metabolism and the increase in production, but milk yield was unchanged when thyroid hormones administration to animal for more than two months (Blaxter et al., 1949).

2.2 Overview of somatotropin

2.2.1 Structure and synthesis

Somatotropin (ST) or growth hormone (GH) was a protein hormone synthesized from somatotroph cell of anterior pituitary gland (Etherton and Bauman, 1998) and comprised up to 45% of pituitary cells which contained a total of 5-15 mg of GH in human (Melmed, 2011). Protein hormones consisted of a linear chain of amino acids. As with any protein, the specific sequence of the different amino acids determined the primary structure and nature of the protein. The structure of bovine ST was a single chain polypeptide comprising 191 amino acids, with molecular mass 21819 daltons as calculated from the amino acid sequence. The molecule had two disulphide bonds at 53-164 and 181-189 positions (Secchi and Borromeo, 1997). Bovine ST and porcine ST shared up to 90% similar amino acid sequence (Table 2.1), while human ST shared only 35% similarity (Bauman and Vernon, 1993; Etherton and Bauman, 1998).

2.2.2 Control of GH secretion

GH was secreted primarily during the period of growth. After adolescent, GH secretion decreased slowly and finally fell to about 25% of the adolescent level in very old age. The normal concentration of growth hormone in plasma of an adult was between 1.5 and 3ng/ml and in a child or adolescent about 6ng/ml (Guyton, 1991). GH release occurs in a pulsatile pattern and this pattern is thought to be regulated by the interplay of the two main hypothalamic peptides; growth hormone releasing factor (GHRF) and somatostatin (SS). The hypothalamic GHRF increases GH release from the anterior pituitary. The production of SS decreases the release of GH (Figure 2.2) (Muller et al., 1999).

Table 2.1 Amino-acid sequence of somatotropins from various species.

	0 10	20	30	40	50
Porcine (pST)	AFPAMPLSSL	FANAVLRAQH	LHQLAADTYK	EFERAYIPEG	QRYSIQNAQA
Bovine (bST)	AFPAMSLSGL	FANAVLRAQH	LHQLAADTFK	EFERAYIPEG	QRYSIQNTQV
Sheep (oST)	AFPAMSLSGL	FANAVLRAQH	LHQLAADTFK	EFERAYIPEG	QRYSIQNTQV
Human (hST)	FPTIPLSRLF	DNAMLRAHRL	HQLAFDTYQE	FEEAYIPKEQ	KYSFLQNPQT
	60	70	80	90	100
Porcine (pST)	AFCFSETIPA	PTGKDEAQQR	SDVELLRFSL	LLIQSWLGPV	QFLSRVFTNS
Bovine (bST)	AFCFSETIPA	PTGKNEAQQK	SDLELLRISL	LLIQSWLGPL	QFLSRVFTNS
Sheep (oST)	AFCFSETIPA	PTGKNEAQQK	SDLELLRISL	LLIQSWLGPL	QFLSRVFTNS
Human (hST)	SLCFSESIPT	PSNREETQQK	SNLELLRISL	LLIQSWLEPV	QFLRSVFANS
	110	120	130	140	150
Porcine (pST)	LVFGTSDRVY	EKLKDLEEGI	QALMRELEDG	SPRAGQILKQ	TYDKFDTNLR
Bovine (bST)	LVFGTSDRVY	EKLKDLEEGI	LALMRELEDG	TPRAGQILKQ	TYDKFDTNMR
Sheep (oST)	LVFGTSDRVY	EKLKDLEEGI	LALMRELEDG	TPRAGQILKQ	TYDKFDTNMR
Human (hST)	LVYGASDSNV	YDLLKDLEEG	IQTLMGRLED	GSPRTGQIFK	QTYSKFDTNS
	160	170	180	190	
Porcine (pST)	SDDALLKNYG	LLSCFKKDLH	KAETYLRVMK	CRRFVESSCA	F
Bovine (bST)	SDDALLKNYG	LLSCFRKDLH	KTETYLRVMK	CRRFGEASCA	F
Sheep (oST)	SDDALLKNYG	LLSCFRKDLH	KTETYLRVMK	CRRFGEASCA	F
Human (hST)	HNDDALLKNY	GLLYCFRKDM	DKVETFLRIV	QCRSVEGSCG	F

Source: Squires (2003)

Growth hormone releasing factor was a 44 amino acid peptide synthesized by cells in the arcuate nucleus of the hypothalamus. It was secreted from neuron secretory nerve terminals and transported to the anterior pituitary gland by the hypophyseal portal system. GHRF bound to a G-linked protein receptor in somatotropes, stimulating in cAMP and activating GH release via the transcription factor. Somatostatin was produced in the periventricular and arcuate nucleus of the hypothalamus. It was 14 amino acids that are found in many tissues outside the hypothalamus. This includes the CNS and delta cells of endocrine pancreas and gut. Somatostatin inhibited the release of GH from the anterior pituitary by reducing cAMP concentrations within the somatotropes (Hossner, 2005).

The GH secretion from the anterior pituitary gland is affected by several physiological factors such as nutritional influences, hormones, sleep, and stress. Nutritional factors in which influenced on GH secretion such as high levels of amino acids in the diet, particularly arginine and leucine, induce GH release. Arginine appeared to decrease somatostatin secretion and thereby stimulate the GH secretion (Alba-Roth et al., 1988). In addition, sleep stimulates GH secretion and 60-70% of daily GH secretion happens during early sleep (Van Cauter et al., 2000).

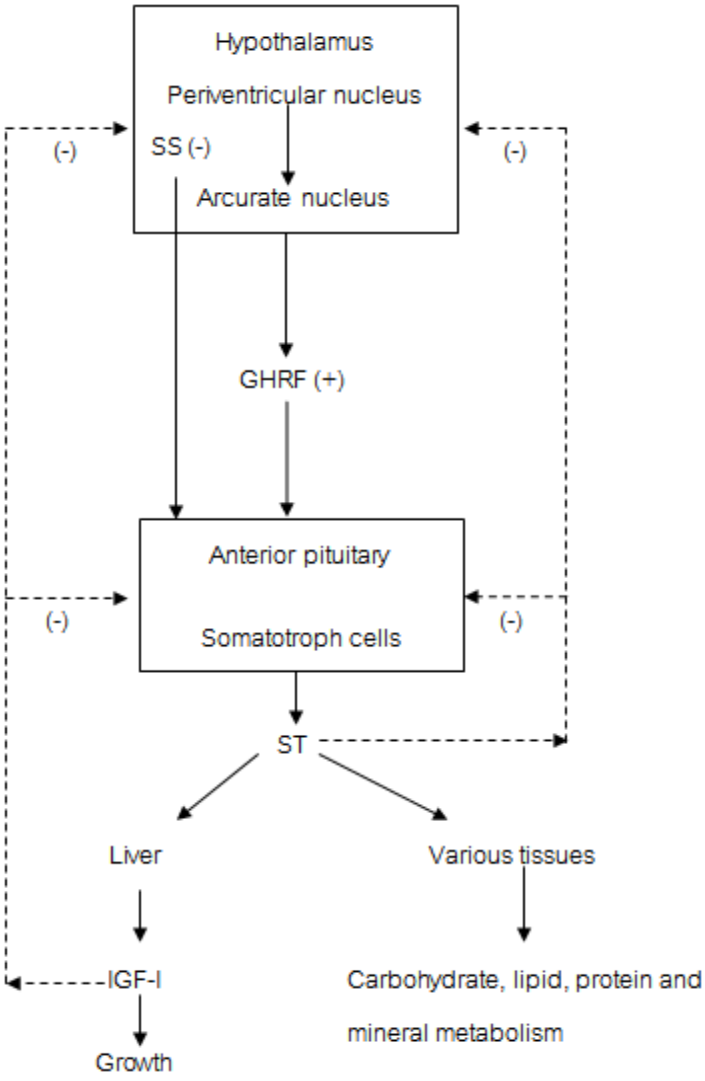

Figure 2.1 Regulation of growth hormone release

Leptin may act as a metabolic signal to regulate GH secretion. Leptin plays an important role in the regulation of body fat mass, food intake and energy expenditure. Plasma leptin concentration reduced rapidly in response to fasting and increased growth hormone secretion, followed by increasing the growth hormone level in circulation (Kelesidis et al., 2010). Conversely, ghrelin is produced by the pituitary and hypothalamus as well as mucosal enteroendocrine cells of the stomach. Ghrelin acts through the hypothalamus and the pituitary to induce pituitary release of GH. The ghrelin mRNA and peptide in the anterior pituitary are increased by GHRF infusion and ghrelin may have a local autocrine or paracrine effect on stimulating GH release (Hossner, 2005).

2.2.3 Mechanism and function of somatotropin

It is well known that ST has broad range of physiological functions including: postnatal growth (bone & muscle), nutrients metabolism, modulation of cell cycle, control of immune system, heart and brain function, mammary gland and lactation. These functions of ST can be divided into somatogenic and metabolic effects. The first effects are the stimulation of cell proliferation while the latter effects change the whole body metabolism by partitioning of all nutrients to support the specific action of ST (Bauman and Vernon, 1993; Etherton and Bauman, 1998). With the wide range of ST function, it suggests that the mechanism of ST action should be tightly regulated. ST mediates its function directly via growth hormone receptors (GHR) or indirectly via insulin like growth factor (IGF) system (Bauman and Vernon, 1993; Brooks and Waters, 2010). The GHR belongs to class I cytokine receptor super-family. The expression of GHR has been demonstrated in many organs or tissues, eg. liver, adipose tissue, heart, brain, kidney etc. This wide distribution of GHR indicates the pleotropic effect of ST (Kopchick et al., 2002).

As mention above, ST mediate its action indirectly via IGF system. One of the most important mediators from ST-IGF axis is IGF-I. IGF-I belongs to a family of insulin-like growth factors (IGFs) that shares close structure homology to the precursor form of

insulin (pro-insulin). Although circulating IGF-I acts as endocrine fashion appear to come mainly from liver, the local production of IGF-I as paracrine or autocrine has been known as well (LeRoith et al., 2001). The ST effect on mammary gland function has been proposed to influence by both plasma and local production of IGF system (Flint and Knight, 1997; Brooks and Waters, 2010).

2.2.4 Production of recombinant somatotropin (rbST)

Recombinant bovine growth hormone (rbGH) or rbST refers to bovine growth hormone that was manufactured in a laboratory using genetic technology. This synthetic hormone is approved by United State of American Food and Drug Administration (US FDA) and marketed to dairy farmer to increase milk production.

To make rbST, the plasmid of a bacterium is cut by enzymes, and then combined with a cow's DNA. It is reintroduced to the bacterium, placed in a fermentation tank and allowed to multiply, then separated and purified before delivery to the farmer (Roush, 1991). This technology permitted the development of rbST, which provided an unlimited source of ST for research and for commercial application. Recombinantly derived bST products differ slightly from bST derived pituitary gland which the manufacturing process can add a few extra amino acids to substitute for the terminal alanine residue (Hammond et al., 1990). The number of extra amino acids differs from 0 to 9, depending on the particular manufacturing process (Table 2.2).

Table 2.2 The products of rbST on the market

Products	Amino acid replace for alanine (191)
Somagrebove	Met-Asp-Gin
Somidobove	Met-Phe-Pro-Leu-Asp-Asp-Asp-Asp-Lys
Sometribove	Met
Somavubove	None

Source: Hammond et al. (1990)

2.3 Effects of recombinant bovine somatotropin on lactation performance

Treatment with rbST has been shown to increase milk yield. Although the exact mechanism that rbST stimulates mammary gland activity is still unclear. Several evidences supported that the pharmacological effect of rbST on lactation apparently mediate mainly via IGF dependent pathway by increasing the plasma IGF-I concentration. Firstly, IGF receptor was successfully demonstrated from mammary tissue, while GHR expression was very scarce at mRNA level and could not be detected at protein level. Secondly, the closed infusion of ST to the mammary artery failed to produce the effect on milk yield, whereas the infusion by IGF-I significantly increased milk production. Supplement with rbST dramatically increased circulatory IGF-I (Etherton and Bauman, 1998). Overall, rbST hormone affected on mammary tissue indirectly via IGF-I hormone.

Milk yield gradually increased in both dairy cattle and goat after treated with rbST. Supplementation with rbST consistently resulted in a higher milk performance and an increased persistency in yield over the lactation cycle in both temperate and tropical zone (Bauman and Vernon, 1993). In dairy cattle, an increased milk yield after rbST administration was found at all parities. However, the magnitude of the increase in milk production differed from the stage of lactation. In general, small response was found

when lactating animals were injected rbST in early lactation prior to peak yield (Etherton and Bauman, 1998). In addition, rbST increased milk yield by 10% when administered in early to mid-lactation, and by 40% in late lactation (Bauman and Vernon, 1993). In Thailand, rbST increased lactation performance by 22% during early lactation (Chaiyabutr et al., 2007).

Similarly, milk yield rose significantly over the entire experimental time when lactating goats supplemented with rbST (Disenhaus et al., 1995; Gallo et al., 1997; Chadio et al., 2000; Sallam et al., 2005). In Thailand, supplement with 250 mg rbST every 2 weeks in dairy goat doubled milk yield during late lactation (Polratana et al., 2004). It remains to be investigated whether milk yield during early period of lactation in dairy goat still response at this dose of rbST. In addition, the milk yield response to rbST is related to the dose and lactation cycle. In lactating cow the maximal milk production achieved at a rbST dose of 100 IU/day (Table 2.3).

The major factor which affects the milk production response to bST is the quality of management. Milk production responses to bST were not dependent on particular diets or unique feed ingredients, but animals must receive adequate amounts of a balance diet (Etherton and Bauman, 1998). Daily nutrient requirements were increased by an amount in equivalent with the increase in milk yield. These results improved the feed efficiency (milk per unit of feed) because a greater rate of nutrient intake was used for milk synthesis (McGuire and Bauman, 1997). Several previous studies found that rbST supplementation effect was a near zero in regarding with milk yield when the management was inadequate. It has been demonstrated by Fetrow (1999) that the hormone could not increase milk production during inadequate diet supply. Similarly, McCutcheon et al. (1989) showed more evidence on the effects of management in response to rbST supplement. They compared the dairy cows which fed on the pasture between spring (good quality pasture) and summer (lower quality pasture). The milk yield response to bST was greater 18% in spring and reduced nearly zero during the summer drought.

Table 2.3 The relationship of milk production responses to dose and stage of lactation.

Variable	Treatment	Milk yield (kg/d)	Dairy animal	References
Stage of lactation				
Early	Control	10.81	Crossbred cows	Boonsanit et al. (2010)
	Somatotropin	12.3		
Mid	Control	9.19		
	Somatotropin	10.44		
Late	Control	8.24		
	Somatotropin	9.73		
Dose response	Control	27.5	Cows	Eppard et al. (1985)
	Somatotropin			
	5 IU/day	29.1		
	10 IU/day	28.6		
	25 IU/day	31.9		
	50 IU/day	35.6		
	100 IU/day	37.6		
	Control	0.78	Lactating goat	Sallam et al. (2007)
	Somatotropin			
	100 mg/ 14 days	0.97		
	200 mg/ 14 days	0.96		
	Control	0.99	Ewes	Fernandez et al. (1997)
	Somatotropin			
	80 mg/ 14 days	1.20		
	160 mg/ 14 days	1.34		
	240 mg/ 14 days	1.30		

2.4 Effects of recombinant bovine somatotropin on milk composition

The composition of milk remained in normal range after rbST treatment (Bauman and Vernon, 1993; Etherton and Bauman, 1998). However, there was a slight increase in milk fat after rbST treatment in dairy cattle (McDowell, 1991; Chaiyabutr et al., 2007). The same response was found in dairy goat after rbST supplemented during first and second week, indicating higher lipolysis for rbST treatment to providing nutrient available for milk synthesis (Disenhaus et al., 1995; Chadio et al., 2000). Overall, the milk composition is unchanged after rbST treatment. The composition is varied depend on other factors including: breed, diet composition, energy status etc. Moreover, there is also no significant effect of rbST to the micro-constituents of milk (Bauman and Vernon, 1993; Etherton and Bauman, 1998).

Composition of milk fat is one of important characters to evaluating the quality of milk, particularly in manufacturing properties. According to Disenhaus et al. (1995), they found that the fatty acids in goat milk were an increase of long chain fatty acids in first and second weeks and short chain fatty acids in third and fourth weeks of rbST supplementation. This finding was similar with study of Chalupa and Galligan (1989). They explained that it just temporarily changed in relation with the energy status and dairy animal in negative energy balance produce milk with higher fat content due to the lipid mobilization from body fat store in order to compensation of the high nutrition require for milk synthesis. The use of body fat store for milk synthesis was followed by the increase in fatty acid oxidation and secretion of long chain fatty acid in the milk. The change in short chain fatty acid in milk mainly comes from feed with the increase of volatile fatty acids in rumen, particularly from acetic acid concentration. In contrast, when bST treated animals were in positive energy balance, body fat mobilization, milk fat level and milk fatty acid composition were unaffected. The mammary gland could not produce fatty acid longer than 16 carbons. The balance between fatty acids synthesized in the mammary gland and those available from the diet or microbial synthesis can be altered by dietary means.

Milk protein from rbST supplemented animals or unsupplemented animals did not differ. Changes in percentage of milk protein mainly resulted from the amount of dietary protein which was consumed by animal. When the animals were in positive nitrogen balance, there was no change in milk protein. Conversely, when cows were in negative nitrogen balance and somatotropin supplementation declined the percentage of milk protein. Because dairy cows were unable to supply adequate the available amino acids to the mammary gland for maintain the protein synthesis (Peel and Bauman, 1987).

2.5 Effects of recombinant bovine somatotropin on feed intake and nutrient digestibility

Supplemented with rbST has been shown to increase dry matter intake (DMI) in dairy animal (Polratana et al., 2004; Boonsanit et al., 2010; Chanchai et al., 2010[a]). However, some experiments have been demonstrated no effect of rbST on feed intake (Disenhaus et al., 1995; Chadio et al., 2000; Sallam et al., 2005; Chaiyabutr et al., 2005; Chaiyabutr et al., 2007). This discrepancy information suggested that these results probably came from the difference in experimental condition and reflected that this behavior was controlled by multiple factors. In crossbred dairy cattle fed in Thailand, there was no significant effect of rbST on feed intake (Chaiyabutr et al., 2007). However, the latter investigation from the same group reported that an increase feed intake in the crossbred dairy cattle treated with rbST compared with control at all stage of lactation (Boonsanit et al., 2010; Chanchai et al,. 2010[a]). In dairy goat and ewes, dry matter intake did not differ significantly between control and rbST treatment (Disenhaus et al., 1995; Chadio et al., 2000; Sallam et al., 2005). However, Polratana et al. (2004) found that dry matter intake of concentrate in rbST injected goat was significantly higher than control group during late lactation. As mention above, the increase feed intake may depend on the increase in milk production, energy status, environmental condition and the nutrients of diet (particularly energy). Overall, dairy animals supplemented with rbST appear to adjust their voluntary feed intake in relation to the additional nutrient required for increased milk yield.

In addition, rbST administration in dairy cows had no effect on nutrient digestibility when compared to control in entire lactation cycle and also no significant difference between cooled cow and non-cooled cow (Chanchai et al., 2010[b]). There were in agreement with other studies that carried out in lactating buffaloes (Khattab et al., 2008). Most of previous studies had been done nutrients digestibility on dairy cattle or buffaloes when administrated with rbST.

2.6 Effects of recombinant bovine somatotropin on nitrogen retention and allantoin level

Previous studies in nitrogen balance reported that beef and dairy cows receiving growth hormone excreted less urinary nitrogen (Bines et al., 1980) and secreted more nitrogen in milk (Bines et al., 1980; Chanchai et al., 2010[a]). Nitrogen retention of growing dairy heifers was increased by rbST administration in positive relationship with daily rbST dose (daily rbST dose: 0, 6.7, 33, 67, 100, 200 μg/kg body weight). The highest dose of rbST (200 μg rbST/kg BW/day) was 23% greater than compared with control (0 mg rbST/kg BW/day). Urinary N excretion decreased from 44 to 35% of N intake in consistent with rbST dose and the highest dose of rbST reduced 20% in excretion of urinary N (Crooker et al., 1990). These results have reflected the improved efficiency of protein used. Decrease urea nitrogen levels in blood was observed, indicating that whole body oxidation of amino acids was decreased with rbST treatment.

Purine derivatives (allantoin, uric acid, xanthine and hypoxanthine) were indicator for microbial protein production in rumen. Microbial organisms in rumen were the major source of protein to ruminants. The yields of microbial nitrogen ranged from 14 to 49g of microbial nitrogen/kg of organic matter (ARC, 1984). There was evidence that the efficiency of microbial protein supply was correlated with the ratio of dry matter intake per body weight (Chen et al., 1992). Besides, Schager et al. (2003) and Chanchai et al. (2010[a]) reported that there was an increase of allantoin secretion in milk after rbST supplementation in dairy cows.

2.7 Safety concerns of recombinant bovine somatotropin on animal and consumer

Recombinant bovine somatotropin raised milk yield and also the problems normally associated with high milk production. These include increased somatic cell counts and clinical mastitis approximately by 19.4% during treatment period (day 60 to 305 of lactation). These may be a result of the increased milk yield rather than a direct effect of rbST (Dohoo et al., 2003). There was also some evidences of increased lameness in rbST treated-cows (Dohoo et al., 2003). This may be due to negative effects of rbST on connective tissue and bone development.

Cows injected with rbST can take longer time to come into estrus after parturition than untreated cows, particularly if their body condition is not appropriate. Proper nutrient should be provided to improve body condition during early lactation. Number of days open also increased a few days in rbST treated cows and was related to the increases in milk yield (Butler and Smith, 1989). Similarly, Hard et al. (1988) summarized some studies with the same design and found that days from parturition to conception rose by 5 days in the rbST group. However, when data were stratified by level of milk yield, days open did not differ between control and rbST supplemented cows. Thus, influences of greater milk yield on reproductive performance are the same regardless of rbST used.

Bovine ST is thought to have no oral activity in adult humans and is destroyed by digestion. However, young infants can absorb intact proteins. Bovine IGF-I has the same sequence as human IGF-I, but levels in milk are only increased by abnormally high doses of rbST and are not above those found in human breast milk (Squires, 2003). Both rbST and IGF-I will be partially denatured by heat during pasteurization. It is estimated that spray-drying and pasteurization process could reduce up to 95% of rbST in milk (Le Breton et al., 2010).

2.8 Role of leptin on feed intake and energy balance

Leptin is protein hormone secreted from the adipose tissue into the circulation. This hormone meets the criteria of adiposity signals which play an important role for the control of feed intake and body weight. Firstly, the concentration of plasma leptin has positive relationship with body fat mass. Secondly, exogenous leptin reduces body weight, feed intake, and rises energy expenditure. Thirdly, leptin signals to the brain after release by the adipocyte and gives information about the status of the body energy reserves. Leptin receptors are located in several hypothalamic nuclei. Leptin not only influenced on feed intake and body weight, but also affected on other physiological functions such as: reproduction, the immune and inflammatory response, angiogenesis by various biological mechanisms (Schwartz et al., 2000; Wood and Seeley, 2000; Kershaw and Flier, 2004). In addition to the extensive investigation of leptin effect found in rat and mouse, the effect of leptin on feed intake has also been studied in dairy animal (Blache et al., 2000; Leury et al., 2003; Liefers et al., 2003; Whitley et al., 2005). Voluntary feed intake decreased approximately one third after 3 days leptin administration in ewes (Henry et al., 1999). However, the anorectic effect of leptin was lost when growing and adult sheep were underfed (Morrison et al., 2001). In crossbred dairy cattle, plasma leptin was lower in rbST treatment compared with control. The lower of plasma leptin in rbST treated cows was associated with an increase of feed intake (Chanchai et al., 2010[a]). These results suggested that the effect of rbST on feed intake appeared to be mediated partly by influence of plasma leptin.

CHAPTER III
MATERIALS AND METHODS

3.1 Location and environmental temperature of the study area

The experiment was carried out at Rajamankala University of Technology, Nan Province, Thailand. The experiment was conducted during January to March 2011. The average temperature in the shed was 20.4 ± 0.5°C at 9:00 am to 31.5 ± 0.7°C at 3:00 pm and temperature humidity index was 68.8 ± 0.7% at 9:00 am to 80.4 ± 0.9% at 3:00 pm throughout the experiment.

3.2 Animals and management

Ten multiparous crossbred Saanen lactating goats, average body weight (BW) 29.2 ± 2.4 kg, were used in this experiment. One month before parturition, all animals were kept in individual metabolic cages for adaptation. They were *ad libitum* fed with total mixed ration, containing corn silage and mixture of concentrate, shown in Table 3.1. They were fed twice daily at 07:30 am and 3.30 pm and had free access to water. After parturition, the animals were randomly divided into two groups, five animals in each group. The first group was injected subcutaneously with 396 mg sesame oil for control, while the second group was injected with 250 mg rbST, suspended in 396 mg sesame oil for prolonged–release action (POSILAC, Monsanto, USA). Both groups were injected at two weeks interval.

Table 3.1 Feed ingredients of the ration

Ingredients	(as% DM basis)
Corn silage	46
Corn ground	30
Soybean meal	20
Molasses	3
Mineral	1
Total	100

3.3 Data collection and measurements

Feed samples were collected once a week throughout the experiment and dried in microwave until constant to determine dry matter. The other subsamples of feed were kept frozen at -20 ^{0}C for later chemical analysis. All feed samples were mixed thoroughly and subsamples were dried at 65 ^{0}C overnight (about 12h) and analyzed for dry matter, nitrogen and ash according to AOAC (1990), NDF and ADF by the procedure of Van Soest et al. (1991). Milk yield was daily recorded twice at 07:00 am and 3:00 pm from parturition to day 35 postpartum.

After parturition all goats were weighed before morning feeding, once a week throughout the experiment. The experiment was done in 35 days, consisting of two periods (Figure 3.1), pre-treatment period from parturition to day 6 postpartum and treatment period from day 7 to day 35 postpartum. Injection of sesame oil and rbST was done on day 7 (first injection) and day 22 (second injection) after parturition. The digestibility measurement was done from day 25 to day 35 by total fecal collection method (Schneider and Flatt, 1975).

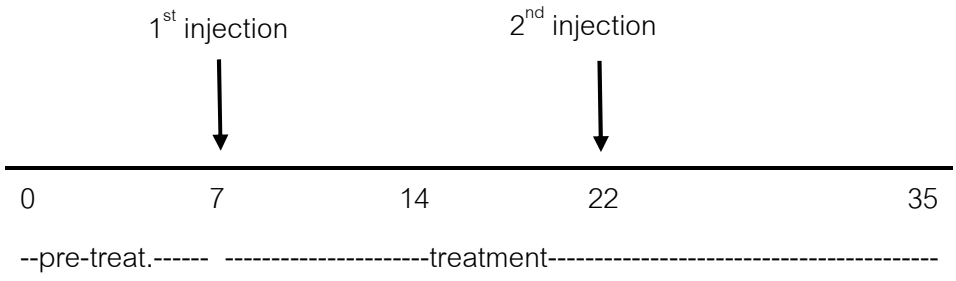

Figure 3.1 Experimental period.

3.4 Determination of dry matter intake, water intake and nutrient digestibility

Feed offered and feed refusals were daily recorded in the morning starting from parturition to day 35 postpartum. Daily dry matter intake was calculated by following formula:

Daily feed intake = feed offered - feed refusals (Dry matter basis)

Water intake (WI) was daily measured from parturition to day 35 postpartum and calculated by an average of each period (pretreatment, first and second injection period). The measurement of water intake was performed by subtracting the weight of water offered with the weight of water refusal.

Total fecal collections were daily performed during day 25-35. Subsamples (about 10% of total amount) of feces were collected and dried in microwave until constant weight to determine dry matter. Another subsample of feces was performed and kept at -20 ^{0}C for later analysis. The fecal samples were analyzed for DM, N, Ash, NDF and ADF levels.

Nutrient digestibility was determined by:

$$\text{Digestibility of nutrients (\%)} = \frac{\text{Nutrients in feed intake} - \text{Nutrients in feces}}{\text{Nutrients in feed intake}} \times 100$$

Efficiency of feed utilization = MY / DMI

Where, MY = milk yield (kg/animal/day), DMI = dry matter intake (kg/day).

3.5 Milk composition

30ml of milk sample was daily collected from each animal at the last five days (d31-d35) of second injection period, preserved with 0.1 g potassium dichromate and kept in the refrigerator until analysis. Milk samples were analyzed for total solids, solid no fat, fat, lactose and protein by using Milkoscan 133B (N. FOSS ELECTRIC. DENMARK).

3.6 Urine collection

Total urine was collected and measured on the same days as feces by using plastic containers with amount 10% sulfuric acid solution (13ml H_2SO_4 10% in 100ml urine) added to prevent nitrogen loss (final pH of urine was kept below 3). The total urine from each day was then collected at 10% of total volume, and 100ml of deionized water was added to the collection vessels to minimize the crystallization of urinary compounds. After that, all samples were kept in a refrigerator and pooled at the end of each period to be analyzed for nitrogen by the Kjeldahl method (AOAC, 1990) and for allantoin by colorimetric method according to Young and Conway (1942).

To determine the nitrogen retention the following formula was performed:
Nitrogen retention = Total nitrogen in feed intake – (total nitrogen in feces + total nitrogen in urine + total nitrogen in milk)

3.7 Determination of plasma glucose, leptin and IGF-I

Blood samples were collected twice a day at 9:00 am and 9:00 pm, starting from a day before the first injection (d6) until d21. The samples were obtained from the jugular vein, placed in EDTA tube, kept in crushed ice and then centrifuged at 3,000 rpm for 10 minutes. Total plasma was removed and plasma glucose was determined immediately by ACCU-CHEK® Advantage glucose meter (Roche Interamericana S.A). The rest of plasma samples were stored at -20 ^{0}C until analysis. Plasma leptin concentrations were determined using a radioimmunoassay kit specific for multi-species

hormone from d6 to d20 (Linco Research, Inc., USA) as described by Zhongmin and coworker (Zhongmin et al., 1996). Plasma IGF-I was determined by the IMMULITE® analyzer (DPC, Los Angeles, CA). The IMMULITE® analyzer is automated chemiluminescent immunoassay for the quantitative determination of the plasma IGF-I concentration before and after 24 hours of first rbST injection.

3.8 Statistical analysis

All data were reported as the mean value ± SE. The data were analyzed using repeated measurement ANOVA model. The mean differences between treatments were tested by the Bonferroni test and Sequentially-Rejective Bonferroni test. Differences were considered significant when $P < 0.05$.

CHAPTER IV
RESULTS

4.1 Chemical composition of experimental diet

The chemical analysis of experimental diet was shown in Table 4.1. The diet contained 23.8% CP, 33.4% NDF, 19.9% ADF and 2.71 Mcal/kg DM of ME (calculated from NRC, 1981) respectively.

Table 4.1 Chemical analysis of experimental diet

Items	(% DM basis)
DM	41.8
CP	23.8
NDF	33.4
ADF	19.9
ME (Mcal/kg DM)*	2.71

* Calculated from NRC (1981)

4.2 Effects of rbST on body weight, dry matter intake, water intake, dry matter intake/body weight and water intake/dry matter intake

The effects of rbST on BW, DMI, WI, DMI/BW were shown in Table 4.2. There was no significantly difference in BW in both treatments. The DMI slightly decreased in rbST group in comparison with control group and ranged from 1.22 to 1.32 kg DM/day. However, significant difference was found in DMI/BW or DMI/$BW^{0.75}$ after both injections ($P<0.05$). DMI/BW for control versus rbST were 46.1 versus 42.3 g/kg BW during first injection and 46.6 versus 41.5 g/kg BW during second injection, respectively. When average 2 days of DMI/BW was calculated, it was found that DMI/BW of rbST treated group was lower than control group ($P<0.05$). Interestingly, the effect of rbST on DMI/BW apparently occurred during the first week after injection. The difference occurred on day 11-12, day 13-14 and day 15-16 for first injection (4-8 days after injection) and day 27-

28, day 29-30 for second injection (6-8 days after injection) (Figure 4.1). No difference was found after 8 days of injection. There was a significant difference between treatments in water intake during second injection period. An increase of WI found in rbST group compared with control group, the control versus rbST were 3.14 versus 3.60 kg/day during first injection and 3.71 versus 4.56 kg/day during second injection, respectively. Water intake/DMI did not statistically differ between treatments at pretreatment and first week after first injection period (Table 4.3). However, the significant difference was found at second week after first injection and second injection period ($P<0.05$). Water intake/DMI in rbST group was greater than the control group, particularly during second injection.

Table 4.2 Effects of rbST on body weight, dry matter intake, water intake, dry matter intake/body weight in control and treated animal (means ± S.E.)

	Pretreatment	Treatment	
		1st injection	2nd injection
Body weight, kg			
Control	28.3 ± 3.5	28.6 ± 3.3	28.3 ± 2.7
Treatment	30 ± 3.6	29.7 ± 3.4	29.1 ± 3.6
P-value	0.7	0.8	0.9
Dry matter intake, kg DM/day			
Control	1.28 ± 0.08	1.32 ± 0.04	1.29 ± 0.03
Treatment	1.32 ± 0.07	1.25 ± 0.05	1.22 ± 0.05
P-value	0.74	0.29	0.28
DMI/BW, g/kg BW			
Control	44.5 ± 1.3	46.1 ± 0.9	46.6 ± 0.6
Treatment	43.8 ± 1.1	42.3 ± 0.7	41.5 ± 0.9
P-value	0.7	0.01	0.01
$DMI/BW^{0.75}$, g/kg BW			
Control	59.4 ± 1.8	61.5 ± 1.4	62.1 ± 1.4
Treatment	58.4 ± 1.4	56.5 ± 1.2	55.3 ± 1.3
P-value	0.7	0.01	0.01
Water intake, kg/day			
Control	2.60 ± 0.17	3.14 ± 0.20	3.71 ± 0.33
Treatment	2.35 ± 0.14	3.60 ± 0.14	4.56 ± 0.24
P-value	0.26	0.06	0.04

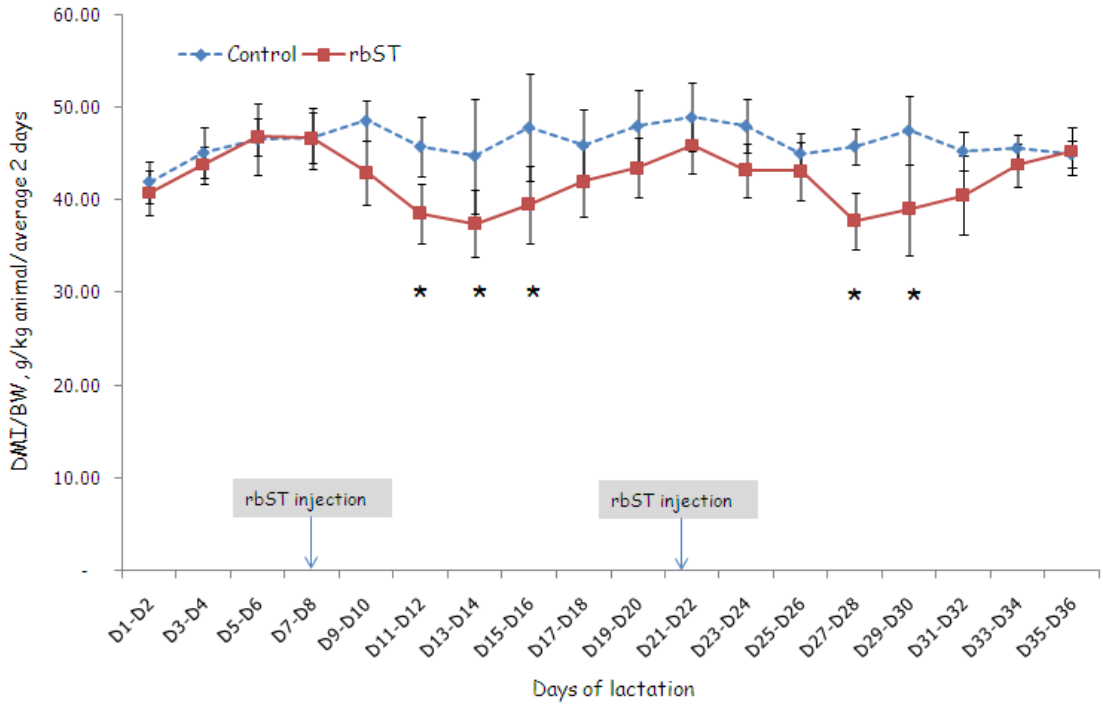

Figure 4.1 Effects of rbST on average 2 days of DMI/BW in dairy goat during early lactation

*: $P < 0.05$

Table 4.3 Effects of rbST on water intake/DMI in treated and control animals

	Week	Control	rbST	P-value
Pretreatment	Wk1	2.12 ± 0.07	1.93 ± 0.06	0.40
First injection	Wk2	2.33 ± 0.07	2.58 ± 0.08	0.12
	Wk3	2.32 ± 0.22	3.43 ± 0.16	0.01
Second injection	Wk4	2.90 ± 0.35	3.58 ± 0.07	0.04
	Wk5	2.76 ± 0.39	3.67 ± 0.20	0.03

4.3 Effects of rbST on milk yield and feed efficiency of lactating goats

The milk yield of treated goats gradually increased after rbST supplementation in first and second injection periods. The average improvement of milk yield in response with rbST supplementation was approximately 4% for first injection up to 15% for second injection in comparison with control group (Figure 4.2). However, the significant difference ($P<0.05$) appeared during last week of second injection period, control versus rbST: 1.20 versus 1.46 kg/day respectively (Figure 4.3). Similarly, feed efficiency was greater in rbST treated animals than in control animals, particularly in second week of first injection and weeks after second injection, control versus rbST: 0.98 versus 1.09 kg milk/kg DMI for first injection; 0.93 versus 1.18 and 0.93 versus 1.20 kg milk/kg DMI for second injection, respectively (Figure 4.4). In addition, average 2 days of feed efficiency differed significantly between treatments ($P<0.05$) and feed efficiency in rbST group was greater than in control group at both injections. The significant difference of average 2 days appeared on day 13-14 and day 15-16 for first injection and from day 23-24 to the last day of second injection (Figure 4.5).

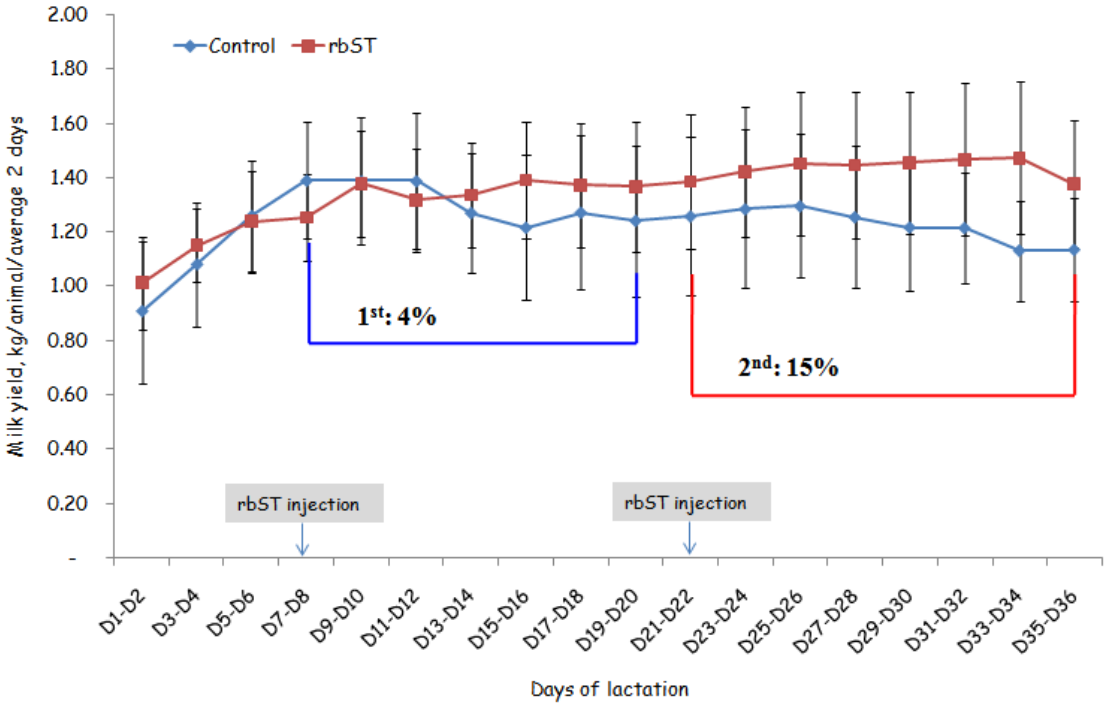

Figure 4.2 Effects of rbST on average daily milk yield in dairy goat during early lactation

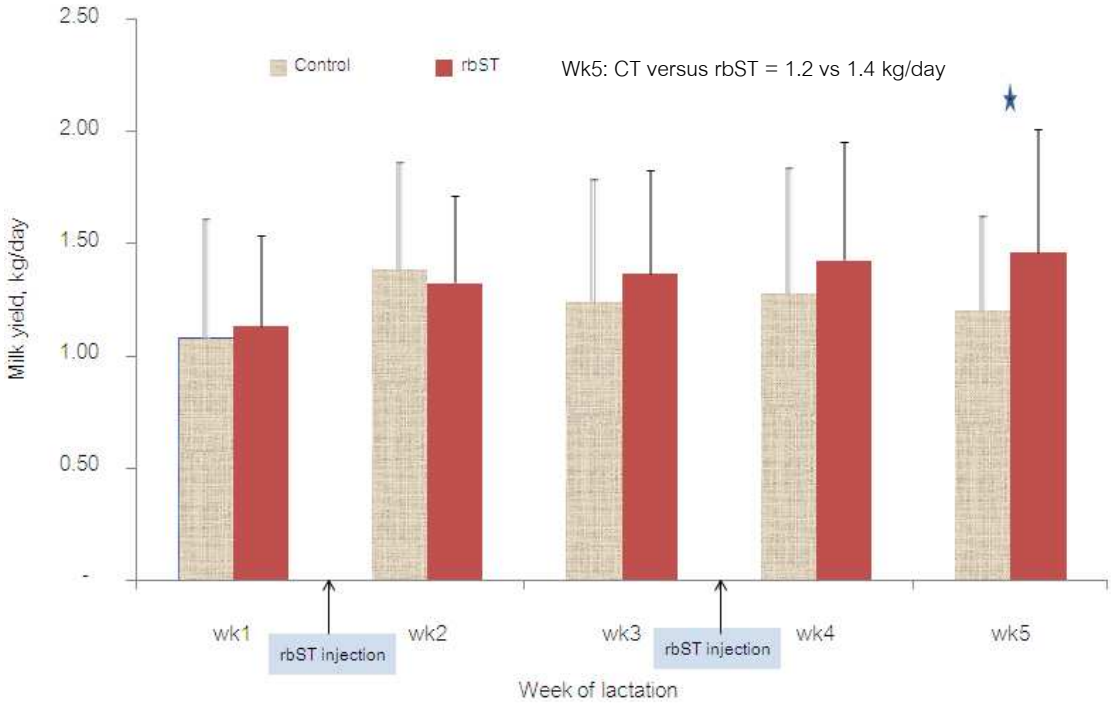

Figure 4.3 Effects of rbST on milk yield (kg/day) of lactating goats

*: P < 0.05

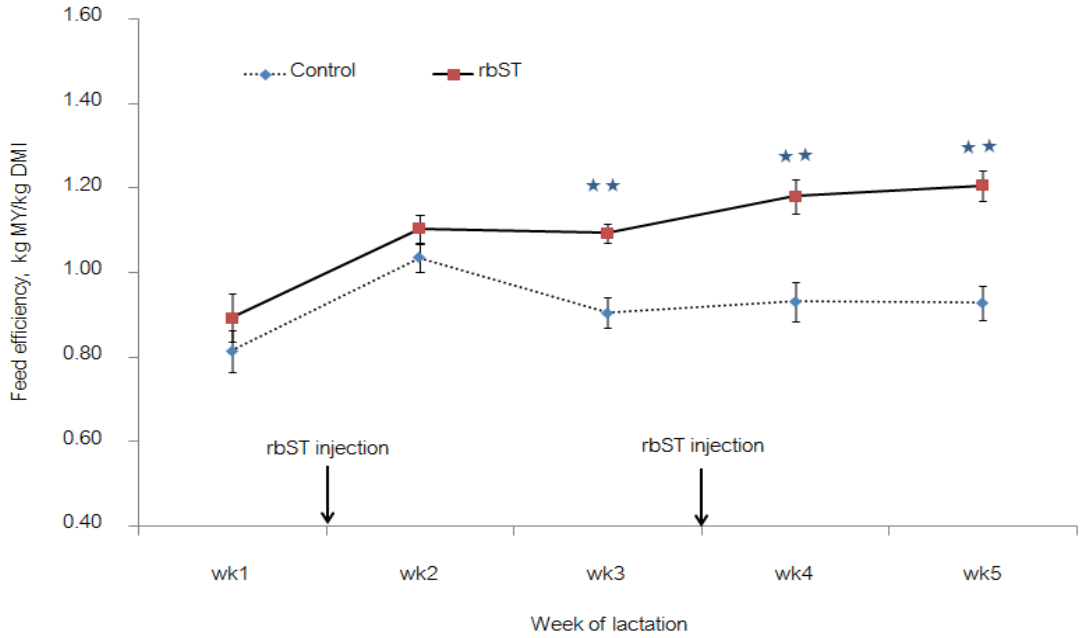

Figure 4.4 Effects of rbST on feed efficiency (kg MY/kg DMI) of lactating goats

**: P < 0.01

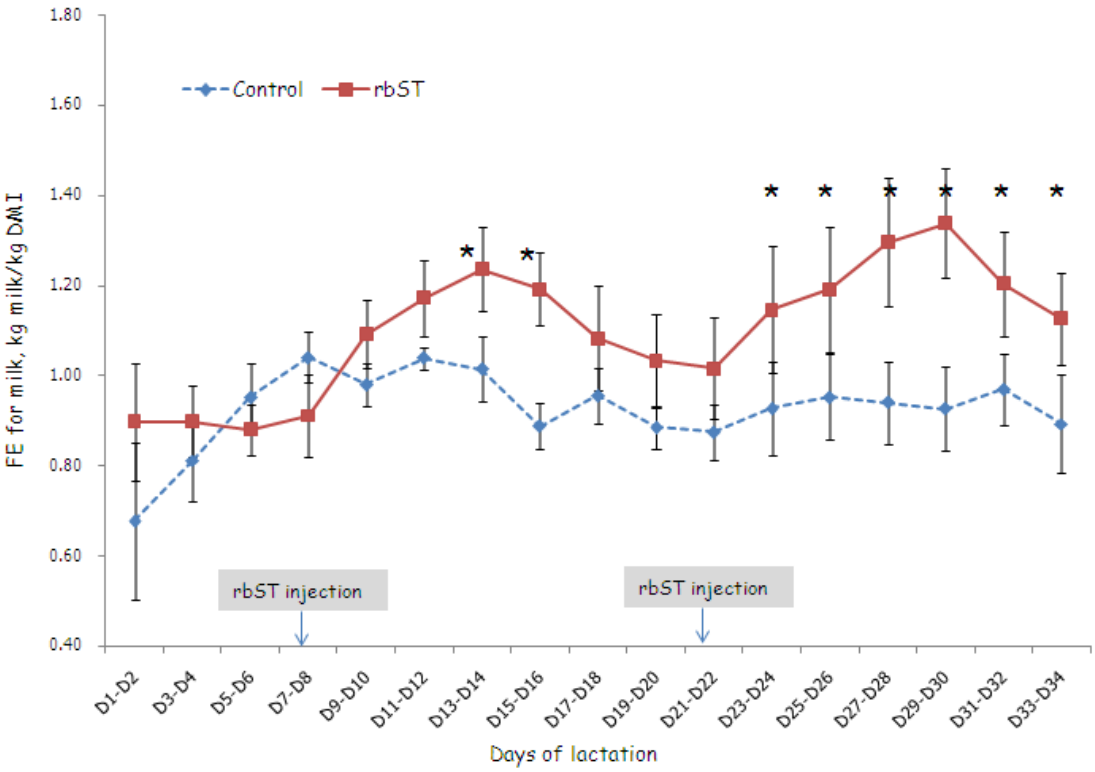

Figure 4.5 Effects of rbST on average 2 days of feed efficiency in dairy goat during early lactation

*: $P < 0.05$

4.4 Effects of rbST on milk composition

The effects of rbST on milk components for control and treated animals were shown in Table 4.4. There were no significant differences in the major milk compositions after rbST supplementation in comparison with control group.

Table 4.4 Effects of rbST on milk components in control and treated animal (means ± S.E.)

Items (%)	Control	Treatment	P- value
Total solid	13.9 ± 0.2	13.8 ± 0.2	0.6
Protein	3.4 ± 0.1	3.4 ± 0.1	0.5
Fat	4.9 ± 0.2	4.9 ± 0.2	0.7
Lactose	4.7 ± 0.1	4.8 ± 0.1	0.4
Solid not fat	8.8 ± 0.1	8.9 ± 0.1	0.3

4.5 Effects of rbST on nitrogen intake, nitrogen utilization and urinary allantoin

Nitrogen intake and utilization in control and rbST treated animal were shown in Table 4.5. rbST treatment had no significant effect on nitrogen intake, absorption, retention and excretion in feces, urine and milk secretion.

Table 4.5 Nitrogen intake and utilization in control and rbST treated animal (means ± SE.)

Nitrogen (g/day)	Control	Treatment	P-value
Intake	48.8 ± 4.3	45.3 ± 7.7	0.7
Fecal excretion	10.9 ± 1.5	9.7 ± 1.6	0.6
Absorbed	37.9 ± 3.2	35.6 ± 6.2	0.8
Urinary excretion	11.9 ± 2.3	13.2 ± 2.7	0.7
Milk secretion	6.6 ± 2.8	7.9 ± 3.2	0.5
Retained	19.3 ± 2.1	14.5 ± 3.9	0.3

Urinary allantoin in control and treated animal were shown in Table 4.6 Supplementation with rbST did not influence urinary allantoin. Urinary allantoin levels for control versus treatment were 370.8 versus 438.7 mg/animal/day or 2.4 versus 2.8 mmol/animal/day, respectively.

Table 4.6 Effects of rbST on urinary allantoin in control and treated animal (means ± SE.)

	Control	Treatment	P-value
Allantoin, mg/animal/day	370.8 ± 40.6	438.7 ± 41.2	0.30
Allantoin, mg/$BW^{0.75}$	28.9 ± 1.8	32.3 ± 2.4	0.26
Allantoin, mmol/animal/day	2.4 ± 0.3	2.8 ± 0.3	0.30

4.6 Effects of rbST treatment on total tract nutrient apparent digestibility

The digestibility of nutrients of diet were unaffected by treatment (Table 4.7). The mean values of apparent digestibility (%) for control and treated groups were 77.5 ± 1.4 to 77.6 ± 1.0 for dry matter, 79.5 ± 1.3 to 79.6 ± 1.0 for organic matter, 77.6 ± 1.9 to 78.3 ± 1.3 for crude protein, 52.8 ± 1.9 to 54.9 ± 2.2 for acid detergent fiber and 58.0 ± 2.3 to 58.3 ± 2.6 for neutral detergent fiber, respectively.

Table 4.7 Effects of rbST treatment on total tract nutrient apparent digestibility (means ± SE.)

Item	Control	Treatment	P-value
DMD, %	77.5 ± 1.4	77.6 ± 1.0	0.9
OMD, %	79.5 ± 1.3	79.6 ± 1.0	0.8
CPD, %	77.6 ± 1.9	78.3 ± 1.3	0.9
NDFD, %	58.3 ± 2.6	58.0 ± 2.3	0.9
ADFD, %	54.9 ± 2.2	52.8 ± 1.9	0.5

4.7 Effects of rbST on plasma glucose, IGF-I and plasma leptin of lactating goats

In this experiment there was no significantly difference in plasma glucose concentration at the pretreatment period, but plasma glucose concentration was higher ($P<0.05$) in rbST treated animals than in control animals for the first and second week after first rbST supplementation (Figure 4.6). Specifically, plasma glucose level significantly differed ($P<0.05$) after 24 hours rbST injection (Figure 4.7). The overall mean of plasma glucose in control group and rbST group were 49.99 versus 52.93 mg/dl, respectively.

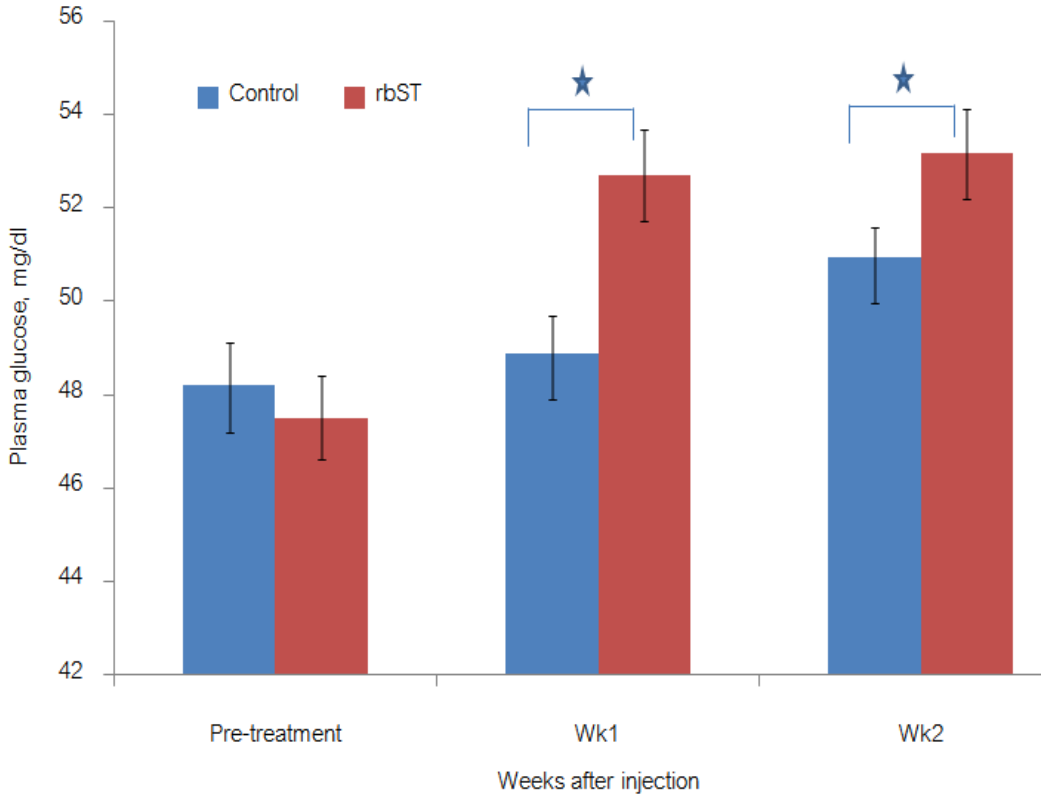

Figure 4.6 Effects of rbST on plasma glucose of lactating goats

*: P < 0.05

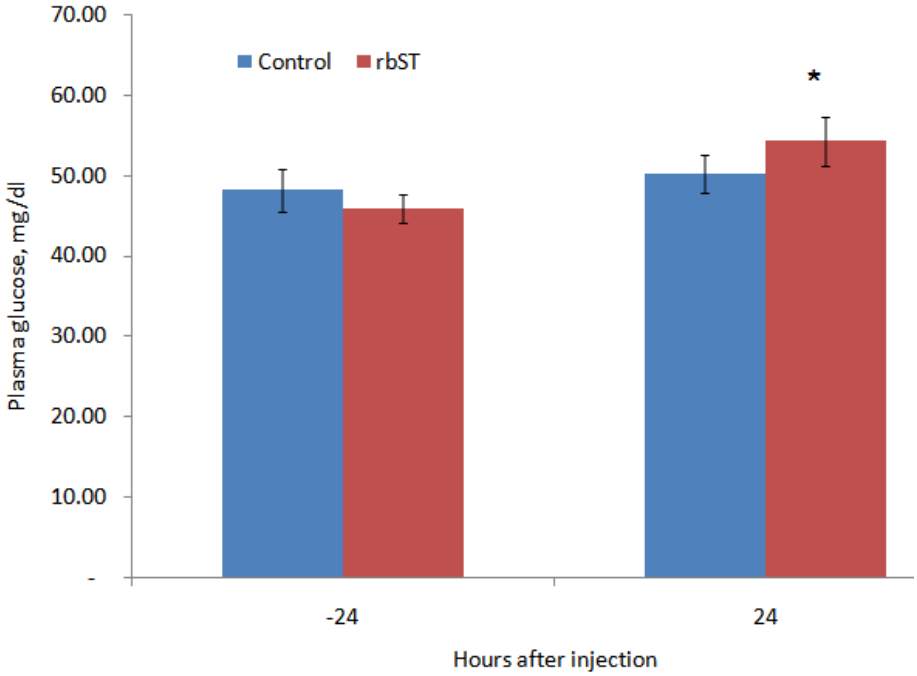

Figure 4.7 Effects of rbST on plasma glucose of lactating goats before and after 24 hours of injection

*: P < 0.05

There was no significant difference of plasma IGF-I level before 24 hours of injection between rbST group and control group, whereas plasma IGF-I concentration in rbST supplementation significantly increased ($P<0.05$) after 24 hours of injection (Figure 4.7). The plasma IGF-I in rbST treatment was higher three times than in control, the control versus rbST: 48.4 versus 160.3 ng/ml, respectively.

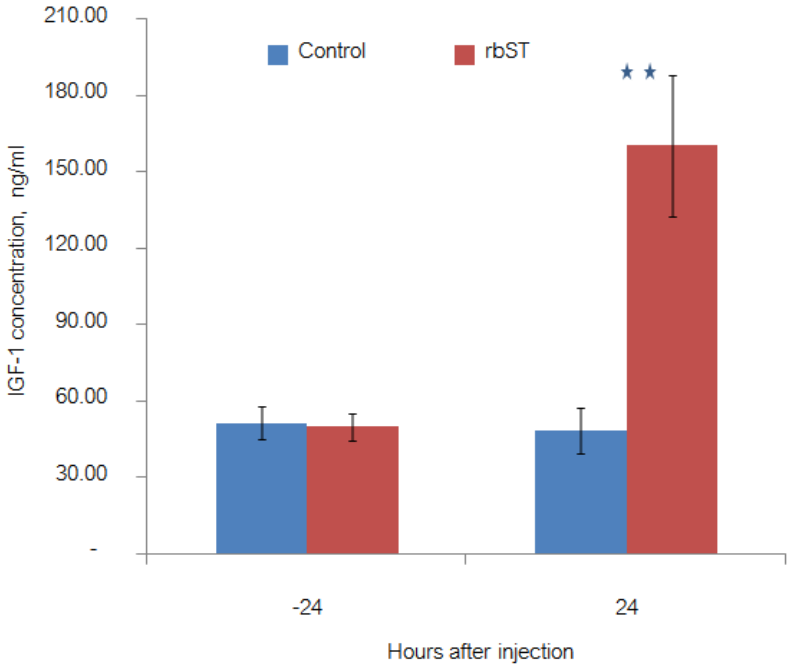

Figure 4.8 Effects of rbST on plasma IGF-I of lactating goats

**: $P < 0.01$

Plasma leptin level did not differ between treatments on day 6 (a day before rbST injection). However, the significant difference appeared on day 9-10, day 15-16 after rbST injection ($P<0.05$) and plasma leptin concentration in rbST group was greater than in control group (Figure 4.9). The overall mean values (day 7-20) of plasma leptin between treatments were 1.54 versus 2.04 ng/ml for control versus rbST, respectively.

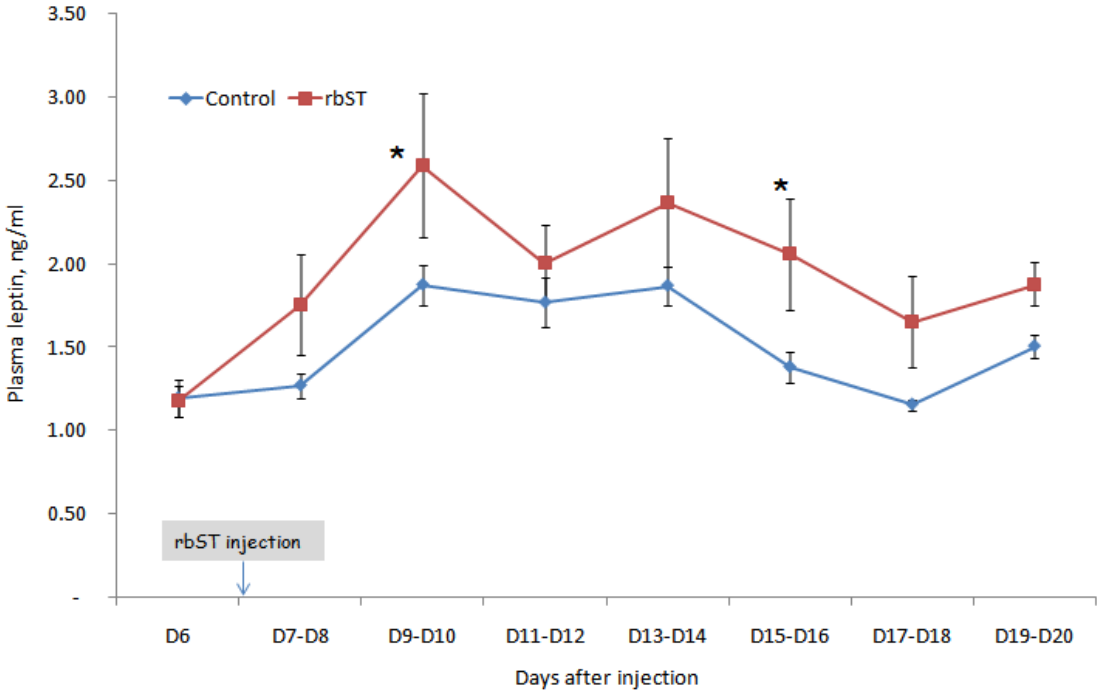

Figure 4.9 Effects of rbST on average 2-days of plasma leptin in lactating goats

CHAPTER V
DISCUSSION

Recombinant bST supplementation animals showed lower DMI/BW than control animals. The lower DMI/BW after rbST supplementation in the present experiment appeared to come mainly from the effect of rbST on feed intake because there was no difference in body weight. In addition, a decrease in DMI/BW was 8% and 11% for the first and second rbST injection, respectively. The present experiment found that the decrease of DMI/BW followed by the increase of plasma leptin concentration after rbST supplementation. High level of plasma leptin would activate the satiety centre in the hypothalamus and this reduced feed intake in the animal (Schwartz et al., 2000). This result was similar to previous report in which supplemented with rbST and clenbuterol during early lactation in dariy cow (Bareille et al., 1997). However, in dairy goat and sheep, rbST supplementation during early lactation had no effect on feed intake (Disenhaus et al., 1995; Sallam et al., 2005). Previous studies in Thailand reported that long term trial with rbST supplementation increased DMI in dairy cattle (Chanchai et al., 2010[c]; Boonsanit et al., 2010) and during late lactation in dairy goat (Polratana et al., 2004). Further, ST supplementation produced either no effect or increased feed intake during the anabolic state in mature rat (Byatt et al., 1993; Azain et al., 1995), whereas ST supplementation decreased feed intake during the catabolic state in old and obese rat (Malmlof et al., 2002). Collectively, supplementation with rbST during lactation may produce no effect, increase or decrease feed intake in animals. The difference outcome of somatotropin effect on feed intake during different physiological status suggested that the effect of rbST supplementation in feed intake depend on multiple factors such as body metabolic status, dietary nutrient, etc.

Somatotropin may influence feed intake by it direct effect via somatotropin receptor or indirectly via IGF system. The transgenic mouse models revealed that the direct effect of somatotropin produced both increase and decrease in feed intake. Bovine somatotropin over expression mice (bGH mouse model) ate slightly less and somatotropin receptor knockout mice ate more than their wild type control (Berryman et

al., 2006). The studies on central nervous system specific over expression of bovine somatotropin (GFAP-bGH) mouse model revealed that the GFAP-bGH mice ate more than their wild type control (Bohlooly-Y et al., 2005). The present experiment did not exclude the possibility that supplementation with rbST had any direct effect on feed intake. Importantly, the present experiment demonstrated that plasma IGF-I and leptin were greater in rbST group than in control group after 24 hours or one day of rbST supplementation, whereas DMI/BW in rbST group was lower than in compare with control group after 3-4 days of rbST supplementation. So, the significant day of plasma IGF-I and leptin were earlier than the feeding behavior effect. This suggested that the effect of rbST on feed intake may describe partly by the effect of somatotropin and IGF-I on adipose tissue and plasma leptin level.

Recombinant bST increased plasma leptin in the present experiment. This was contrast with previous experiment in dairy cows. The long-term treatment of dairy cows with rbST decreased plasma leptin (Chanchai et al., 2010[a]). The reason for this contradiction may be due to the different period of time for rbST injection. In the present study, the animals were injected rbST on day 7 after parturition, whereas Chanchai et al. (2010[a]) and Boonsanit et al. (2010) injected rbST for their animal on day 60 postpartum. Chanchai et al. (2010[a]) found that rbST supplementation in dairy cow reduced plasma leptin level and followed by the increase of feed intake. Collectively, the current results revealed that rbST supplementation on day 7 postpartum increased plasma leptin and subsequently decreased feed intake.

There are many factors that affect water intake in dairy animal such as environmental temperature and milk yield. In addition, milk components contained about 87% of water. The finding by Dewhurst et al. (1998) reported that milk yield was positive relationship with water intake. Thus, it appears that higher milk yield would require higher water intake. Higher water intake was found in rbST group in the current study, particularly in the second injection ($P<0.05$).

It is evident that GH influences a lot of physiological processes in many tissues and organs so that more nutrients could be used for milk synthesis and fewer nutrients were deposited in adipose tissue (Etherton and Bauman, 1998). In the current experiment, the milk yield was significant difference during last week of second injection. The increment of milk yield was not a direct response to the DMI because DMI slightly decreased in rbST treated animals. The present study found that the increase of milk yield followed by an increase of IGF-I after rbST injection. It has also been demonstrated that infusion of IGF-I to the mammary artery significantly increased milk production (Etherton and Bauman, 1998). Another reason was that the amount of mammary parenchyma was increased by bST treated goats (Vernon et al., 1995). In addition, rbST supplementation increased mammary blood flow and nutrient uptake (Chaiyabutr et al., 2005 and 2007). The increase of milk yield in the current experiment appeared to explain by GH-IGF axis. The rbST response to milk yield in this study was similar with study of Chadio et al. (2000). They reported that the administration of rbST in dairy goat during early lactation increased milk yield by 12.6% from day 56 to day 84 after parturition experiment. However, Moallem et al. (1997) found that milk yield did not differ between treatments during the first 60 days postpartum when the cows were injected with 500 mg of bST/14 days from 10 to 150 after parturition. It is likely that rbST may not influence milk yield in treated animals during early stage of lactation which was similar to the first injection of this study (from day 7 to day 21 postpartum). In addition, average milk yield response to rbST supplementation in this study was lower than study of Disenhaus et al. (1995) (15% versus 28.6%). This difference may be due to the different breeds used or time for rbST injection, at one week of lactation for this study versus eight week of lactation for Disenhaus's experiment respectively.

The nutrient requirements for maintenance and the nutrient requirements per unit of milk produced by the dairy animals were not changed by rbST treatment (Sechen et al., 1989). However, the increase in milk production resulted in an overall increase in feed efficiency. This study demonstrated that the feed efficiency was higher in rbST group than in control group ($P<0.05$) (control versus rbST: 0.98 versus 1.10 for first injection and 0.93 versus 1.21 kg of milk/kg DMI for second injection, respectively). The

reason for increase of feed efficiency in rbST group mainly came from lower feed intake in rbST group. Because milk yield did not significantly differ between treatment from parturition to week 4 postpartum. Similar finding reported by Chadio et al. (2000), they injected 160 mg rbST at 14-day interval starting from 8th week of lactation and showed that rbST treatment produced more fat corrected milk (FCM) than control group. The increase in FCM resulted in enhanced feed efficiency for rbST goats, as dry matter intake did not increase above those of control goats. Overall, this experiment indicated that rbST supplementation in crossbred dairy goat could improve the feed efficiency during early lactation.

The rbST administration did not affect on milk composition in current study and consistent with earlier studies (Etherton and Bauman, 1998; Polratana et al., 2004). Similarly, Santos et al. (1999) found that milk components in dairy cows were not affected by treatment for 45 days after parturition. However, other studies found that there was a slight increase in milk fat followed by the slight increase of plasma NEFA concentration after rbST treatment in dairy cattle (McDowell, 1991; Chaiyabutr et al., 2007) and dairy goat after rbST supplemented during first and second week (Disenhaus et al., 1995; Chadio et al., 2000). In addition, the temporary increase in milk fat could relate to an increase in the mobilization of long chain fatty acids from body reserves when dairy cows were in negative energy balance, whereas milk fat did not affect by rbST supplementation when dairy cows were in positive energy balance (Bauman, 1992). In the present study, body weight did not alter between treatments during the experimental period. It suggested that dairy goats in this study were not lost their body weight via mobilization of body reserve for milk synthesis. Overall, the change in milk components after rbST supplementation could involve several factors such as stage of lactation, energy status and dietary nutrition (Etherton and Bauman, 1998)

In the present study, nitrogen intake and utilization did not differ between treatments. These findings were similar with previous studies (Peel et al., 1981; Sechen et al., 1989). Nitrogen utilization was partly influenced by urinary nitrogen excretion. A previous experiment in lactating cows found that bST supplementation was lower than

the urinary nitrogen output (Tyrrell et al., 1988). The absence of a significant decrease in urinary excretion during rbST injection may be due to a higher water intake followed by higher volume of urine in this experiment (Table 1 in appendix). Although, the increase in milk nitrogen secretion was not significant. It has been known that growth hormone increased more nitrogen output in milk (Bines et al., 1980). Because milk yield in rbST group did not statistically differ during first injection and first week of second injection in the present study and greater milk yield in bST injection was found in study of Bines et al. (1980).

In dairy animal, allantoin can be found from two sources, milk and urine. The urinary allantoin excretion was the main source and the amount of milk allantoin accounted for only minor ratio of the total allatoin excretion, ranging 0.60 to 2.34% (Giesecke et al., 1994; Gonda and Lindberg, 1997). Moreover, the urinary excretion of allantoin is considered as a method to determine microbial protein supply in ruminants. In the current study, amount of urinary allantoin excretion in treated animal was slightly higher than in control animal, however the difference was not significant. In addition there was not difference in urinary allantoin concentration between treatments. The higher urinary allantoin excretion in rbST group appeared to come from the higher urine volume (Table 1 in appendix). Other studies in dairy cattle found that there was an increase of milk allantoin secretion after rbST supplementation. In addition, the same experiments also reported that there was an unchanged or increased DMI between treatments (Schager et al., 2003; Chanchai et al., 2010[a]). However, there was lower DMI/BW in rbST group in the current experiment. The difference in allantoin excretion between previous and the current experiment may be due to difference in DMI. Chen et al. (1992) found that the efficiency of microbial protein supply was positively correlated with the ratio of DMI:BW.

No significant difference in diet digestibility has been found between treatments when the animal fed the same diet in the present study and the results was similar to the previous studies in dairy cows (Chanchai et al., 2010[b]) or lactating buffaloes (Khattab et al., 2008). The lack of rbST effect on diet digestibility suggested that there were

unchanged of the rate of passage, rumen microbial numbers and fermentation pattern by bST supplementation (Cheli et al., 1998).

The present result was shown that rbST enhanced plasma glucose concentration in treated animals. The increase in plasma glucose was observed 24 hours after rbST injection without any increase in milk yield and voluntary feed intake. The reason for higher plasma glucose in rbST group apparently came from 2 major mechanisms. First, it has been demonstrated that rbST supplementation increased gluconeogenesis process. Second, rbST supplementation decreased the ability of insulin effect and caused to induce insulin resistance. Insulin resistance caused reduction in glucose, acetate uptake and glucose oxidation by adipose tissue as well as skeletal muscle (Etherton and Bauman, 1998). Somatotropin induced-insulin resistance was caused partly by the higher IGF-I that can compete with insulin on insulin receptor (Burton et al., 1994). The present result was consistent with obvious studies (McDowell, 1991 and Knapp et al., 1992). In contrast, Disenhause et al. (1995) found that plasma glucose concentration remained unchanged in rbST treated goats.

Somatotropin was known to be the main hormonal factor to activate IGF-I secretion (Clemmons and Underwood, 1991). The plasma IGF-I level was increased within 24 hours rbST supplementation in the present study. This result was consistent with study of Cohick (1989). They reported that plasma IGF-I concentration began to increase about 6 to 12 hours after initial rbST injection and reached maximum concentration in approximately 48 hours. Similarly, other studies found that IGF-I level increased 2 or 3 folds after rbST supplementation (Zhao et al., 1994 and Castigliego et al., 2009).

In the current experiment plasma leptin concentrations significantly increased after rbST injection. Increase density of the nutrient in diet has been shown to have an effect on plasma leptin (Blache et al., 2000). However, plasma leptin concentrations were not influenced by the nutritional level in the diet in the current experiment. The same diet was used for the animals in the current experiment. In addition, Sauerwein et

al. (2004) reported that plasma leptin concentrations after GH treatment were decreased by 20% for pregnant cows, but not for non-pregnant cows. In current experiment, all animals were not pregnant. Thus, different leptin concentration in this experiment was mainly caused by rbST supplementation. The mechanisms that rbST increase plasma leptin appeared to be influence by plasma insulin and glucose. It has been demonstrated in insulin and glucose infusion experiment (hyperinsulinemic euglycemic clamp model) that insulin infusion increased approximately 13% of plasma leptin level during early lactation of dairy cows (Leury et al., 2003). However, Chanchai et al. (2010[a]) found that rbST supplementation in dairy cows reduced plasma leptin concentration. In Chanchai et al. (2010[a]) study, blood samples were collected on day two after the third rbST injection (long-term experiment), whereas in the present study blood samples were taken immediately after first rbST injection (short term experiment). So, the present experiment revealed the acute effect of rbST on leptin secretion.

In conclusion, the present study revealed that rbST supplementation in dairy goats during early lactation increased milk yield, feed efficiency, water intake. In addition, the increase of plasma IGF-I, glucose and leptin concentration was revealed after first rbST injection. However, supplementation with rbST did not affect on body weight, milk composition, nutrient digestibility, nitrogen and allantoin metabolism. The increment of milk yield was not a direct response to the DMI because the DMI/BW reduced in rbST treated animals. It was suggested from the results that the reduction in feed intake came indirectly from the mechanism that rbST supplementation increased plasma leptin level.

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APPENDIX

APPENDIX

Table 1 Volume of urine in rbST and control animals

Treatment	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	Mean
Control	0.53	0.71	0.74	1.10	0.83	0.95	0.58	0.73	0.84	1.04	0.81
Control	0.56	0.38	0.42	0.58	0.45	0.52	0.44	0.52	0.43	0.45	0.48
Control	4.20	3.24	4.94	4.52	3.74	6.40	5.39	1.72	4.64	4.36	4.32
Control	0.46	0.61	0.47	0.58	0.64	0.60	0.74	0.98	1.24	1.06	0.74
Control	0.34	1.66	1.22	1.00	2.52	1.72	2.06	2.11	2.31	1.00	1.59
rbST	3.47	1.92	2.02	3.16	2.02	3.04	2.74	2.84	3.32	4.48	2.90
rbST	1.65	2.18	2.07	1.59	0.82	1.08	1.20	2.29	2.16	2.30	1.73
rbST	2.56	2.11	2.28	2.08	1.28	0.54	0.51	0.84	1.88	2.14	1.62
rbST	1.52	1.18	4.18	4.32	4.82	5.36	4.20	4.77	3.88	3.73	3.80
rbST	0.83	2.14	1.37	2.58	1.91	1.26	1.09	1.84	1.78	2.27	1.71

BIOGRAPHY

Mr Nguyen Thiet was born on 24 December 1982 in Thai Thuy district, Thai Binh province, Vietnam. He attended university in 2000 and earned his Bachelor degree in Animal Husbandry that was awarded on 15 August 2005 by Rector of Can Tho University, Can Tho city, Viet Nam. After graduation, Mr Thiet has been worked at Department of Animal Husbandry, College of Agriculture and Applied Biology, Can Tho University. He has been working there as a researcher and teaching assistant as well as a technician for animal feed laboratory. In 2009, he applied and was awarded a master scholarship from Chulalongkorn University, Thailand in the program of “Scholarship Programs for Neighboring Countries”. He studied in the field of Animal Nutrition at Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University. In order to fulfill requirements for his master degree he carried out the thesis entitled “THE EFFECT OF BOVINE SOMATOTROPIN ON MILK PRODUCTION, NUTRIENT DIGESTIBILITY AND PLASMA LEPTIN OF LACTATING GOAT DURING EARLY LACTATING PERIOD” as a partial need.