Mammary Gland Indices at the End of Lactation in Javanese Thin-tail Ewes with Different Litter Sizes

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ABSTRACT : Twenty-four Javanese thin-tail ewes (11, 9, and 4 ewes giving birth to 1, 2, and 3 lambs, respectively) with similar body weight and age at breeding were used to study serum progesterone concentrations during pregnancy, milk production during lactation, and mammary gland indices at the end of lactation (3 months postpartum).

The results of the experiment showed that averages serum progesterone concentrations during pregnancy in the ewes giving birth to twin and triplet lambs were higher ($p < 0.01$) than those giving birth to a single lamb. Ewes giving birth to 3 lambs had higher ($p < 0.01$) mammary dry fat-free tissue (DFFT) (by 31 and 34%), DNA concentration (by 25 and 16%) and RNA concentration (by 29 and 16%) at the end of lactation than those giving birth to 1 and 2 lambs. There was no difference in mammary collagen, protein and glycogen concentrations at the end of lactation among litter sizes. Ewes giving birth to 3 lambs had higher ($p < 0.01$) total mammary DNA content (by 64 and 61%) and RNA content (by 69 and 53%) at the end of lactation than those giving birth to 1 and 2 lambs. There was no difference in total mammary collagen, protein and glycogen contents at the end of lactation among litter sizes. Even though ewes with higher litter size had numerically higher milk production, there was no significant difference in milk production per 4 h among litter sizes.

The results of the experiment indicated that ewes having higher litter size had greater mammary cell number and synthetic activities at the end of lactation. The results suggested that ewes with higher progesterone concentrations and better developed mammary glands during pregnancy could maintain higher cell number and activities throughout lactation.

(**Key Words**: Litter Size, Mammary Gland Indices, Lactating Sheep)

INTRODUCTION

Milk production during lactation is affected by the number of functional mammary epithelial cells (degree of mammary glands development) at the beginning of lactation (Anderson, 1985), the availability of nutrients as precursors of milk components in the mammary epithelial cells (Collier, 1985), and the rate of mammary glands cells involution (Wilde and Knight, 1989). Mammary gland development during pregnancy is determined by the degree of mammogenic hormones stimulation (Tucker, 1985). Ewes with higher litter size had higher average serum progesterone concentrations during pregnancy, and better developed mammary glands at parturition (Manalu and Sumaryadi, 1998b). Higher average maternal serum progesterone concentrations during pregnancy, as a representative of mammogenic hormones, is associated with the higher mammary gland growth and development at the beginning of lactation as indicated by the mammary dry fat-free tissue, DNA, RNA, collagen concentrations per unit weight and total contents in the mammary gland (Manalu and Sumaryadi, 1998a).

The objective of the present study was to document mammary gland indices at the end of lactation (3 months postpartum) in the ewe having different litter sizes and averages serum progesterone concentrations during pregnancy.

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MATERIALS AND METHODS

Environmental conditions and animal description

This experiment was conducted during the hot and wet season of the year in the humid tropic of Indonesia. Average daily temperature and relative humidity ranged from 25 to 32℃ and from 70 to 80%, respectively. The Javanese thin-tail sheep used in the experiment is an indigenous breed with meat production type and is well known for its high prolificacy (Bradford et al., 1986).

Experimental design and protocol

Experimental animals were twenty-four Javanese thin-tail ewes (11, 9, and 4 ewes giving birth to 1, 2, and 3 lambs, respectively) with similar body weight (20 to 22 kg) and age (2 to 3 years) at breeding. The experimental ewes were injected twice with $PGF_{2\alpha}$ (i.m) with an 11-day interval. Three days after the last prostaglandin injection, the experimental ewes were mated naturally by colony breeding. Blood samples were drawn one day after the last prostaglandin injection as week 0 of pregnancy, and then 10 days after the last prostaglandin injection (seven days after the predicted ovulation, as the end of week 1 of pregnancy). Blood samples were drawn weekly (at the same time of a day) until parturition (week 20 of pregnancy). During lactation, the experimental ewes were milked by hand milking for 4 h every other week. During milk yield measurements, the lambs were separated from the ewes for 4 h. The udder was emptied by hand milking prior to measurements. Prior to milking, the experimental ewes had been injected with oxytocine to ensure milk harvesting. At the end of lactation, the experimental ewes were sacrificed for determination of mammary gland indices.

Blood sampling and processing

Ten ml of blood samples were drawn with plain vacutainer or sterile syringes from the jugular vein at 09:00 to 10:00 h Thursday weekly. Bleeding time was conducted prior to feeding, and the day of the week was chosen based on the 7-day interval from the predicted ovulation day (three days after the last prostaglandin injection). Blood samples were allowed to clot in a cool ice box and transported to the laboratory for further separation of serum by centrifugation. The serum then was kept frozen for progesterone analyses.

Progesterone analyses

Concentration of serum progesterone in duplicate was measured by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) with a slight modification to accommodate wide ranges of progesterone concentrations in pregnant ovine (Manalu et al., 1996). Radioactivity of progesterone-bound tubes was counted with an automatic gamma counter. Concentrations of standard progesterone used to construct the standard curve ranged from 0.1 to 20 ng/ml. A sample volume of 100 μl serum was used in the assay of samples with progesterone concentrations ranged from 0.1 to 20 ng/ml. For samples with progesterone concentrations lower than 0.1 ng/ml, sample volume was increased to 200 μl to bring the progesterone concentrations to the range of standard used. Progesterone concentrations in the serum samples were within the range of concentrations of standard progesterone used to construct the standard curve. Inter- and intra-assay coefficients of variations were 9, and 4.0%, respectively. Concentrations of progesterone were parallel in the sample volumes of 50, 100, and 200 μl.

Mammary gland indices measurements

The mammary gland indices are dry fat-free tissue (DFFT), DNA, RNA, collagen, protein and glycogen. The DNA, RNA, collagen, protein and glycogen are expressed both in concentration (μg/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 the udders 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 glands free of fat and dried in 50℃ for 24 h to obtain DFFT. The DFFT was then ground to make a fine powder to be used for mammary DNA by p-nitrophenylhydrazine reaction (Webb and Levy, 1956), RNA by orcinol reaction (Albaum and Umbreit, 1947), collagen by measuring hydroxyproline (Woessner, 1961), protein by Lowry method (Lowry et al., 1951), and glycogen by anthrone method (Seifter et al., 1950).

Statistical analyses

Data were analyzed with analysis of variance to test the means differences among litter sizes. Averages serum progesterone concentrations during pregnancy were correlated with mammary gland indices at the end of lactation by using linear regression and correlation

analysis (Snedecor and Cochran, 1982).

RESULTS

As an indication of mammary gland growth and development at the beginning of lactation, averages serum progesterone concentrations during pregnancy are presented (table 1). Averages serum progesterone concentrations during pregnancy in the ewes giving birth to twin lambs were higher ($p < 0.01$) than those giving birth to a single lamb. Concentrations serum progesterone during pregnancy in the ewes giving birth to 1, 2, and 3 lambs were 13.70 ± 1.28, 18.73 ± 1.30, and 20.53 ± 1.70 ng/ml, respectively.

Mammary DNA and RNA concentrations per unit weight of mammary DFFT at the end of lactation in the ewes giving birth to 3 lambs were higher ($p < 0.01$) than those in giving birth to 1 and 2 lambs (by 25 and 16%, and 29 and 16%, respectively). There was no difference in mammary DNA and RNA concentrations at the end of lactation between ewes giving birth to 1 and 2 lambs. Concentrations of mammary DNA and RNA per unit weight of mammary DFFT at the end of lactation in the ewes giving birth to 1, 2, and 3 lambs were 36.43 ± 1.53, 39.25 ± 2.77, and 45.55 ± 1.93, and 19.04 ± 1.50, 21.23 ± 1.49, and 24.56 ± 1.27 μg/mg DFFT, respectively (table 1).

There was no difference in mammary collagen, protein and glycogen concentrations per unit weight of mammary DFFT at the end of lactation among litter sizes. Concentrations of mammary collagen, protein, and glycogen per unit weight of mammary DFFT at the end of

Table 1. Average maternal serum progesterone concentrations during pregnancy and mammary DNA, RNA, collagen, protein and glycogen concentrations at the end of lactation[1]

Litter size		Progesterone[2] (ng/ml)	Mammary indices concentrations				
			DNA	RNA	Collagen	Protein	Glycogen
			(ug/mg DFFT)				
1	Mean	13.70[a]	36.43[a]	19.04[a]	24.97[a]	636.41[a]	2.91[a]
	SE	1.28	1.53	1.50	2.30	9.85	0.21
2	Mean	18.73[b]	39.25[a]	21.23[a]	23.82[a]	644.96[a]	2.53[a]
	SE	1.30	2.77	1.49	2.46	13.15	1.18
3	Mean	20.53[b]	45.55[b]	24.56[b]	21.11[a]	627.28[a]	3.11[a]
	SE	1.70	1.93	1.27	2.97	19.73	0.47

[1] Means and SE of 11, 9, and 4 ewes giving birth to 1, 2, and 3 lambs at parturition, respectively.
[2] Averages serum progesterone concentrations during pregnancy.
[a,b] Different superscripts in the same column refer to difference between litter size ($p < 0.01$).

Table 2. Mammary DFFT and total mammary DNA, RNA, collagen, protein, and glycogen contents at the end of lactation[1]

Litter size		Milk yield (ml/4 h)	Total mammary chemical indices content					
			DFFT	DNA	RNA	Collagen	Protein	Glycogen
			g					
1	Mean	124.57[a]	15.44[a]	0.56[a]	0.29[a]	0.39[a]	9.83[a]	0.045[a]
	SE	5.89	1.03	0.05	0.03	0.05	0.67	0.004
2	Mean	135.90[a]	15.04[a]	0.57[a]	0.32[a]	0.36[a]	9.84[a]	0.037[a]
	SE	14.38	1.46	0.05	0.04	0.05	1.10	0.005
3	Mean	158.35[a]	20.22[b]	0.92[b]	0.49[b]	0.42[a]	12.81[a]	0.058[a]
	SE	34.14	3.80	0.18	0.09	0.09	2.50	0.008

[1] Means and SE of 11, 9, and 4 ewes giving birth to 1, 2, and 3 lambs at parturition, respectively.
[a,b] Different superscripts in the same column refer to difference between litter size ($p < 0.01$).

lactation in the ewes giving birth to 1, 2, and 3 lambs were 24.97 ± 2.30, 28.32 ± 2.46, and 21.11 ± 2.97, 636.41 ± 9.85, 644.96 ± 13.15, and 627.28 ± 19.73, and 2.91 ± 0.21, 2.53 ± 1.18, and 3.11 ± 0.47 μg/mg DFFT, respectively (table 1).

Mammary DFFT at the end of lactation in the ewes giving birth to 3 lambs was higher ($p < 0.01$) than those in giving birth to 1 and 2 lambs (31 and 34%, respectively). There was no difference in mammary DFFT at the end of lactation between ewes giving birth to 1 and 2 lambs. Mammary DFFT at the end of lactation in the ewes giving birth to 1, 2, and 3 lambs were 15.44 ± 1.03, 15.04 ± 1.46, and 20.22 ± 3.80 g, respectively (table 2).

Ewes giving birth to 3 lambs had higher ($p < 0.01$) total mammary DNA and RNA contents at the end of lactation than those giving birth to 1 and 2 lambs (by 64 and 61%, and 69 and 53%, respectively). There was no difference in total mammary DNA and RNA contents at the end of lactation between ewes giving birth to 1 and 2 lambs. Total mammary DNA and RNA contents at the

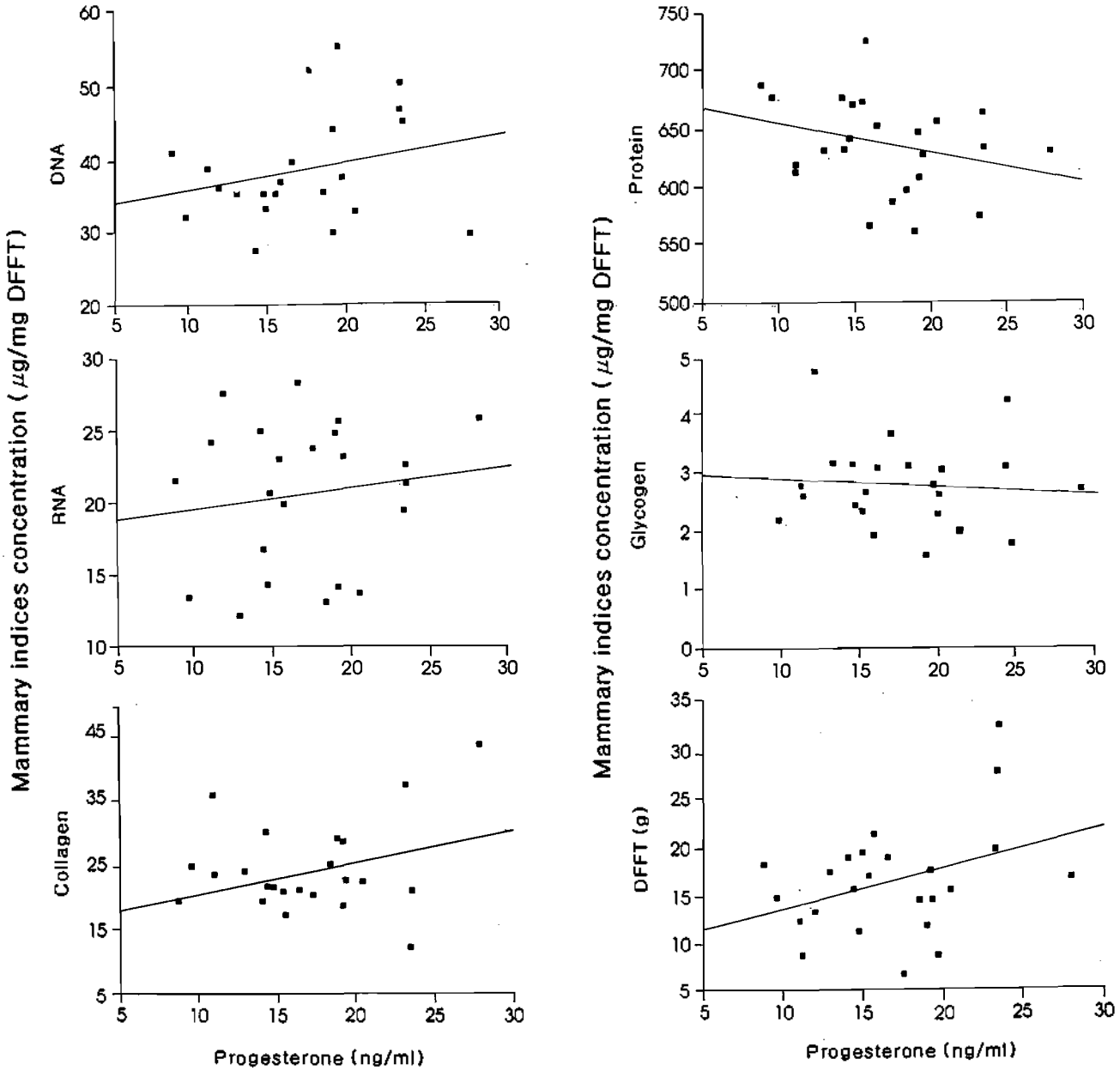

Figure 1. Mammary indices (DNA, RNA, Collagen, Protein, Glycogen) concentrations (μg/mg DFFT) and DFFT (g) at the end of lactation within various ranges of serum progesterone concentrations during pregnancy in Javanese thin-tail sheep.

end of lactation in the ewes giving birth to 1, 2, and 3 lambs were 0.56 ± 0.05, 0.57 ± 0.05, and 0.92 ± 0.18, and 0.29 ± 0.03, 0.32 ± 0.04, and 0.49 ± 0.09 g, respectively (table 2).

There was no difference in total mammary collagen, protein and glycogen contents at the end of lactation among litter sizes. Total mammary collagen, protein, and glycogen contents at the end of lactation in the ewes giving birth to 1, 2, and 3 lambs were 0.39 ± 0.05, 0.36 ± 0.05, and 0.42 ± 0.09, 9.83 ± 0.67, 9.84 ± 1.10, and 12.81 ± 2.50, and 0.045 ± 0.004, 0.037 ± 0.005, and 0.058 ± 0.008 g, respectively (table 2).

There was no difference in milk production per 4 h among litter sizes. However, milk production per 4 h was numerically increased with the increased litter size. Milk production in the ewes giving birth to 1, 2, and 3 lambs were 124.57 ± 5.89, 135.90 ± 14.38, and 158.35 ± 34.14 g, respectively (table 2).

There were positive correlations, but not statistically significant, of averages maternal serum progesterone

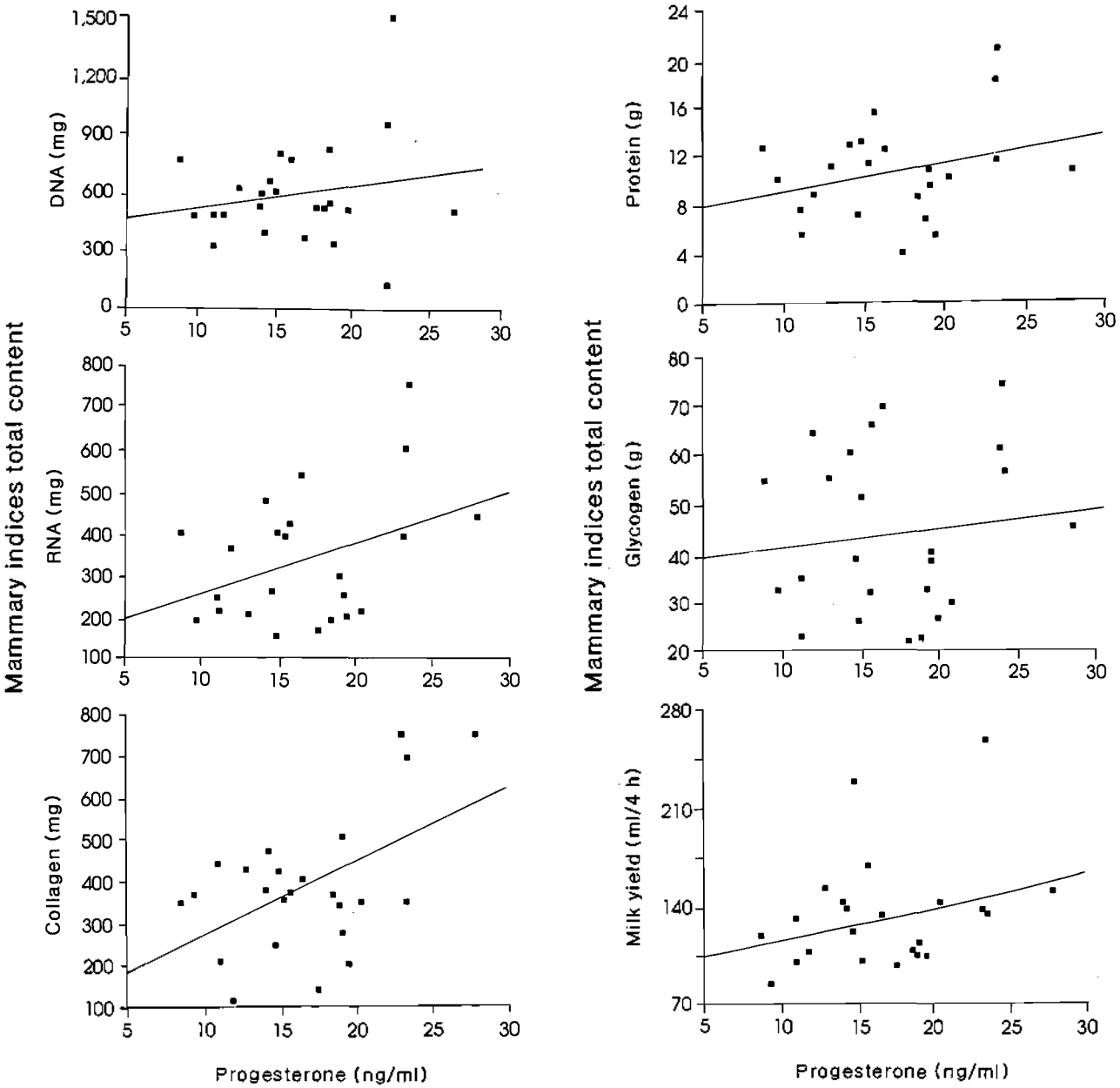

Figure 2. Mammary indices total (DFFT, DNA, RNA, Collagen, Protein, Glycogen) contents at the end of lactation and average milk yield (ml/4 h) during lactation within various ranges of serum progesterone concentrations during pregnancy in Javanese thin-tail sheep.

concentrations during pregnancy with mammary DFFT, mammary DNA, RNA, collagen concentrations per unit weight of DFFT at the end of lactation. However, mammary protein, and glycogen concentrations per unit weight of DFFT at the end of lactation were negatively correlated, but not significant, with the averages serum progesterone concentrations during pregnancy. Mammary DFFT, mammary DNA, RNA, collagen, protein, and glycogen concentrations per unit weight of DFFT at the end of lactation within various averages serum progesterone concentrations during pregnancy are presented in figure 1.

Averages serum progesterone concentrations during pregnancy were positively correlated with the total mammary DNA, RNA, collagen, protein, and glycogen contents at the end of lactation and milk production during lactation. Mammary DFFT, mammary DNA, RNA, collagen, protein, and glycogen contents at the end of lactation within various averages serum progesterone concentrations during pregnancy are presented in figure 2.

DISCUSSION

The results of this study indicated that ewes giving birth to multiple lambs, which had higher averages serum progesterone concentrations during pregnancy, had greater mammary gland indices (DNA and RNA concentrations per unit weight of DFFT, and mammary DFFT, total mammary DNA and RNA contents) at the end of lactation. Since ewes with higher litter size had higher averages serum progesterone concentrations during pregnancy and better developed mammary glands at the beginning of lactation (Manalu and Sumaryadi, 1998a,b), the experimental ewes used in this experiment could have a similar pattern of mammary glands growth and development at parturition. The results of this experiment suggested that ewes with better developed mammary glands during pregnancy (at the beginning of lactation) maintained greater mammary indices throughout lactation.

As an indication of mammary gland growth and development at the beginning of lactation averages serum progesterone concentrations during pregnancy are presented. The ranges of progesterone concentrations measured in this experiment were similar and still in the ranges of those found in the other experiments (Manalu and Sumaryadi, 1998a,b). Based on these progesterone data during pregnancy, it was assumed that the mammary gland growth and development at the beginning of lactation in these experimental ewes might be similar to those previously reported in this breed of sheep (Manalu and Sumaryadi, 1998a). These mammary gland growth indices at parturition were then used as a standard to evaluate mammary gland involution during the course of 3-month lactation.

At the end of lactation mammary DNA and RNA concentrations per unit weight of mammary DFFT did not change as compared to those at the beginning of lactation (Manalu and Sumaryadi, 1998a). Mammary RNA concentrations per unit weight of mammary tissue at the end of lactation were slightly higher than those at the beginning of lactation. Mammary protein and glycogen concentrations per unit weight of mammary DFFT and total mammary protein and glycogen contents were constantly higher at the beginning of lactation than at the end of lactation at respective litter sizes.

These data indicated that the number of cells and synthetic activity per unit weight of mammary glands barely reduced at 3 months postpartum in the sheep. The reduced milk production at the end of lactation seems to be due to the decreased total cells number and synthetic activities as indicated by the reduction in mammary DFFT (by 41, 63, and 64%, in the ewes giving birth to 1, 2, and 3 lambs, respectively), total mammary DNA contents (by 39, 52, and 57%, in the ewes giving birth to 1, 2, and 3 lambs, respectively), and total mammary RNA contents (by 2, 61, and 58%, in the ewes giving birth to 1, 2, and 3 lambs, respectively) as compared to that at the beginning of lactation (Manalu and Sumaryadi, 1998a). The decreased total cell number and activity was also corroborated by the increased mammary collagen concentrations per unit weight of mammary tissues without significant changes in total mammary collagen contents at the end of lactation as compared to those at the beginning of lactation. When compared to mammary collagen concentrations at the beginning of lactation, mammary collagen concentrations per unit weight of mammary DFFT at the end of lactation increased by 115, 116, and 142%, in the ewes giving birth to 1, 2, and 3 lambs, respectively.

The data of this experiment suggested that ewes with better developed mammary gland at the beginning of lactation had greater mammary glands involution after the peak of lactation. This probably the reason why milk production at the end of lactation (3 months postpartum) was similar in all litter sizes, even though total milk production was higher in the ewes with higher litter size. Why mammary glands involution was greater in the ewes having better developed mammary glands? Probably

nutrients availabilities in the mammary epithelial cells (Wilde and Knight, 1989) could be a limiting factor. This could, partly, be a reason for the lower lambs' preweaning growth and survival with the higher litter size. It is suggested that feeding standard in lactating ewes, especially in the small-holder farmer, should be based on litter size to meet nutrients requirements for milk synthesis to nourish the increased number of lambs.

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