Mammary Gland Indices at the End of Lactation in the Superovulated Javanese Thin-Tail Ewes

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ABSTRACT : Thirty lactating Javanese thin-tail ewes (12 ewes had been injected, prior to mating, with 700 IU pregnant mare serum gonadotropin, and 18 ewes with saline as a control) were used to evaluate the effect of superovulation on milk production during lactation and mammary chemical indices at the end of lactation. Thirteen ewes (9 control and 4 superovulated ewes) were fed at low and the other 17 ewes (9 control and 8 superovulated ewes) were fed at high quality ration. Superovulated ewes, either fed at low or high quality ration, had dramatically higher milk yields (57%). At the end of lactation, superovulated ewes had higher mammary dry fat-free tissue, mammary DNA concentration, total mammary DNA and RNA contents than nonsuperovulated ewes. Superovulation did not affect mammary RNA and collagen concentrations, and total collagen content. Ration quality did not significantly increase milk production during lactation and mammary chemical indices at the end of lactation. The observed increase in milk production in the superovulated ewes was probably due to the increased mammary secretory cell number and their synthetic activities during lactation as a result of the increased endogenous hormonal stimulation of mammary growth and development during pregnancy. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 4 : 440-445*)

Key Words : Superovulation, Mammary Gland Indices, Lactating Sheep

INTRODUCTION

Milk yield during lactation is affected by the number of functional mammary epithelial cells at the beginning of lactation (Anderson, 1985; Forsyth, 1996), the availability of nutrients as precursors of milk components in the mammary epithelial cells (Collier, 1985). Mammary gland development is determined by the degree of mammogenic hormones stimulation during pregnancy (Tucker, 1985).

Major mammogenic hormones are secreted by the ovary, corpus luteum and placenta during pregnancy. Ewes with higher litter size have higher serum progesterone concentrations during pregnancy, and have better developed mammary glands at parturition (Manalu and Sumaryadi, 1998a, c) and at the end of lactation (Manalu and Sumaryadi, 1998b). Superovulation of ewes prior to mating increases corpora lutea number and maternal serum progesterone concentration (Manalu et al., 1998). Superovulated ewes have better mammary growth during pregnancy (Manalu et al., 1999b), and higher mammary lactose synthetase activity at the end of lactation (Frimawaty and Manalu, 1999). The objective of the present study was to evaluate mammary gland indices at the end of lactation (3 months postpartum).

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MATERIALS AND METHODS

Environmental conditions and animal description

This experiment was conducted during the hot (25 to 32℃) and wet (70 to 80% relative humidity) season in the humid tropics of Indonesia. Experimental animals were sixty Javanese thin-tail ewelambs with live weights ranging from 14 to 16 kg, and from 1 to 1.5 years of age. The Javanese thin-tail sheep is a meat-type indigenous breed that is well recognized for its high prolificacy. Prior to the experiment, the ewes had been raised in a semigrazing system, grazed in the field during the day and were housed at night, without concentrate supplementation. During the experiment, the ewes were maintained in experimental pens and fed with a complete mixed ration provided in the form of pellet. The experimental ewes were adapted to the experimental conditions and rations for 2 months prior to treatment.

Experimental design and protocol

Eighty ewes were used at the commencement of the experiment. Forty ewes were fed with a low quality ration (12% CP and 65% TDN) and the others (forty ewes) were fed a high quality ration (15% CP and 75% TDN) during the adaptation period and throughout the experiment. Compositions and chemical analyses of rations used are presented in table 1. At the end of adaptation period, the experimental ewes were injected twice (11 days apart) with 7.5 mg PGF_2

(i.m) to synchronize the estrous cycle. Forty of the experimental ewes (20 ewes receiving a low quality ration and 20 ewes receiving a high quality ration) were injected with 700 IU PMSG (Folligon, Intervet, North Holland) at the time of the last prostaglandin injection, to stimulate superovulation. The remainder (40 ewes; 20 ewes receiving a low quality ration and 20 ewes receiving a high quality ration) were injected with a saline as a control. Two days after the last prostaglandin injections, at the onset of estrous cycle, the experimental ewes were mated as a group (with 1 ram for 3 ewes). The experimental ewes were maintained in their respective feeding group throughout pregnancy.

Table 1. Composition and chemical analysis of the experimental rations

Constituent	Low quality ration (12% CP & 65% TDN)	High quality ration (15% CP & 75% TDN)
Dry elephant grass, %	49.21	19.67
Corn, %	27.29	35.07
Ricebran, %	12.27	10.01
Soybean meal, %	5.13	14.44
Coconut meal, %	5.62	19.95
Bone meal, %	0.15	0.19
Fish meal, %	0.15	0.49
Premix, %	0.18	0.18
Total	100.00	100.00
Crude protein, %	12.12	15.20
Crude fiber, %	15.91	11.88
Calculated TDN, %	65.00	75.00
Ether extract, %	5.51	5.80
Nitrogen-free extract, %	43.75	43.71
Ash, %	9.48	9.50
Calcium, %	0.88	1.02
Phosphorus, %	0.61	0.73
Gross energy, MJ/kg	17.41	16.13

During gestation, the experimental ewes were fed twice daily. Feed intake was restricted in early pregnancy, during the first 7 weeks the ewes were fed at maintenance level (average consumption of 0.4 kg/d), and feed on offer increased gradually until week 15 of pregnancy when average consumption was 0.56 kg/d. From week 15 to parturition, the ewes were fed ad libitum and average intake was 1.0 kg/d. During lactation, the experimental ewes were fed twice a day and the ewes had free access to feed and water.

During the first week postpartum, lambs were allowed to suckle. One week after parturition, lambs were separated from the ewes and milk was collected twice a day until 91 days postpartum. This was done by hand milking after a prior injection of 3 IU oxytocin (i.m.). On day 92 postpartum, the experimental ewes were slaughtered, and the mammary glands were excised for determination of chemical indices.

Mammary gland indices measurements

A number of measurements were made on mammary tissue including dry fat-free tissue (DFFT), DNA, RNA, and collagen. The DNA, RNA, and collagen are expressed both in concentration (g/mg DFFT), and total content (concentration multiplied with weight of DFFT). Dry fat-free tissue of the mammary gland was measured by modification of method described by Anderson (1975). Half of each udder was excised and the mammary gland was isolated by trimming the skin and subcutaneous fat and removing milk inside the gland. The isolated mammary gland was frozen for easy slicing. The thinly sliced mammary gland was soaked in ethanol for 48 hr and then with diethyl ether (48 hr) until the gland was free of fat. It was then dried at 50℃ for 24 h to obtain DFFT. The mammary DFFT was then ground to a fine powder to be used for mammary DNA by p-nitrophenylhydrazine reaction (Webb and Levy, 1955), RNA by orcinol reaction (Albaum and Umbreit, 1947), and collagen by measuring hydroxyproline (Woessner, 1961).

Statistical analyses

Since each cell of the experimental unit did not contain enough number of single and twin bearing ewes, litter size was not included as a main factor. However, separated analysis indicated that litter size did not affect milk production and mammary chemical indices. Therefore, litter size was pooled, and data were analyzed as a completely randomized design with a factorial arrangement 2×2 (unequal n) using superovulation and ration quality as main factors. Analysis of variance using General Linear Model procedure of SAS (1985) was performed to test the effects of main factors (superovulation and ration quality) and their interaction.

RESULTS

During the predicted week of parturition, of 40 nonsuperovulated ewes only 20 ewes (9 low quality [5 single and 4 twin bearing], and 11 high quality ration [9 single and 2 twin bearing]) were pregnant and gave birth during the week of predicted parturition. Of 40 superovulated ewes only 24 ewes (9 low quality [5 single and 4 twin bearing], and 15 high quality ration [9 single and 6 twin bearing]) were pregnant and gave birth during the week of predicted parturition. The remainder of the ewes were nonpregnant. Of the superovulated ewes fed on the low quality diet, 4 gave birth to multiple lambs, 2 sets of twins, and 2 sets of triplets. Of forty four ewes giving birth, 14 ewes were slaughtered at parturition to measure

Table 2. Mean live weights ± SE at the end of lactation, mean daily milk production, and mammary indices at the end of lactation in the control and superovulated ewes fed on a low or high quality ration

Variables	Ration quality				Level of significance		
	Low		High		Super-ovulation	Ration quality	Inter-action
	Control	Superovulation	Control	Superovulation			
Live weight[1], kg	21.56 ± 0.72	24.63 ± 1.38	25.22 ± 1.26	25.25 ± 1.71	ns	ns	ns
Milk yield, g/d	269.64 ± 17.71	438.21 ± 22.30	323.21 ± 9.35	486.07 ± 23.10	**	ns	ns
Mammary DFFT[2], g	9.86 ± 0.52	15.84 ± 1.38	12.04 ± 1.27	14.26 ± 1.23	**	ns	ns
Mammary DNA							
Concentration, μg/mg DFFT	33.90 ± 4.35	51.40 ± 6.38	35.95 ± 5.20	42.55 ± 2.64	*	ns	ns
Total, mg	334.76 ± 47.61	792.12 ± 61.84	427.25 ± 69.49	619.41 ± 74.17	*	ns	*
Mammary RNA							
Concentration, μg/mg DFFT	14.12 ± 1.42	16.54 ± 2.47	16.04 ± 1.71	18.20 ± 2.14	ns	ns	ns
Total, mg	139.95 ± 17.78	254.06 ± 23.71	195.17 ± 37.25	253.77 ± 28.23	**	ns	ns
Mammary collagen							
Concentration, μg/mg DFFT	18.40 ± 1.28	17.02 ± 1.24	17.38 ± 1 1.70	14.22 ± 2.12	ns	ns	ns
Total, mg	180.80 ± 13.06	266.92 ± 22.59	207.17 ± 28.38	193.00 ± 22.69	ns	ns	*

[1] Live weight at the end of lactation (91 days postpartum). [2] DFFT=dry fat-free tissue.
* $p<0.05$; ** $p<0.01$; ns=nonsignificant.

mammary gland development. Thirty ewes, 9 control fed on a low quality ration (6 singletons, and 3 twins), 9 control fed on a high quality ration (7 singletons and 2 twins), 4 superovulated fed on a low quality ration (4 singletons and none twin), and 8 superovulated fed on a high quality ration (5 singletons and 3 twins), were maintained until 91 d postpartum. Therefore, litter size could not be included in the analysis of the data as a main factor with superovulation and ration quality. Prior and separated analysis indicated that litter size did not affect milk production and mammary gland indices. Therefore, litter size was pooled, and the analysis of data was performed to test the effects of superovulation and ration quality, and their interaction.

Live weights of the experimental ewes at the end of an 84-d lactation period were not significantly different. Therefore, any difference in mammary DFFT was not related to live weight. In evaluating the mammary gland indices at the end of lactation, average daily milk production during 84-d observation in the experimental ewes is presented. More comprehensive analyses on milk production, compositions, and energetic efficiency of milk synthesis from the same experimental animals were explained (Manalu et al., 1999a). Briefly, superovulation prior to mating significantly increased (by 59%) average daily milk production during 84-d lactation ($p<0.01$); 296.43 and 470.12 g/d in the control and superovulated ewes, respectively, regardless of ration quality. Ration quality, however, did not significantly increase milk production ($p=0.06$). Ewes fed on a high quality ration had numerically higher milk production (by 24%) than those fed on a low quality ration (321.55 vs. 399.88 g/d). Superovulation and ration quality did not have a significant interaction effect on average daily milk production (table 2). Lactation curves of nonsuperovulated and superovulated ewes and of receiving a low and a high quality rations during 84-d lactation period are presented in figures 1 and 2, respectively. Superovulated ewes had consistently higher daily milk production during a 84-d lactation period. Ewes receiving a high and low quality rations had similar milk production ($p>0.05$) during lactation.

At the end of a 84-d lactation period, superovulated ewes had a greater mammary tissue as was indicated by a 37% higher weight of mammary DFFT ($p<0.01$) than nonsuperovulated ewes (10.82 and 14.86 g, in the nonsuperovulated and superovulated ewes, respectively). Ration quality and its interaction with superovulation did not have significant effects on mammary DFFT. The weights of mammary DFFT in the ewes fed on a low and a high quality rations were 11.70 and 13.17 g, respectively.

Superovulated ewes had some 30% higher mammary cells number at the end of a 84-d lactation period as was indicated by the mammary DNA concentration per unit weight of mammary DFFT ($p<0.05$). Mammary DNA concentrations per unit weight of mammary DFFT were 34.93 and 45.50 g/mg DFFT in the nonsuperovulated and superovulated ewes, respectively. Ration quality and its interaction with superovulation did not affect mammary DNA concentration. Mammary DNA concentrations in the ewes fed on a low and a high quality ration were 39.27 and 39.07 g/mg DFFT, respectively. Total

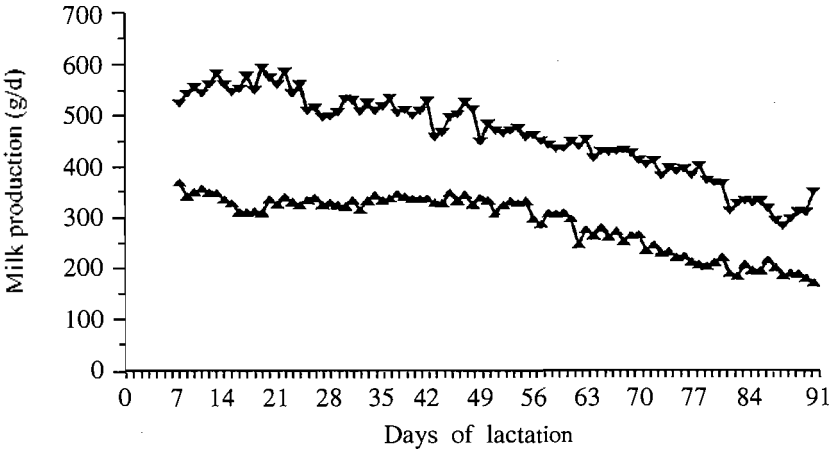

Figure 1. Mean daily milk production of control (▲) and superovulated ewes (▼) during 84 days of lactation. Standard error of mean daily milk production ranged from 15 to 36 g/d in the nonsuperovulated ewes, and from 16 to 56 g/d in the superovulated ewes

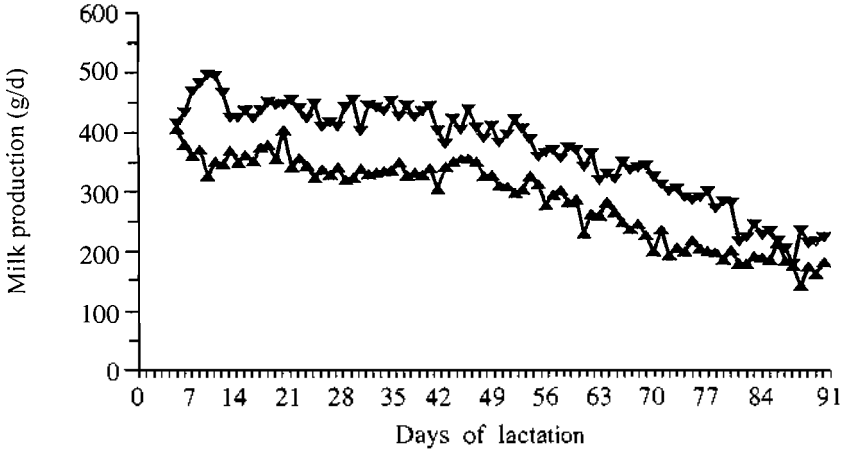

Figure 2. Mean daily milk production of ewes receiving low (▲) and high (▼) quality ration during 84 days of lactation. Standard error of mean daily milk production ranged from 7 to 49 g/d in the ewes receiving low quality ration, and from 22 to 52 g/d in the ewes receiving high quality ration

mammary cells number at the end of a 84-d lactation period, as was indicated by the total content of DNA in the mammary DFFT, in the superovulated ewes was higher by 79% ($p<0.01$); 380.54 and 676.98 mg in the nonsuperovulated and superovulated ewes, respectively. Ration quality, however, did not affect total DNA content of mammary DFFT; 475.49 and 517.19 mg in the ewes fed on a low and high quality ration, respectively. Interaction of superovulation and ration quality, however, significantly affects total DNA content of the mammary DFFT ($p<0.05$). Superovulated ewes receiving a low quality ration had a higher total DNA content of mammary DFFT (by 28%) than those receiving a high quality ration. Nonsuperovulated ewes receiving a high quality ration had significantly higher total DNA content of mammary DFFT (by 28%) than those receiving a low quality ration. Total DNA contents of mammary DFFT in the nonsuperovulated and superovulated ewes receiving a low quality ration were 334.76 and 792.12 mg, respectively, while in those receiving a high quality ration were 427.25 and 619.41 mg, respectively (table 2).

Synthetic activity per unit weight of mammary gland as was indicated by the mammary RNA concentration per unit weight of mammary DFFT was not significantly influenced by superovulation, ration quality, or their interaction. Mammary RNA concentrations per unit weight of mammary DFFT in the nonsuperovulated and superovulated ewes were 15.03 and 17.65 g/mg DFFT, respectively. In the ewes fed on a low and high quality rations, mammary RNA concentrations per unit weight of mammary DFFT were 14.79 and 17.06 g/mg DFFT, respectively. However, total synthetic activity of the mammary glands at the end of a 84-d lactation period, as was indicated by the total RNA contents of the mammary DFFT, in the superovulated ewes was higher by 56%

($p<0.01$). Total RNA contents of the mammary DFFT in the nonsuperovulated and superovulated ewes were 167.56 and 253.87 mg, respectively. Ration quality or its interaction with superovulation did not significantly affect total RNA content of the mammary DFFT. Ewes fed on a high quality ration had a numerically higher (by 27%) total RNA contents of the mammary DFFT as compared to those fed on a low quality ration (175.06 and 222.74 mg in the ewes fed a low and a high quality ration, respectively).

Superovulation prior to mating, ration quality or their interaction did not significantly affect the mammary connective tissue concentration per unit weight of mammary DFFT at the end of a 84-d lactation period, as was indicated by the mammary collagen concentration. Mammary collagen concentrations in the control (nonsuperovulated) and superovulated ewes were 15.16 and 17.91 g/mg DFFT, respectively. Mammary collagen concentration in the ewes fed on a low and a high ration quality were 18.00 and 15.90 g/mg DFFT, respectively. Superovulation prior to mating did not significantly affect, but tended to increase ($p=0.09$), total collagen content of the mammary DFFT at the end of a 84-d lactation period; 193.99 and 217.64 mg in the nonsuperovulated and superovulated ewes, respectively. Ration quality did not significantly affect total collagen content of the mammary DFFT; 207.30 vs. 200.50 mg in the ewes fed a low and a high quality ration, respectively. However, the interaction between superovulation treatment prior to mating and ration quality significantly influenced total collagen content of the mammary DFFT at 84-d ($p=0.02$). Nonsuperovulated ewes receiving a high quality ration had higher ($p<0.05$) total mammary collagen (by 15%) than those receiving a low quality ration. Superovulated ewes receiving a low quality ration had higher total collagen content of the mammary DFFT (by 38%) than those receiving a high quality ration. Total collagen contents of the mammary DFFT in the nonsuperovulated and superovulated ewes receiving a low quality ration were 180.80 and 266.92 mg, respectively, while in those receiving a high quality ration were 207.17 and 193.00 mg, respectively.

DISCUSSION

The results of this study indicated that injection of 700 IU PMSG prior to mating to stimulate superovulation in the ewes had a dramatic effect on the increased milk production during lactation (by 59%) without a significant difference in live weight at the end of lactation. Chemical analysis of the mammary glands indicated that superovulated ewes had a greater mammary cell number (as was indicated by the total DNA content of the mammary DFFT) and total synthetic activities (as was indicated by the total RNA content of the mammary DFFT) even at the end of lactation. The effect of superovulation on milk production during lactation and the mammary cells number was far greater than that of ration quality. The increased crude protein and TDN of the ration from 12 and 65% to 15 and 75%, respectively, increased milk production by 24%. The nonsignificant effect of ration quality on milk production ($p=0.06$) in this study was probably due to a great variation of milk production (29%) among animals (superovulated and nonsuperovulated ewes) within ration. Ration quality did not have a significant effect on the mammary cells number and total synthetic activity at the end of lactation, even though there was a significant higher mammary cell number and total collagen content in the superovulated ewes receiving a low quality ration than in those receiving a high quality ration (table 2). The nonsignificant effect of ration quality on total mammary cells number and synthetic activity at the end of lactation was corroborated by the milk production level at the end of lactation (figure 2).

How did superovulation prior to mating increase milk production during lactation? Superovulation prior to mating increased number of corpora lutea and progesterone concentration as an indicator of mammogenic hormone levels during pregnancy (Manalu et al., 1998) which associated with a greater mammary growth during pregnancy (Manalu et al., 1999b) and at parturition (Manalu et al., 1999a). Enzymatic activity of the mammary cells as was indicated by the lactose synthetase activity at the end of lactation were also increased in the superovulated ewes (Frimawaty and Manalu, 1999). Ewes having higher progesterone concentrations during pregnancy had a greater mammary growth and development at parturition and at the end of lactation (Manalu and Sumaryadi, 1998a, b, c). The results of the present study suggested that superovulated ewes had a greater mammary cell number and synthetic activities throughout lactation.

The greater increase in milk production observed in the superovulated ewes in the present study was due to the increased mammary cells number and total mammary synthetic activity stimulated by superovulation. Close observation of lactation curve in figure 1 clearly indicated that superovulated ewes had consistently higher milk production throughout lactation. While lactation curve in figure 2 indicated that improved ration quality could not maintain higher milk production throughout lactation. Superovulated ewes receiving a low quality ration increased milk production by 63%. Combination of superovulation and improved ration quality increased milk production by 80%.

The results of this experiment indicated that superovulation prior to mating could maintain higher mammary cells number and synthetic activities throughout lactation. The implication of the technique

is great. Single injection of PMSG prior to mating could have a long lasting effect on higher mammary cells number and their synthetic activities and milk production during lactation. The effect single injection of PMSG on milk production during lactation in the ewes fed a low quality ration was 63%, while feeding a high quality ration throughout lactation in the nonsuperovulated ewes only produced 20% increase in milk production. Milk production is influenced by the degree of mammary growth and development attained at parturition (the number of mammary secretory cells during lactation) (Forsyth, 1996), and nutrient availability in the secretory cells (Collier, 1985). In the superovulated ewes receiving a low quality ration, the increased mammary cells number could utilize available nutrients to synthesize more milk. This implies that superovulation increased milk production and the efficiency of milk synthesis.

This technique has a promising use in improving milk production without improvement in ration quality as was true for somatotropin and other metabolic modulator used in dairy industry. The technique, however, should be tested in the dairy animals and in high producing dairy animals that were genetically selected for better mammary growth and synthetic activities, since the animal used in the present experiment are meat type breed of sheep. Nevertheless, the technique still has a promising use in meat producing animals since superovulation prior to mating in sheep could improve fetal growth (Manalu et al., 1998) during pregnancy and lamb birth weight at parturition, especially in the ewes carrying multiple fetuses (Manalu et al., 1999c). Combination of better lamb birth weight and maternal milk production during lactation could improve preweaning growth and finally total weaning weight per ewes.

However, there is a great work to be done before the technique could be applied in the animal industry. In addition to the requirement of testing in different species and breeds of animals stated above, the effects of superovulation on reproduction efficiency merits further studies. The technique becomes effective when the superovulated animals are pregnant and the pregnancy is maintained until parturition.

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