

# SUPPLEMENTATION BOVINE SOMATOTROPIN EFFECTS ON MILK PRODUCTION, LEVELS OF METABOLITES AND HORMONE METABOLISM ON LACTATION HOLSTEIN COWS KEPT IN THE TROPICAL HIGHLANDS

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## Abstract

*The use of recombinant somatotropin (bST) recommended to increase milk production has not been used widely in Indonesia so it is necessary to study somatotropin supplementation effects on milk production, metabolite and hormone metabolism. A total of 24 Holstein cows in lactation maintained in the tropical highlands has been used in this study. The study by using randomized complete block design with 3x2 factorial pattern should have been elaborated. The first factor of bST 3 level mentioned with was composed of non bST or control group and daily bST (36 mg/head/day) and biweekly bST (500mg/head/14 days). Bovine somatotropin was injected by intramuscular. The second factor is the feeding consisting of 2 levels. The first level is usually food consume or standard feed (feed from Research Center) and the second is a standard feed plus 25% concentrate. The parameters measured include the production of milk, glucose, triglycerides, blood urea nitrogen, cortisol, T4, T3 in the blood, and body condition score. There is interaction between supplementation of somatotropin and the addition of concentrated 25% on milk production and a change of body weight. Somatotropin supplementation followed by the usual food consumption can increase milk production by 17-26%, but somatotropin supplementation did not affect significantly the levels of metabolites and hormone metabolism and body condition score*

**Key words:** recombinant somatotropin, milk production, metabolite, hormone metabolism

## INTRODUCTION

Main problem in the humid tropical countries like Indonesia is height of environment temperature, relative humidity and rain fall was affect direct or indirect toward animal productivity. Environmental changed will arise a series of biochemistry process in the bodies which affect an cell activity and the work of organ which finally case a lot of energy used only for maintaining for survival, in turn at the finally decrease productivity.

Cows include homoitherm animal, which always maintainance the balance between heat production and sourounding. Therefore rearing cows from subtropis will show optimal productivity in area 750 until 1.250 m altitude at sea level or in area which temperature about 17 - 220C and relative humidity about 55% (Atmadilaga, 1979).

In Indonesia, most of cow population is Holstein cow, which originally is from subtropical countries so rearing cow is only in certain areas, particullary the one of has temperature and humidity near enviroment which suitable for this kind of cows whereas the less average of milk production only reaches 8-10 kg/head/day (Direktorat Jenderal Peternakan, 2006)

Recently, some publication about commercial aplication about Somatotropin, that is result of biotechnology promosing DNA that increase whitout being balance special diet or different nutrient (Gulay, 2003), at the level of farmers show the average of milk production increase until 5 kg/day or about 15 and 20% without bring about metabolic diseases or change no significant of milk quality (Manalu, 1994). The reseach report of increasing of milk production can

reach 30 -41%, even 60% depending on doses of use (Akers, 2002, Gluckman et al, 1987, Rose and Obara, 2000)

Its nesenary that about somatotropin to study of learning the effect of supplementation on milk production and the level of metabolite and hormone metabolism on lactation Holstein which are kept on tropical high in Indonesia.

### Animal and treatment

The Holstein cows used have adapted in Indonesia environment. The number 24 cows from lactation 1,2,3, and 4 and the fifth month lactation have weight about 430 - 462.5 kg and average actual milk production milk about 13 -16 kg/head/day. The research was design by using Randomized Block Design with 3 x 2 factorial pattern The first factor is bST injection with 3 level. The first factor is the injection of bST is composed of 3 levels, namely nonbST (placebo with 1 mL of sesame oil) or G0, Daily bST injections at a dose of 36 mg / head / day (G1) and Biweekly BST injections at a dose 500 mg / head / biweekly or (G2). The second factor is the feed which consists of 2 levels. Standard feed (F1), which is commonly consumed food in the Research Center, and the standard feed plus 25% concentrate (F2). Addition of 25% concentrate (= 2 kg / head / day) calculated from the usual amount given concentrates (8 kg / head / day). The placebo, bST injections daily, and biweekly injections given intramuscular (im) after the morning feeding, at 09.00. a.m. Ingredients of feed studies presented in Table 1. Performed feeding 3 times / day, ie 06.00 a.m after milking, 11:00

a.m in noon, and afternoon at 4.00 p.m after milking. Milking done 2 times a day Milking done 2 times a day The treatment given for 12 weeks. Placebo, daily injection of BST and BST-weekly injections have been injected through the intra-muscular (im) after the morning feeding, ie at 09.00. Feed composition is presented in Table 1. The feed was given 3 times / day, ie after 06:00 in the morning milking, at 11:00 noon, and 4:00 o'clock in the afternoon after a dairy. milk milked 2 times a day and provided treatment for 12 weeks. Blood sampling for analysis of blood metabolites and hormones was performed two weeks. Blood was taken by 5 mL of the base of the tail by using venoject, and stored ± 2 hours at room temperature. After that disentrifuse blood samples at 3, 000 rpm for 10 to 15 minutes. Furthermore the serum separated into a special tube and stored in a Freezer (temperature of -20°C) until further analysis.

### Actual Milk Production

The daily actual milk production is sum from the total morning milking and day milking. Milking is done by using portable milking machine. It result of milking was weighed by using Nagata Scale capacity 20 kg with smaller scale 200gr.

### Blood Metabolite

The analyzed blood metabolite was glucose, triglyceride and blood urea nitrogen. Analyzed concentration of glucose was measure by Wedermeyer and Yatasuke method (1977) but triglyceride and blood nitrogen urea were done at the end of result by using commercial kit.

Table1. Composition ration of Research Center Cikole Lembang

Ingredient	Amount of (kg)	Composition (%)
Rumput Gajah	35	
Ampas Bir	3	
Field Grass	5	
Concentrate	8	
- Pollard		40.4
- Bungkil Kelapa		15.2
- Tepung Jagung		24.3
- Dedak		10.1
- Bungkil Kedelai		9.9
- Mixed Mineral		0.5
- Kapur		0.5

Source : Research Center , 2005

Table 2. Nutritional research feed, the balance of green forege and concentrate feed, and the number of research cow nutrient needs.

Ingredient	F1 (kg)							F2 (kg)						
	DM	CP	CF	L	MWN	Ash	TDN	DM	CP	CF	L	MWN	Ash	TDN
Elephant grass	7.77	0.68	2.51	0.21	3.4	0.99	4.5	7.77	0.68	2.51	0.21	3.4	0.9	4.5
Field grass	1.22	0.10	0.39	0.02	0.54	0.18	0.68	1.22	0.10	0.39	0.02	0.54	0.18	0.68
Concentrate	7.49	1.11	2.69	0.91	2.24	0.53	3.77	9.36	1.39	3.37	1.13	2.8	0.67	4.71
Barkley	1.45	0.24	0.77	0.16	0.23	0.06	1.09	1.45	0.24	0.7	0.16	0.23	0.06	1.09
Total	17.93	2.13	6.36	1.29	6.41	1.76	10.04	19.8	2.41	7.03	1.52	6.97	1.9	10.98
Green Forage	50%							45%						
Concentrate	:							55%						
Nutrient Requirement (kg)	12.15	1.97	-	-	-	-	7.85	12.15	1.97	-	-	-	-	7.85
Nutrient surplus (%)	39.87	8.12	-	-	-	-	27.90	62.96	22.34	-	-	-	-	39.87
Description :	DM : Dry Matter CP : Crude Protein CF : Crude Fiber							L : Lipid MWN : Material Without Nitrogen TDN : Total Digestible Nutrient						

**Glucose**

Concentration of blood glucose was measure by Wedermeyer and Yatasuke method (1977) by using spectometre (spectroonic Hitachi-U-2001).

Levels of glucose are calculated by the formula:

$$(BG) = \frac{Abs\ Sp}{Abs\ St} \times (G\ St)$$

Description:

[BG] = Levels of Glucose (mg/100 mL)

Abs Sp = Absorbance Sample

Abs St = Absorbance of Standard

[G St] = Standard Glucose Levels (mg/100 mL)

**Trigliceride**

The serum triglyceride concentration was measured by using enzymatic techniques using a commercial kit from Human. The range of standards used to measure the concentration of triglycerides in serum was from 0 to 200 mg / dL. All concentrations of triglycerides in the blood sample used the standard range. Triglyceride concentration is read by spectrophotometer (Spectronic Hitachi U-2001).

**Blood Urea Nitrogen**

Blood Urea Nitrogen concentration measured using an enzymatic technique using commercial kit Human products. The range of

standards used to measure serum concentrations of Blood Urea Nitrogen is from 0 to 80 mg / dL. All Blood Urea Nitrogen serum concentrations within the range used standard. Blood Urea Nitrogen concentration contained in the serum is read by spectrophotometer (Spectronic Hitachi U-2001).

**Metabolism Hormone**

Hormones were measured tetraiodotironin, and triiodotironin and cortisol. Concentrations of the three hormones were conducted using the kit radioimmunoassai solid phase techniques.

**Tetraiodotironin (T4)**

Tetraiodotironin concentration in serum is determined using the kit radioimmunoassai solid phase techniques which are arranged in a monoclonal antibody and a radiolabel using 125I (Diagnostic Products Corporation. Los Angeles, CA). The range of standards used to create a standard curve ranging from 0 to 24 mcg / dL. Tetraiodotironin concentrations were analyzed directly in the blood serum with a volume of 0.25 μL. Hormone-bound radioactivity with gamma counter reads.

**Triiodotironin (T3)**

The serum triiodotironin concentration is determined using the kit radioimmunoassai solid phase techniques which are arranged in

a monoclonal antibody and a radiolabel using 125I (Diagnostic Products Corporation. Los Angeles, CA). The range of standards used to create a standard curve ranging from 0 to 24 mcg/dL. Triiodotironin concentrations analyzed directly in the blood serum with a volume of 0.25 μL. Hormone-bound radioactivity with gamma counter reads.

### Cortisol

Concentration of the hormone cortisol in serum is determined using the kit radioimmunoassay solid phase techniques which are arranged in a monoclonal antibody and a radiolabel using 125I (Diagnostic Products Corporation. Los Angeles, CA). The range of standards used to create a standard curve ranging from 0 to 24 mcg / dL. Cortisol concentrations were analyzed directly in the blood serum with a volume of 0.25 μL. Hormone-bound radioactivity with gamma counter reads.

### Body Weight

Body weight measurements performed using a measuring tape directly converted into kilograms. Measurements were taken once a month covering the body weight at the start of the study and body weight at the end of the study.

### Consumption of Feed And Dry Matter Intake

The feed used in this study consisted of two types of feed, which is a standard feed (P1), which is used to feed given by Research Center, and the standard feed plus 25% concentrate (P2). Feed consumption is calculated based on the amount of green forage and concentrate feed offered minus the rest (of any provision). Consumption of dry matter is calculated by multiplying the amount of consumed dry ingredients with the dry material content of the feed.

### Body Condition Score (BCS)

Body condition score is determined using the method of Wildman et. al. (1982). Values range from 1-5, value 1 shows the cow is very thin and 5 shows the cow is fatter. The point of focus were assessment in the area back, waist, haunch, roof of tail, hip bone,

horn and waist rib. Its assessment was conducted every 4 weeks.

### Statistical Analysis

The experimental design was based on Randomized Block design on a 3x2 factorial design. There is two factor of treatment. The first factor is three level of bST injection (Placebo, daily and biweekly) and second factor is two level food (standard feed and standard plus 25% concentrate. Data obtained for 12 weeks observation the resulting linear model for efficacy variable is follow:

$$Y_{ijk} = \mu + K_k + A_i + B_j + AB_{ij} + C_{ijk}$$

Description :

$Y_{ijk}$  : Response was observed cow k in treatment bST and Food

M : Overall mean

$K_k$  : Influence Group to - k

$A_i$  : The influence of bST injection to the way - i

$B_j$  : The influence of Feeding to - j

$AB_{ij}$  : The Influence of the interaction between injected bST to -i and level feeding to the j

$C_{ijk}$  : Residual Error

### RESULTS AND DISCUSSION

Total actual milk production, body weight changes, body condition score and dry matter intake

Total actual milk production, body weight, body condition score and dry matter intake from the test cows which were given an injection bovine somatotropin for 12 weeks are presented in Table 3. Actual milk production of cows tested ranged from  $1096.00 \pm 107, 17$  to  $1411.18 \pm 252.35$  kg / head / week or  $13.04 - 16.79$  kg / head / day. Followed by changes in body weight with a wide range, that is -  $10.75 \pm 18.96 - 36 \pm 6.36$  kg, and dry matter intake ranged from  $17.80 \pm 0.09 - 0.39 \pm 19.94$  kg / head / day. BSC averaging during 12 weeks showed a narrow variation range, i.e.  $2.60 \pm 0.20 - 2.94 \pm 0.19$ .

Table 3. Total actual milk production, body weight, body condition score, dry matter intake

Variable	bST						Int (GxF)
	Control (G0)		Daily (G1)		Biweekly (G2)		
	F1	F2	F1	F2	F1	F2	
Total Actual Milk prod (kg)	1120.92±106.54	1289.30±87.69	1308.63±109.70	1277.05±69.35	1411.18±252.35	1096.00±107.17	*
Initial Body Weight (kg)	462.5 ± 71.59b	457.8 ± 31.38b	440.5 ± 39.08b	448.5 ± 59.27b	430.0 ± 33.66b	448.0 ± 63.00b	-
Final Body weight (kg)	475 ± 77.27c	447 ± 18.96c	462 ± 46.28c	434 ± 39.20c	446 ± 40.04c	494.7 ± 59.48c	-
Change of Body Weight (kg)	+ 12.25 ± 7.80	-10.75 ± 18.96	+21.75 ± 17.42	-15 ± 30.80	+16 ± 6.97	+36 ± 6.36	*
Body condition score	2.71 ± 0.20d	2.65 ± 0.14d	2.75 ± 0.20d	2.69 ± 0.12d	2.60 ± 0.20d	2.94 ± 0.19d	
Dry matter intake	17.80±0.09a	19.72 ± 0.03b	17.81 ± 0.07a	19.94 ± 0.39b	17.86 ± 0.03a	19.71 ± 0.01b	

Description: (\*) show interaction between bST x Feed

The same letter to the row showing not significantly different (P>0.05).

F1 is the usual standard feed given by Research Center, F2 is a standard feed plus 25% concentrate (2 kg of concentrated feed used on the standard)

Table 4. Average levels of metabolites and hormones metabolism in Holstein cows were injected BST and concentrate for 12 weeks

Parameter	bST						Observation
	Control (G0)		Daily (G1)		Biweekly (G2)		
	F1	F2	F1	F2	F1	F2	
Glucose	39.43 ± 8.95 a	33.40 ± 5.38a	39.69 ± 6.14a	38.48 ± 6.85a	35.85 ± 5.73a	40.46 ± 7.00a	
Triglycerida (mg/dL)	99.37 ± 16.09b	96.20 ± 2.71b	100.52 ± 19.52b	92.80 ± 15.05b	93.47 ± 27.63b	103.14 ± 29.42b	
Urea Nitrogen (mg/dL)	115.17 ± 10.31c	95.67 ± 0.01c	107.65 ± 0.03 c	102.14 ± 0.07c	101.72 ± 0.03c	105.38 ± 0.15c	
Cortisol (µg/ dL)	0.731 ± 0.06d	0.612 ± 0.01d	0.669 ± 0.03d	0.574 ± 0.15d	0.592 ± 0.03d	0.665 ± 0.07d	
T4 (µg/dL)	3.281 ± 0.34e	2.993 ± 0.07e	2.935 ± 0.15e	2.805 ± 0.29e	2.811 ± 0.11e	2.620 ± 0.14e	
T3 (ng/dL)	111.45 ± 2.67f	99.86 ± 7.35f	100.73 ± 6.00f	100.92 ± 2.80f	100.66 ± 9.47f	96.17 ± 0.31f	

Description :The same letter to the row showing not significantly different (P>0.05).

F1 is the usual standard feed given by Research Center, F2 is a standard feed plus 25% concentrate (2 kg of concentrated feed used on the standard)

Total actual milk production during the 12 weeks of observation showed an increase caused by the interaction between the factors bovine somatotropin injection and feed. Bovine somatotropin injections with standard feed showed increased production by 17 -26%. Cows that were treated biweekly injection of bovine somatotropin had a higher production compared with production in cows that daily injection of bovine somatotropin, but the way of injection (daily or biweekly) are not significantly different ( $P > 0.05$ ). On contrary, both biweekly injection and daily injection of bovine somatotropin plus 25% concentrate showed decline in total actual milk production, especially in biweekly injection that showed sharp decline (22%), but compensated by an increase in body weight as the interaction between bovine somatotropin injection factor and feed. Supplementation bovine somatotropin daily or bi-weekly does not have an impact on milk production. This situation can be explained that no difference in milk production seems more likely due to the use of prolong release bovine somatotropin, so daily and biweekly treatment as indicated by the G1 is not different. This is in accordance with the workings of prolong release bovine somatotropin work which more slowly than the ones made for daily injection. Bovine somatotropin facility is still difficult to obtain in Indonesia.

Bovine somatotropin injection will increase the concentration of somatotropin in the blood and reached a peak after 8 hours of injection (Manalu, 2001). Initially, elevated levels of somatotropin will stimulate the work of the heart rate to pump blood flow, in response lactation cows are needed to bring subtract and precursor to the cells where the activity alveoli milk synthesis. Activity of alveoli cells are highly dependent on continuity supply of nutrients or subtract and precursor. In this case, somatotropin act as homeorhetic agent that fit the process of body metabolism, especially in nutrient-raising for milk synthesis (Key et al, 1997; Akers, 2002). Bovine somatotropin treatment on lactation cows will decrease the oxidation process in the entire network and the entire nutrient nonmammary focused on the synthesis in the

udder gland. (Akers, 2002). Somatotropin also stimulate the liver cells to secrete IGF-I which will mediate the role of somatotropin on the mammary gland (Manalu, 2001).

Cows in early lactation are in negative energy balance, as a result of decreased consumption of intake, while milk production has increased, it is suspected that in postpartum cows have the relatively high concentration of somatotropin that is closely related to milk production need for the child, so for the purposes of milk synthesis, it will break up the body reserve in the form of glycogen, or adipose tissue (Akers, 2002). This pattern also occurred in lactation cows which receiving exogenous somatotropin. Several times after the injection of bovine somatotropin, it will participate directly in nutrient partitioning in the settings need to supply milk synthesis Etherton

The results of observation using exogenous bovine somatotropin for 12 weeks, the tested cows has entered the fifth month of lactation so that the energy status of cows were in positive energy balance, at the start of treatment occurs dismantling the body energy reserves for milk synthesis (Manalu, 2001) but later using feed from the results of the digestion process is 5-6 weeks after injection of bovine somatotropin treatment went so it allows the body weight and the depreciation body condition score.

In this study, increased milk production was not accompanied by a depreciation of body weight, it is supported by data changes in body weight over 12 weeks of treatment that was not significantly different ( $P > 0.05$ ) and the average body condition score (BCS), which looks not significantly different ( $P > 0.05$ ). Increased production of 17 -26% shown in the treatment of injection bovine somatotropin and the standard feed, while injection of bovine somatotropin plus 25% concentrate, especially on the biweekly injections bovine somatotropin actual milk production seen higher milk production compared with that given daily injections or nonbovine somatotropin. Previous experiment revealed that the superiority of the somatotropin as a nutrient partitioning, showed that the use of bovine somatotropin in milk production increase could reach 10 - 15% without requiring the

addition of feed and this can take place during the first 6 weeks of delivery of exogenous somatotropin (Gulay, 2003)

High and low concentrations of somatotropin in the blood stimulates the synthesis of IGF-I in the liver, and IGF-I mediate this will increase the role of somatotropin in milk synthesis (Eherton & Bauman, 1998). But it has to be remembered that somatotropin and IGF-I acutely sensitive to the nutritional status of animal, especially in ruminants (Gluckman et al, 1987; Prosser and Mempham, 1989). Observe from the consumption between the treatments of dry material showed significantly different ( $P < 0.05$ ).

In this study, food was designed differently in order to anticipate the increase in milk production. The data based on the consumption of feed dry matter intake, just the standard feed (17,82 kg) meanwhile the standard food plus 25% concentrate (19,73 kg). In these observations, it seems clear that bovine somatotropin treatment plus 25% indicate nutritional status of these cows in excess (Table 3). Observations for the treatment for 12 weeks showed that the cow is given an injection of somatotropin and cows feed plus the standard 25% concentration showed that the lower milk production compared with the injection of somatotropin and feed cows just standard. In this study, animals injected with a placebo plus 25% concentrate showed increase milk production up to 15%. The situation shows that excessive feeding cows given bovine somatotropin injection unfortunate, it will only add additional costs due to a disturbed somatotropin responsibility even tends to support the lipogenesis process (Vernon, 1989) and this situation will only spur increased work activity of insulin, (Kamil et al, 2001; Manalu, 2001) in turn will affect the increase in body weight.

Body weight changes apparent in the tested cows that received bovine somatotropin biweekly injection plus 25% concentrate, as the interaction factor is bovine somatotropin injection and feeding, in which actual total milk production was low but gained body weight changes in a high enough compared with the control treatment or another. In the case of a daily injection of

bovine somatotropin and the addition of 25% concentrate did not show significant differences ( $P > 0.05$ ), as it has been stated before, it is more likely due to the use of inappropriate bovine somatotropin.

In early lactation, cows usually experience shrinkage 0,5-1 kg body weight per day as a result of the dismantling of the body's energy reserves that have been used to increase milk production, which is 1 kg of body weight is equivalent to 7 kg of milk. Things will be different when the cow had entered the middle period of lactation, energy balance has become positive, in this period will begin to happen to gain weight gradually. In this observation, the interaction between bovine somatotropin and feed factors to changes in body weight support increased body weight in cows that received an injection test bovine somatotropin bi-weekly plus 25% concentrate. While body condition score is an indicator of changes in body weight accurately enough, the change means that 1 unit of BCS has been reform of the body reserves of 57 kg which is used to synthesize milk. In this observation as a whole does not indicate a change in the BCS, this situation is closely related to actual increase in milk production is achieved only 2-3 kg, in addition to the status of the test cows were in positive energy balance. Although the test cows have a higher BCS, but not statistically significantly different ( $P > 0.05$ ) compared to other test cows.

#### **Metabolite levels**

Observation levels of metabolites are presented in Table 3. Metabolite levels, blood glucose showed the range of  $33.40 \pm 5.38$  -  $40.46 \pm 7$  mg / dL, triglycerides in the range  $15.05 \pm 92.80$  -  $103.14 \pm 29.42$ , and Blood Urea Nitrogen ranged from  $95.67 \pm 0.01$  -  $10.31 \pm 115.17$  mg / dL. The range of glucose, triglycerides and blood urea nitrogen during 12 weeks of observation indicates that blood metabolite levels are not significantly different in statistics ( $P > 0.05$ ).

Glucose is the main precursor required for milk lactose synthesis. Blood glucose levels in dairy cows ranged from 40 to 60 mg / dL (Preston and Lang, 1987; Sutardi 1981), but on

lactation cows are usually lower because nearly 80 % of blood glucose used for synthesis of lactose and it is often associated with take up glucose for lactose synthesis (Akers, 2002). Specifically on dairy cows, propionic acid (C4) which is the result of fermentation in the rumen is providing an important role as a precursor for lactose. Energy reserves stored in the liver in the form of glycogen is converted into glucose. Glucose is a carbohydrate derived Volatile Fatty Acid which include acetic acid (C2), butyric acid (C3) and propionic acid (C4), and therefore volatile fatty acid used as an indicator energy balance, especially after partus.

Average blood glucose was observed in the range 33.40 - 40.46 mg%, it is lower than that had reported by Sutardi (1981). Allegedly, fast take-up rate of glucose has a positive relationship with the rate of increase in milk production in cows that are injected daily and biweekly of bovine somatotropin has a milk production higher than control group, but specifically biweekly injections of bovine somatotropin showed the highest production. The act of somatotropin on lactation cows work directly as anti insulin (Kamil et al. 2001; Manalu 2001) and its influence will appear several hours after the first injection and after the daily injection (Manalu 2001) on the whole extra mammary tissue, it will work to adjust metabolism and homeostasis. Take up glucose and use of glucose in the non-mammary tissue of milk is low (Etherton & Bauman 1998), which is characterized by reduced expression of glucose transport in the network, but there is an increase lipolysis stimulation (Zhao et al. 1996) which will lead to low concentrations of insulin in the blood. Low blood glucose levels will have an impact on insulin concentrations and work in order to facilitate glucose entry into cells.

Triglycerides are energy reserves that always available and ready to use, which come from fat for energy needs in the body. As energy needs increase, the fat will be mobilized primarily from adipose tissue and oxidized to produce energy, so that the process of deposition and mobilization is always going on. Deposition will occur when the cows are in the condition of positive energy balance

while mobilization will occur when the negative energy balance (Bondi, 1987). Observation for 12 weeks showed that triglyceride levels was not different ( $P > 0.05$ ) from all treatments, but the range of triglyceride levels in this study is much higher when compared with the results reported by Soderholm et al. (1988), namely the use of bovine somatotropin dose 0; 10.3; 20.6 and 41.2 mg / day derived triglyceride levels, respectively, 20.2; 24.2; 26.7 and 25.4 mg / dL. High levels of triglycerides obtained from tested cows closely related to the energy status of the cow it-self, which is in positive energy balance. Feed consumed will be fermented to produce VFA in the rumen (propionic acid), after that it is absorbed by the abomasum that would bring the blood turned into glucose in the liver. The glucose will be stored as glycogen or converted into 3-phosphate LGliserol used for the synthesis of triglycerides (Sumardi, 2006). Bovine somatotropin injection on treatment, especially early treatment will be followed by reform of body energy reserves in the context of metabolic adaptation, therefore a standard feed that has been designed according to the needs of lactation cows and plus 25% concentrate has not use. Therefore it will change in form of triglyceride and will flow with the blood.

Increased milk production is not balanced by an increase in feed consumption will lead to the dismantling of reserve nutrients in the body. Urea nitrogen is a product of protein metabolism, and urea nitrogen levels use as a standard indicator of protein requirement level during lactation. Increase levels of urea describe the use of proteins, especially the reform of protein to meet energy needs in the synthesis of milk. During pregnant and lactation, the concentration of urea nitrogen can also increase with the increase use of proteins and protein consumption of feed, and will decrease when the concentration of proteins and protein consumption decreased. Cows that received a daily injection of bovine somatotropin, and cows have biweekly injection bovine somatotropin in a row amounted to 108.18; 99.71 and 105.42; 104.89; 101.52 mg / dL

Observations showed that injection of bovine somatotropin and the addition of concentrates did not affect ( $P > 0.05$ ) blood urea nitrogen concentration. Urea nitrogen levels observed in a ranged between 0.96 and 1.16 mg / mL or equivalent to 15.95 and 19.20 mmol / L. The range levels of blood urea nitrogen was much higher than that reported by Soderholm et al. (1988), the cow that received injection of bovine somatotropin with successive doses of 0; 10.3; 20.6 and 41.2 mg / day obtained in the levels of blood urea nitrogen, respectively, for 17.8; 16, 4; 17.1 and 15.2 mg / dL or equal to 0.178; 0.164; 0.171 and 0.152 mg / mL.

Meanwhile, levels of blood urea nitrogen injected cows bovine somatotropin and concentrate supplementation on the energy levels and high crude protein showed levels of urea nitrogen 5.7 mmol / L for the control cows, and 4.0 mmol / L for cows injected bovine somatotropin (McGuire et al. 1992). Blood urea nitrogen levels are dependent on the feed conditions, not because there is a removal of proteins for high-energy requirements, so the obtained fluctuation figures illustrate the increase use of protein. Urea nitrogen level inconsistent cells during lactation and changes in concentration of blood proteins and metabolites are not fully mediated by hormones (Mège 2004). Improved observations of urea nitrogen due to alleged excessive supply of feed protein that excess protein must be deamination to form ammonia and most of ammonia transform back into urea and then enter the bloodstream to be excreted through urine (NRC 1988).

#### Levels of hormone metabolism

Tetraiodothyronine (T4) concentration range is between  $2620 \pm 0.34 \pm 0.14$  -3281 mcg/dL whereas triiodothyronine (T3) levels ranged from  $96.17 \pm 0.31$ - $111.45 \pm 2.67$  η / dL. Average cortisol levels range from  $0574 \pm 0.15$  -  $0.06 \pm 0731$  mcg / dL. Average levels of hormone metabolism of all treatments showed not significantly different ( $P > 0.05$ ).

Thyroid hormones play a role in set galactopoietics and mammary gland function. It is always associated with calorigenesis, especially in the process of metabolism.

Tetraiodothyronine (T4) is a prohormone that has low biological activity in the circulation, whereas triiodothyronine (T3) is the active form of T4 that have been experienced deiodination process by the 5'deiodinase enzyme (5'D) (Kahl et al.1995). 5'deiodinase enzyme plays an important role in regulating various physiological statuses (Akers 2002). The results of observations showed that cows receiving injections bovine somatotropin (daily and biweekly) and 25% concentrate addition did not affect the concentration of T4 and T3. However, it seems there is a tendency that the concentration of T4 and T3 on bovine somatotropin injected cows is relatively lower than the control cows. This indicates that thyroid hormones in extra-mammary gland cells is low, which reflects the decrease in metabolic activity. The situation is similar with the results of research by Kahl et al. (1995) that bovine somatotropin injections can reduce levels of thyroxin on the extra-mammary gland and to maintain the status euthyroid (normal) in the mammary gland, the above situation meant to give priority to the mammary gland metabolic.

In lactation cows, extra-mammary gland in the state thyroid levels low so that the concentration of T3 as an agent calorigenic is decreased. Triiodothyronine is a hormone that is closely related to the provision of ATP though not yet clear how the mechanism (Kahl et al. 1995). Decrease in serum T3 concentration will reduce calorigenesis effects. It is often caused by T3 in turn will help the livestock in the process of homeostasis (Johnson et al 1991). This strengthens the statement Riis (1983) that there is a negative correlation between milk productions with the concentration of thyroxin in the blood.

Observations in cows receiving bovine somatotropin injection (daily and biweekly) showed no different than controls. These observations are strengthen reported by Johnson et al. (1991) that the cow gets injected in the summer with bovine somatotropin (farm) has T4 and T3 levels are lower (respectively for 36.20; 1.32 ng / mL) compared to control cows (ie by 32.60; 1, 21 ηg / mL).

The cortisol hormone is always associated with glucose and its function in regulating physiological and biochemical processes of glucose (Djojosoebagio 1990). Excess cortisol will stimulate the production of glycogen and glucose in the liver by increasing the conversion of pyruvate into glycogen, which is its action in regulating the supply of blood sugar through the process of gluconeogenesis (Djojosoebagio 1990). In addition, cortisol is the hormone that responsible for stress response (Schmidt 1971). Observations showed that injection of bovine somatotropin and the feed does not affect blood cortisol levels ( $P > 0.05$ ). Cortisol levels will be lower when glucose supplies are adequate, or in other words, cortisol is a glucose sparing effect. In addition, alleged that subcutaneous injections done every day in the control cows and cows on a daily injection of bovine somatotropin make the cow not comfortable or experience stress. Cortisol levels obtained in these observations are under reported results Peel (1983), namely 9.90 ng / mL for the control treatment, and 8.20 ng / mL for injection bovine somatotropin Johnson et al. (1991) reported that cortisol levels obtained from cows injected in the summer bovine somatotropin (farm) is 7.50 ng / ml was lower than the control cows, in the amount of 10.20 ng / ml.

## CONCLUSION

Bovine somatotropin supplementation followed by adequate feeding can increase milk production by 17-26%, without showing changes in metabolite levels and hormone metabolism or body condition score.

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