The Influence of *Sauropus androgynus* leaves on the Blood Serum Volatile Fatty Acids Concentration in Lactating Sheep

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ABSTRACT

The leaves of *Sauropus androgynus* (SA) plant can stimulate milk production in lactating ruminants. It has however not been established if the SA leaves influence milk production through the improvement of nutritional factors or hormonal factors. This experiment was conducted to elucidate the influence of either the powder from SA leaves (SAp) or SA leaf alcohol extract (SAx) on the profiles of blood serum volatile fatty acids (VFAs) in lactating sheep.

Thirty-five lactating ewes were divided into four groups and fed with concentrate and dry elephant grass at approximately 880.50 (±45.75) g and 460.80 (±38.35) g respectively for 35 days. Each group was given orally twice a day SA leaf extract solution at 1.89 g d^{-1} ewe^{-1} as SAx-group (10 ewes), SA leaf powder suspension at 7.44 g d^{-1} ewe^{-1} as SAp-group (10 ewes), distilled water as control-group (10 ewes), and untreated-group (5 ewes). Blood serum VFAs concentration were measured using the gas chromatography technique. The study showed that SAp administration had higher contribution to the increase in nutrient supply to the mammary gland (as indicated by total VFAs concentration in the portal vein) than SAx administration, with differences of 55.86 % vs 20.81 % of total VFAs respectively, compared to the control. The possible reason of the biological effects is that the active substances in the SA leaves might improve the fermentative processes in the rumen environment.

Keywords: *Sauropus androgynus*, Blood, VFAs, Sheep

Introduction

Sauropus androgynus, a member of *Euphorbiaceae* family, is a leafy shrub found in Malaysia, Indonesia, South-west China and Vietnam. This plant is commonly used as a vegetable. In Indonesia, many people believe that this plant supports lactation in human beings. Mothers eat or drink SA leaves and preparations respectively, in order to increase their breast feeding capacity (SOEPARTO, 1994). In animal husbandry, farmers occasionally use SA leaves or mixtures of SA and other leaves as additives in dairy cow diets with the purpose of improving milk yield (IIRR, 1994).

The benefit of SA leaves as a stimulant of milk yield have already been proven scientifically in lactating ruminants (SUPRAYOGI, 1993; SANTOSO *et al.*, 1997). In the ruminant digestive system, the rumen fermentative processes produces volatile fatty acids (VFAs) and microbial protein (CUNNINGHUM, 1997). There is a close relationship of these nutrients with milk synthesis in the mammary gland, although the hormonal factors also play an important role in the milk synthesis.

The investigation according to the VFAs production associated with the consuming SA leaves under *in-vitro* conditions using concentrate fermented with rumen liquor have already reported by SUPRAYOGI *et al*. (2000). They reported that the SA leaf alcohol extract or the powder from SA leaves increase partial and total VFAs production from concentrate fermented with rumen liquor under incubated anaerobic conditions. Probably, the chemical substances in the SA leaves such as *monomethyl succinate*; *methylpyroglutamate* and *cyclopentanol,2-methyl-,acetate,cis* (AGUSTA *et al.*, 1997) play an important role in the enhancing fermentative processes by stimulating the metabolic activities and microbial growth in the rumen liquor.

The influence of consuming SA leaves on the fermentative processes in rumen liquor and blood serum VFAs concentration under *in-vivo* conditions have not been established up to now.

This experiment was conducted to elucidate the influence of either the powder from SA leaves (SAp) or SA leaf alcohol extract (SAx) on the profiles of blood serum volatile fatty acids (VFAs) in lactating sheep.

Method

This experiment was conducted during the hot (25.50 $\pm$ 3.02 ^{o}C) and humid (69.85 $\pm$ 9.05 % relative humidity) season. Thirty-five first lactating Javanese Thin-Tailed ewes with a mean body weight of 20.20 $\pm$ 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.

Each ewe was fed a mixed concentrate and dry chopped elephant grass at approximately 880.50 ($\pm$45.75) g and 460.80 ($\pm$38.35) g respectively for 35 days. 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-Indonesia was dried in an automatic oven at 60^{o}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^{o}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.

Experimental Design:

Thirty-five lactating ewes were divided into four groups. 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) for 35 days. The experimental design was completely randomised (Steel & Torrie, 1980).

All solutions were given by oral administration twice a day in the morning (at 07:30 hours) and in the afternoon (at 16:30 hours). After two weeks of administration, 15 ml of blood samples from portal vein was taken from 5 ewes of each group by abdominal surgery procedure using an anaesthetizer (Rompun®, Xylazin). This procedure was repeated after 5 weeks of administration on the remaining animals.

Blood serum volatile fatty acid (VFAs) analysis:

In a tube with 8 ml 0.2 N H_2SO_4, 2 ml of blood serum sample was added. After shaking for 10 minutes, 2 ml of 10 % Na-tungstate was added to the mixture, which was filtered so that a protein-free filtrate was produced. From the filtrate, the sodium salts of the fatty acids were formed by addition of 0.1 ml of 3 N NaOH to 5 ml of the protein-free filtrate. The salts were acidified with 0.2 ml of 1.6 N H_2SO_4 and thoroughly mixed for about 10 minutes. The solution was then directly used for individual VFA analysis using the gas chromatography technique (ERWIN *et al*., 1961).

One ml of the solution from each ewe was injected into the gas chromatography. Peaks were standardized by injection of volatile fatty acid standard.

Data Analysis:

Analysis of variance (ANOVA) was used to determine the difference between the treatment means (SNEDECOR and COCHRAN, 1982). A probability (P) value less than 0.05 was accepted as significantly different. Duncan's multiple range test (STEEL and TORRIE, 1980) was used to determine differences between the treatment means.

Results and Discussion

The oral administration of SAx and SAp solution for 35 days tended to influence acetic, propionic, n-butyric, isobutyric, isovaleric acid, n-valeric acid, and total VFAs concentration. This data is presented in Figures 1 and Table 1. The administration of these solutions for 14 days show a tendency of an increase in the individual and total VFAs concentration ($P>0.05$), while the continuing administration up to 35 days show the same VFAs concentration across treatment. The highest enhancement of 55.86 % in total VFAs concentration occurred in the SAp group after 14 days. The increase in total VFAs concentration after 14 days treatment also occurred in the SAx group, it was approximately 20.81 % compared to the control.

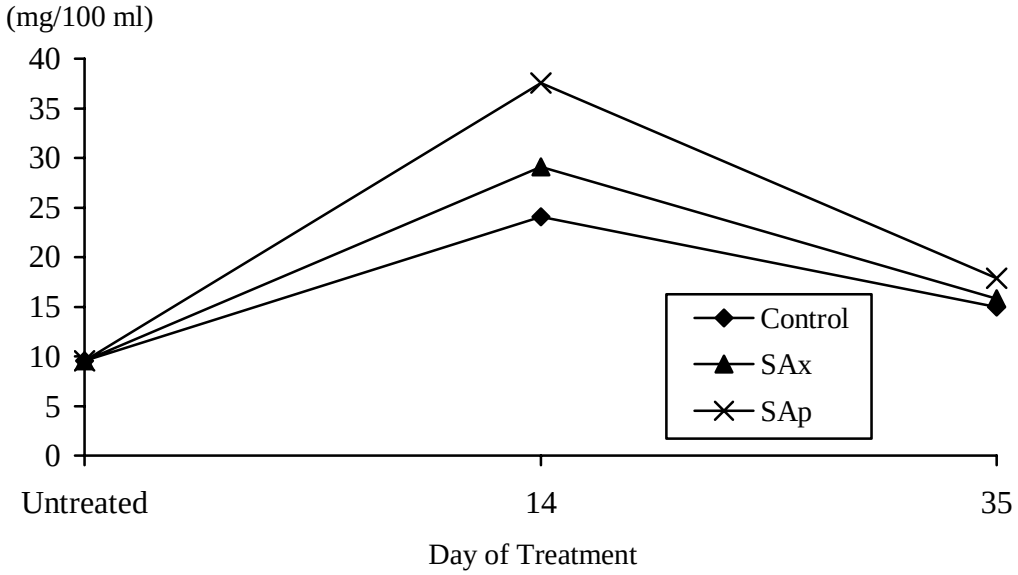

Figure 1: Mean serum total volatile fatty acid concentration (mg/100 ml) in the portal vein of lactating ewes given SAx and SAp

Table 1: Mean serum volatile fatty acid concentration (mg/100 ml) in the portal vein of lactating ewes given SAx, SAp, and SAp

Duration of treatment	Group	Mean VFAs concentration (mg/100 ml ± SD)*				
		Acetic acid	Propionic acid	n-Butyric acid	Isobutyric acid	Total VFAs
Untreated		6.79 ± 1.86	1.58 ± 1.46	1.14 ± 1.42	0.98 ± 1.35	11.48 ± 5.94
14 Days	Control	14.80 ± 7.45	4.35 ± 2.10	1.88 ± 1,26	1.04 ± 1.12	24.08 ± 10.95
	SAx	20.26 ± 14.45	4.65 ± 5.16	3.71 ± 4.08	0.33 ± 0.74	29.09 ± 22.79
	SAp	23.34 ± 18.09	5.14 ± 7.29	2.30 ± 3.41	4.85 ± 7.11	37.53 ± 36.75
35 Days	Control	10.60 ± 3.58	1.89 ± 1.76	0.82 ± 1.25	0.81 ± 1.16	14.94 ± 6.13
	SAx	11.12 ± 1.10	1.83 ± 1.68	1.10 ± 1.54	1.06 ± 1.46	15.81 ± 3.70
	SAp	12.53 ± 3.91	2.66 ± 0.61	1.15 ± 1.57	1.28 ± 1.29	17.88 ± 4.50

*: Isovaleric and n-Valeric acid were not found in the assay.

This experiment showed that the partial and total volatile fatty acids concentration in the portal vein has a tendency to increase as a result of the SA leaves administration, although the enhancement in the SA leaf powder form was higher than in the SA leaf alcohol extract. The increase in VFAs concentration in the portal vein is a consequence of the enhancement of their absorption from the digestive tract, due to the fermentative processes in

the rumen liquor is enhanced by administration of SA leaf alcohol extract or the powder (SUPRAYOGI *et al*., 2000). In the liver these will be converted to acetate, glucose, and ß-hydroxybutyric acid (BHBA). In the post hepatic circulation these nutrients are important as precursors in milk synthesis, for such components as lactose, fatty acid, and amino acids in the mammary epithelial cells (BAUMAN and DAVIS, 1974; COLLIER, 1985; PRESTON and LENG, 1987).

Conclusions

The study indicated that the consuming of SAp had higher contribution to the increase in nutrient supply to the mammary gland (as indicated by total VFAs concentration in the portal vein) than consuming of SAx , with differences of 55.86 % vs 20.81 % of total VFAs respectively, compared to the control. The possible reason of the biological effects is that the active substances in the SA leaves might improve the fermentative processes in the rumen environment.

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