POPULATION OF SECRETORY CELLS AND SYNTHETIC ACTIVITIES IN MAMMARY GLAND OF LACTATING SHEEP AFTER CONSUMING *Sauropus androgynus* (L.) Merr. LEAVES

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ABSTRACT

Thirty-five lactating ewes were divided into four groups and given *Sauropus androgynus* (SA) leaf extract solution at 1.89 g/day orally twice a day (SAx-group), SA leaf powder solution at 7.44 g/day (SAp-group), distilled water (control-group), and untreated group. Milk yield was measured. After 14 and 35 days, half of the udders were excised and the DNA and RNA determined. SAp administration increased total milk yield for 35 days administration (P>0.05), compared to the control values. The population of secretory cells (total DNA) and synthetic activities (total RNA) were also increased significantly (P<0.05) by SA leaves administration for 14 days.

Keywords : Sauropus androgynus, milk synthesis, DNA, RNA

Sauropus androgynus (SA), a member of *Euphorbiaceae* family, is a leafy shrub found in Malaysia, Indonesia, South-west China and Vietnam (Padmavathi & Rao, 1990). In Indonesia, the leaves of this plant are commonly used as a vegetable and have properties as stimulants in mother's breast feeding (Soeparto, 1994).

The SA leaves can also stimulate milk production in lactating ruminants (Suprayogi, 1993). It has however not been established if the SA leaves influence milk synthesis process through improving the population of secretory cells and synthetic activities in the mammary gland or through an improvement in the nutrient supply to the mammary gland. Besides, the chemical investigation on the active compounds in SA leaves is still scarce.

This experiment was carried out to determine the influence of either the powder of SA leaves (SAp) or SA leaf alcohol extract (SAx) on the population of secretory cells (as indicated by total deoxyribonucleic acid, DNA) and synthetic activities (as indicated by total ribonucleic acid, RNA) in the mammary gland, and its effect on milk yield.

METHOD

Animals and Housing

This experiment was carried out from March 1998 until July 1999 at the Faculty of Veterinary Medicine and Life-Sciences Interuniversity Center, Bogor Agricultural University, Bogor-Indonesia. This experiment was conducted during the hot (25.50 ± 3.02 °C) and humid (69.85 ± 9.05 % relative humidity) season.

Thirty-five first lactating javanese Thin-Tailed ewes with a mean body weight of 20.20 ± 2.03 kg and age of 1.5 to 2.0 years at the first week of lactation were used. All ewes had twins at parturition, and were placed in individual cages. The Javanese thin-Tailed sheep is normally used as a model of lactating ruminants (Sumaryadi, 1997; Manalu & Sumaryadi, 1998), although it is a meat-type, it is well recognised for its high prolificacy (Bradford *et al*., 1986; Sutama *et al*., 1988).

Animal Feeding

Each ewe in the individual cage was fed a mixed concentrate and dry chopped elephant grass *ad libitum*. Water was available freely. Elephant grass was given at 06:30 h until mid-day and then was replaced with concentrate until late afternoon, and finally it was replaced again with the elephant grass overnight.

Preparation of SA Leaf Powder and Extract Solution

Fresh SA leaves from the local markets around Bogor were dried in an automatic oven at 60°C overnight. The dry leaves were ground to powder (SAp), and the powder was extracted to produce the thick SA leaf extract (SAx).

Extraction method of SA leaves was as described by Santoso *et al*. (1997), using 70 % alcohol as a solvent. The extract was evaluated using the pharmaceutical test standard (Yuliani & Marwati, 1997), and was found to have no toxic effects on the laboratory animal from acute and subacute doses (Santoso *et al*., 1997).

This method uses a maceration technique, as follows: 88 grams of SA leaf powder were mixed with 1 litter of 70 % alcohol, stirred for up to 9 hours and then the mixture was stored for 24 hours. The mixture was filtered and the liquid extract was evaporated using a rotary-evaporator at the temperature of 50°C to produce the thick extract.

SA leaf extract solution (5 %) was made by dissolving 5 g of SA leaf extract in 100 ml distilled water and SA leaf powder solution (18 %) was made by dissolving 18 g of SA leaf powder in 100 ml distilled water.

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Experimental Design

Thirty-five lactating ewes were divided into four groups and fed concentrate and dry elephant grass for 35 days. Each group was given either SA leaf extract solution at 1.89 g/day orally twice a day (SAx-group; 10 ewes), SA leaf powder solution at 7.44 g/day (SAp-group; 10 ewes), distilled water (control-group; 10 ewes), or untreated (5 ewes). The experimental design was completely randomised (Steel & Torrie, 1980).

Milk Yield Measurement

Milk yield was measured using a glass-measuring cylinder. The ewes were hand milked, twice a day at 07:00 h and at 16:00 h.

Mammary Total DNA and RNA Measurement

After 14 and 35 days the ewes were slaughtered, half of the udders were excised. The mammary gland was isolated by trimming the skin, subcutaneous fat, and removing milk inside the gland. The isolated mammary gland was frozen to facilitate slicing. The thinly sliced mammary gland samples were soaked in 90 % ethanol for 48 h., and then with diethyl-ether for a further 48 hours until the glands became free of fat. The fat-free sliced mammary gland samples obtained were dried at 52°C for 48 h., and then ground to make a fine powder for use in the determinations of mammary chemical indices.

DNA of mammary gland was determined by *p*-nitrophenylhydrazine (Sigma: N-8880, 1996) reaction. RNA of mammary gland was determined by the orcinol (Sigma: O-1875, 1996) reaction as described by Manalu & Sumaryadi (1998).

Data Analysis

Analysis of variance (ANOVA) was used to determine the difference between the treatment means (Snedecor & Cochran, 1982). A probability (P) value less than 0.05 was accepted as significantly different. Duncan's multiple range test (Steel & Torrie, 1980) was used to determine differences between the treatment means.

RESULTS AND DISCUSSION

The concentrate and elephant grass intakes were 880.50 (±45.75) and 460.80 (± 38.35) g of dry matter, respectively. The daily nutrient intake was close to the nutrient requirements of ewes per day, according to NRC (1985) recommendations for lactating ewes suckling twins in the first 6-8 weeks of lactation.

SAp and SAx administration increased total milk yield for 35 days by 7.75 % and 0.89 % (P>0.05), respectively compared to the control values of 17.89 litres (Table 1). The mammary cell numbers (total DNA) and synthetic activities (total RNA) were also increased significantly (P<0.05) by SA leaf administration for 14 days. The SAp administration had a higher contribution to the increase in total DNA and RNA than SAx administration of respectively, 72.84 % vs 25.93 % in DNA and 112.97 % vs 47.28 % in RNA, compared to the control values of 0.81 g in total DNA and 2.39 g in total RNA (Table 2).

The possible reason of the enhancement of milk yield was due to the increase in the proliferation of mammary gland cells and their synthetic activities as indicated by an increase in total mammary DNA and RNA (Manalu & Sumaryadi, 1998). Probably, the SA-leaf active compounds might directly or indirectly modulate, prior to lactogenesis and lactation, hormones such as prolactin (PRL), growth hormone (GH), glucocorticoids, thyroid hormone, prostaglandin, and oxytocin. These hormones directly stimulate the synthesis of DNA and RNA in the lactating mammary secretory cells (Shiu & Friesen, 1980). Further studies should be carried out to identify chemical substances in the SA leaves, which play an important role directly or indirectly in milk synthesis.

Table 1. Weekly mean milk yield, total yield for 35 days, and percentage increase in yield of lactating ewes given SA leaf extract (SAx) and SA leaves powder (SAp).

Mean Weekly Yield	Mean daily milk yield per ewe (litres ± SD)		
	Control Group	SAx-Group	SAp-Group
0 week*	0.43±0.06	0.43±0.09	0.42±0.05
1st week	0.50±0.06	0.50±0.09	0.49±0.04
2nd week	0.53±0.07	0.51±0.08	0.56±0.07
3rd week	0.52±0.05	0.51±0.09	0.57±0.06
4th week	0.50±0.10	0.52±0.08	0.55±0.05
5th week	0.44±0.10	0.48±0.10	0.52±0.05
Total Milk Yield For 35 days	17.89 ± 2.36	18.05 ± 3.12	19.27 ± 1.50
(%) Increase in Milk Yield	0.00	0.89	7.75

* Mean milk yield before starting treatment, or mean milk yield for the first week of lactation.

Table 2. Mean mammary total deoxyribonucleic acid (DNA), and total ribonucleic acid (RNA) of lactating ewes given SAx, SAp, and PPV

Duration of treatment	Group	Mean mammary total DNA and RNA (g ± SD)*	
		DNA	RNA
Untreated (1st week lactation)		0.60 ± 0.19	1.01 ± 0.73
14 Days	Control	0.81 ± 0.05[a]	2.39 ± 0.82[a]
(3rd weeks -	SAx	1.02 ± 0.26[a]	3.52 ± 1.10[a]
lactation)	SAp	1.40 ± 0.43[b]	5.09 ± 1.66[b]
35 Days	Control	0.96 ± 0.20	4.57 ± 2.76
(8th weeks -	SAx	0.98 ± 0.22	4.58 ± 1.60
lactation)	SAp	1.10 ± 0.27	4.88 ± 2.38

* Means with no common superscript ([a], [b]) in the same column are significantly different (P<0.05)

The reason for the lower milk yield by SAx administration compared to SAp administration is that the chemical active compounds in the 70 % alcohol extract are not quantitatively and qualitatively completely available as in the powder form. Therefore the biological effect of the active compounds, in the SAx were not maximal. Probably, addition of higher doses of the SAx could improve biological response.

CONCLUSIONS

The enhancement of milk yield in the lactating mammary gland can be due to an increase in the population of secretory cells (total DNA) and the synthetic activities (total RNA). The higher contribution on the biological effect occurred from SAp administration than SAx administration. Further studies on the influence of the SA leaves administration on the improvement of nutrient supply to the mammary gland needs to be conducted.

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