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Effect of Katu Leaf (*Sauropus androgynus*) Extract Supplementation on Milk Quality and Yield of Bali Cow Fed Rice Straw and Natural Grass Basal Diet

Ketut Suriasih[1*], Nyoman Sucipta[2],wayan Siti[3], Made Suci Sukmawati[4]
1, 3, 4. Faculty of Animal Husbandry, Udayana University, Denpasar, Indonesia
2. Faculty of Agricultural Technology, Udayana University, Denpasar, Indonesia

Abstract
Milk yield and quality of bali cows given supplementation of katu leaf (*Sauropus androgynus* L Merr) extract were investigated. A completely randomized design with three treatments and three replications were used in this experiment. The result showed, milk yield of bali cows in treatments B (0.5% w/bw supplementation of katu leaf extract) and C (0.1% w/bw supplementation of katu leaf extract) were respectively 43.22% and 9.63% higher than that of bali cows in treatment A (0% w/bw supplementation of katu leaf) (P>0.05%). Protein content, fat percentage, lactose content and total solids of milk of bali cows given katu leaf extract supplement were ranging from 3.42-3.55%; 6.37-6.67%; 3.77-3.93% and 16.67-16.95% respectively. There were no significant differences in protein, fat, lactose and total solid percentages of bali cows milk in treatments A, B and C (P>0.05)
Keywords: *milk yield, quality, bali cow, katu leaf*

1. Introduction

Almost 70% of Indonesian milk consumption, come from abroad, due to low domestic milk production. Much of the milk produced in Indonesia harvested from dairy cattle, the rest come from goat and buffalo. Bali cattle was a beef cattle that yield high quality meat. This cattle yield milk of about 1.1 kg/head/day on natural grass of traditional feeding regime (Liwa, 1992). Bath *et al*. (1978) stated that 70% milk production was affected by environment including feeding regime. In 1996 Sarini *et al*. (1998) found that milk yield of bali cattle increase of about 45.45% as concentrate was added to basal feeding navier grass. Sukarini (2000) found that milk yield of bali cattle was 2.2 kg/head/day as concentrate, *Gliricidia sepium*, hibiscus leaf and Zn mineral was added to basal feeding navier grass. Putra (2006) stated that milk yield of bali cow may reach 4-5 kg/head/day in peak lactation. Better quality and quantity of feed offered to the cow will increase the availability of milk precursors in blood so that increase the milk yield.

Katu leaf (*Sauropus androgynus* L.Merr) was known, capable of increasing milk yield of human, goat and dairy cattle (Marwah *et al*., 2010; Garantjang and Mide, 2011) and was grow naturally in farm and wild field. Soka *et al*. (2011) claimed that katu leaf supplementation increase blood prolactin and oxytocin concentration in mice Balb/C, as well as their milk yield. Schmidt (1971) and Tucker (1985) explained that, in addition to the availability of milk precursors in blood enter the mammary gland, milk production was also affected by the occurrence of some hormones that regulate milk synthesis and milk let down. Prolactin was a hormone that responsible in milk synthesis in the mammary gland, while oxytocin was a hormone that squeezed the milk, drain out from the alveoli, down to the gland cistern of the mammary gland. This processes call “milk let down”, which emptied the lumen of the alveoli. Furthermore Schmidt (1971) stated that full milk content ion lumen of alveoli will retard milk synthesis in secretory cells and milk synthesis start again, as the milk in the lumen of alveoli was drawn out. So that in the presence of oxytocin and prolactin the secretory cells of the mammary gland could synthesized milk continuously.

The present study was intended to find out milk yield of bali cow given basal feeding rice straw, natural grass and pollard, supplemented with katu leaf (*Sauropus androgynus* L. Merr) extract. It is hoped that the increase in milk yield of bali cows could be used for human consumption.

2. Materials and Methods

2.1 Animal and Feed

Nine, 4-5 years old bali cows were used and allocated to 3 group of 3 animals each in a completely randomized design. The animals were subjected to three treatments namely: A (without supplementation of katu leaf extract or control); B (0.05% (w/bw) supplementation of katu leaf extract); C (0.1% (w/bw) supplementation of katu leaf extract) and three replications. These animals were hired from farmers at Tabanan Regency, province of Bali, Indonesia. All cows were given a basal diet composed of rice straw, natural grass, pollard, and katu leaf extract (*Sauropus androgynus L Merr*) supplementation. Rice straw procured from nearby paddy field was chopped manually. Natural grass was also procured from nearby fields. Pollard bought from nearby poultry shop. The ingredient composition of the diet is presented in Table 1.

Table 1. Ingredient and chemical composition of diet used in this experiment

Ingredients/Nutrients	Treatments		
	A	B	C
Rice Straw	59.08	59.04	59.04
Natural grass	17.73	17.73	17.69
Pollard	23.19	23.19	23.19
Katu leaf (*Sauropus androgynus L.Merr*)	0.00	0.04	0.08
Total	100	100	100
Nutrients			
Dry Matter	11.319	11.323	11.331
Crude Protein	0.977	0.98	0.982
Total digestible nutrient	4.80	4.801	4.801
Crtude fiber	2.83	2.86	2.92

Note: A: 0% (w/bw) katu leaf extract supplementation; B: 0.05% (w/bw) katu leaf extract supplementation; C: 0.1% (w/bw) katu leaf extract suppolementation

2.2 Preparation of katu leaves extract.

Katu leaf was weigh and blended with tap water to obtained katu leaf extract with a concentration of 30%. Then 500 ml and 1000 ml of the extract were then offered daily to bali cows in treatment B and treatment C respectively. Katu leaf extract was offered a week before parturition and continued for a period of 9 weeks lactation.

2.3 Milk collection

Milk from each of the lactating bali cow was collected every week and commenced 7 days after calving, to allow collostrum intake by the calf. A day before milking at 16.00 PM and during milking the calf was separated from the cow. Milking was done once a day at 07.00 AM. The milk obtained was offered to the calf after it is weighing. The weight of the milk was divided by the time (hours) from calf separation to the milking time, then multiplied by 24 to get daily milk yield.

2.4 Chemical evaluation

2.4.1 Proximate analyzes

Proximate composition of the katu leaf extract and experimental diets were carried out according to the procedure of AOAC (1990).

The crude protein was determined by the Kjeldahl method as described by AOAC (1990). Put I g ground sample into Kjeldahl digestion tube. Add sufficient catalyst tablets to supply 7 g K_2SO_4 , 0.8 g $CuSO_4$ and 12 ml sulfuric acid. Place the tube in block digester in an acid fume hood which was preheated to 410^{o}C. Digest about 60 minutes. Remove tube and let cool for about 10-20 min in a fume hood. Add deionized water till the total volume of 80 ml. Add about 50 ml of 40% NaOH (w/w), then conduct distillation. Placed 250 m titrating flask containing about 25 ml 4% boric acid added 3 to 4 drops methyl red indicator, on the receiving platform, with tube from condenser extending below the surface of the solution. Attach digestion tube to distillation unit and steam distill until 150 ml distillate was collected. Remove titrating flask from distillation unit, titrate with 0.1 N HCl to purple end point. Record volume of acid (VHCl) required for the titration. Titrate reagent blank similarly.

Calculations:

$$\% N = \frac{(VA - VB) \text{ x N HCl x } 1.4007}{W}$$

VA = volume , in mL of HCl required for sample; VB = volume, in mL of HCl required for blank

NHCl = Normality of HCl; 1.4007 = milliequivalent weight of N x 100; W = sample weight in grams

% protein of feed = % N x 6.25

% protein of milk = % N x 6.38

Determination of crude fiber was done according to AOAC (1990). Put 1 gram of prepared sample (C) into test tube, add 60 mL of 0.3 *N* H_2SO_4 solution, then turn Agitate and Heat for 30 minutes. Add 30 ml of 1.5 *N* NaOH, then again turn Agitate and Heat for 30 min. Drain ans exhaust the hot solution using vacuum pump. Rinse using 100 ml hot water, 100 ml 0.3 *N* H_2SO_4, 100 ml hot water and 100 ml alcohol, then dry in oven at 105^o±2^{o}C for 8 hours. Remove tubes from oven, place directly into a desicator for 30 min, then weigh (A grams). Then ash the entire tube with sample for 3 hours at 600 ± 15^{o}C, cool in desicator for 30 min and weigh (B grams).

Calculations :

$$\%\ Crude\ Fiber\ = \frac{A\ -\ B}{C}\ x\ 100\ \%$$

A = sample weight after drying in oven; B = sample weight after heat at 600°C; C = sample weight in grams

2.4.2 Milk analyzes

Chemical analyzes of milk was carried out according to AOAC (1990), AOAC (1975) and Bradley *et al.* (1992). The chemical analyzes include milk fat percentage, protein content as well as lactose percentage. Determination of protein was carried out by Kjeldahl method (AOAC, 1990), as described on point 2.4.1 above.

Milk fat determination was done by Babcok methods according to AOAC (1975). With a pipet, transfer 17.6±0.05 ml prepared sample at 38°C to milk-test Babcock bottle. Add the 17.5 ml 91-92% H_2SO_4 solution in one delivery. Immediately shake by hand rotation until all traces of curd disappear. Place bottles in heated centrifuge, counterbalance, and after proper speed is reached, centrifuge 5 minutes. Add soft H_2O at 60±1°C until bulb of bottle is filled. Centrifuge 2 minutes. Add soft H_2O at 60±1°C until fat column top approaches the 8% mark of the graduated neck of the Babcock bottle. Centrifuge 1 minute longer at about 60°C. Transfer bottle to warm H_2O bath kept at 57.5±1°C, immerse it to level slightly above the top of fat in column, and leave until column is in equilibrium and lower fat surface assumes final convex form (>5 minutes). Remove one bottle from bath, wipe it. The length of the fat layer, as read off on graduation, was give at once per cent of fat.

3. Result and discussion

3.1 Milk yield.

Table 2 represents average daily milk yield and nutrient intake of bali cows in this experiment. Average daily milk yield of bali cows given katu leaf extract supplement were not significantly different (P>0.05) throughout the experimental. This is not in line with Marwah *et al.* (2010), Suprayogi *et al.* (2013) and Garantjang and Mide (2011) who stated that supplementation of katu leaf extract were significantly increase milk production of goat and Friesien Holstein (FH) cow. However, although milk yield of bali cows in this experiment was statistically not different, but biologically milk yield of bali cows given 0.05% katu leaf extract supplement was 43.22% higher than that bali cows in control group. This is in line with Suprayogi *et al.* (2013) in that the increase in milk yield of FH cow given 100g, 150g and 200g /head/day katu leaf extract were 30%, 40% and 34%.

Milk production was about 70% affected by environment, especially feeding (Bath *et al.*,1978). Sukarini (2000) found that improve feeding quality increased milk yield of bali cows of about 36.6 – 126.5%. Furthermore, Campbell and Marshall (1978) stated that milk yield was affected by milk precursors available in blood circulate to the mammary gland, which were resulted from metabolisms of nutrient intake. Since the nutrient intake of bali cows in treatment A, B and C were not significantly different (P>0.05) (Table 2), so that milk yield of those bali cows were also not significantly different (P>0.05). This is in accordance with Sucipto (2005) who found that average nutrient intake of FH cow given katu leaf powder was not significantly different. This is because katu leaf supplementation did not increase the palatability of the feed in this experiment.

Table 2. Milk yield of bali cow given rice straw and natural grass supplemented with *katu leaf* extract

	A	B	C
Milk yield (kg/head/day)	0.696[a]	0.997[a]	0.763[a]
Nutrien Intake			
DMI (kg/head/day)	11.291[a]	11.308[a]	11.300[a]
Crude Protein (kg/head/day)	0.905[a]	0.92[a]	0.91[a]
Total digestible nutrients (kg/head//day)	4.69[a]	4.76[a]	4.72[a]
Crude fiber (kg/head/day)	2.80[a]	2.79[a]	2.80[a]

Note: A : no supplementation; B: 0.05% (w/BW) katu leaf supplementation; C : 0.1% (W/BW) katu leaf supplementation; values followed by same superscript in the same row were not significantly different (P>0.05)

Beside feeding regime, milk production was also affected by the physiological activity of the animals namely hormonal control. Suprayogi (2000) claimed that katu leaf has 7 active compounds that contribute to the increase in milk production. The 7 active compounds were: (1) octadenoic acid , (2) 9-eicosine, (3) 5, 8, 11-Heptadecatrienoic acidmethyl ester,(4) 9, 12, 15- Octadecatrienoic acid ethyl ester, (5) 11, 14, 17 eicosatrienoic acid methyl ester, (6) androstan-17-one,3-ethyl-3-hydroxy-5 alpha and (7) 3, 4-Dimethyl-2 oxocyclopent-3-enylacetatic acid. The first 6 active compound collectively act as (a) a precursor and is involved in compound biosynthesis of eicosanoids (prostaglandins, lipoxins, thromboxin, prostacyclin, leukotrienes), (b) as precursor in synthesis of hormones compounds such as progesterone, estradiol, testosterone, and glucocorticoid (c) modulator of lactation hormone, lactogenesis and other physiological activities.

Furthermore Schmidt (1971) and Tucker (1985) stated that synthesis of prostaglandins and steroid hormone and their occurrence in circulation will stimulate the anterior and posterior pituitary gland to release prolactin, growth hormone and oxytocin which synergizely affect the secretory tissue of the mammary system through

increasing the alveolar population as well as milk synthesis.
In addition Suprayogi (2000) stated that, active compound of katu leaf increase the availability of milk precursor in blood circulated to the mammary gland. Moreover, in digestive tract of monogastric and ruminant the active compounds such as 3,4-dimethyl-2-oxocyclopent-3-enylacetic acid, monomethyl succinate, phenylmalonic acid, cyclopentanol,2-methyl-acetate, and methylpyroglutamate, may hydrolyzed to succinate, malonic acid, acetate and glutamate. These three compounds biochemically and physiologically contribute to metabolisms of carbohydrate, protein and fat to increase volatile fatty acids (VFAs) yield through citric acids cycle in Krebs cycle, rumen microbial protein synthesis. The increase in VFAs production mean more milk precursor available in blood circulated to the mammary gland. Further more Suprayogi (1995) stated that the active compound of katu leaf increase glucose metabolisms for lactose synthesis, which caused an increase in breast milk yield. This is in line with Sukarini (2000) who explain that lactose is the limiting factor in milk synthesis in the alveoli, for it was in correlation to osmotic pressure in the alveoli. The increase in lactose quantity in the alveoli will increase water transport into the alveoli, which caused the increase in milk produced.
On the other hand, milk production of bali cows given 0.1% (w/bw) katu leaf extract supplement was lower than that given 0.05% (w/bw) katu leaf extract. Katu leaf supplementation will increase prolactin secretion of the anterior pituitary gland (Suprayogi, 2000). Soka *et al.*(2011) stated that prolactin secretion has its own control through a short feedback mechanisms. Increase serum level of prolactin increases hypothalamic dopamine synthesis and the concentration of dopamine in hypothalamo-hypophysial portal blood. These statements lead to an explanation on the higher gene expression level of prolactin at the lower dosage compared to the higher dosage. The higher dose of *S. androgynus* leaf extracts is given, the higher papaverine is consumed, which enhances prolactin secretion, thus enhances dopamine secretion. A higher dopamine secretion causes an inhibiton of prolactin secretion which means lower milk produced

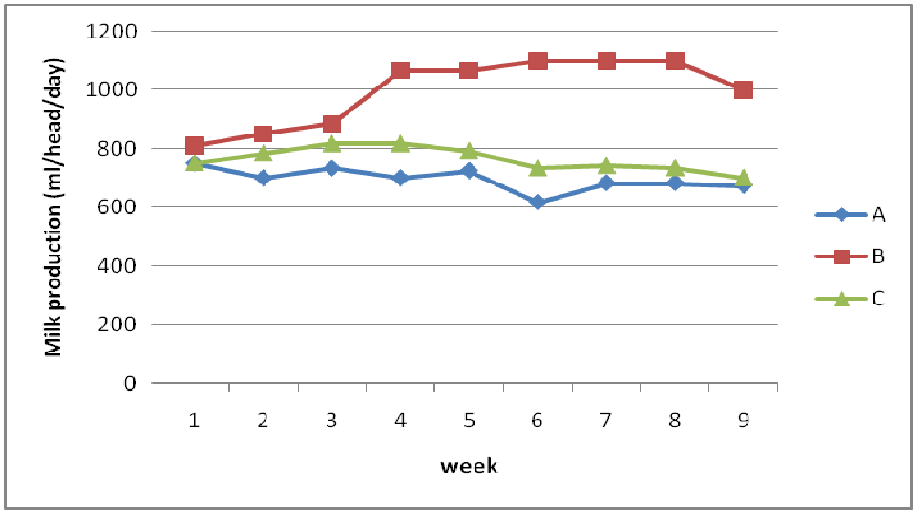

Figure 1. Milk yield of bali cow given katu leaf extract supplementation to the rice straw and natural grass basal diets

Weekly milk yield of bali cow was presented on Figure 1. Milk yield of bali cows offered 0.05% (w/bw) katu leaves extract supplement gradually increase from week 1 to week 3, then increase markedly in week 4.The milk yield was stable until week 8 then starting to decrease. The result was not in accordance with Sukarini (2000) who found that a reduction in milk yield of bali cow given *Gliricidia sepium* and navier grass from week 1 to week 17 of lactation, while bali cow that offered gliricida sepium, navier grass and concentrate showed reduction in milk yield from week 1 to week 5, but then increase until week 9. The stable increase in milk yield of bali cows offered katu leaves extract supplement may be due to continuous secretion of prolactin hormone that stimulate the alveolar activity of the mammary gland to increase secretory cell population as well as milk synthesis. Reduction in milk yield in week 9 was caused by reduction in prolactin secretion. Tuck (1985) stated that naturally quantities of prolactin release at each milking gradually diminish as the milking period proceed.

3.2 Milk quality

Table 2. presented the compositional quality of milk from bali cow offered katu leaf extract supplement. Average milk fat percentage of bali cows milk subjected to treatment A,B and C were statistically not different (P>0.05). This is in line with Marwah *et al.* (2010) who found that katu leaf supplementation in goat did not affect milk fat content. The fat content of milk was affected by acetic acid production in rumen. Since feed intake of the animal was not different, the acetic acid production was also the same.

Table 3. Milk composition of bali cow given basal diet rice straw, natural grass, supplemented with katu leaf extract

Treatment	Milk component (%)			
	Protein	Fat	lactose	Totgal solid
A	3.55 ± 0.26[a]	6.47 ±1.84[a]	3.93 ± 0.52[a]	16.95 ±2.27[a]
B	3.43 ± 0.32[a]	6.67 ± 1.16[a]	3.77 ± 0.51[a]	16.67 ± 2.21[a]
C	3.52 ± 0.22[a]	6.37 ± 1.58[a]	3.89 ± 0.64[a]	16.78 ± 2.12[a]

Note : A: 0% supplementation of katu leaf extract; B: 0.05% supplementation of katu leaf extract; C: 0.1% supplementation of katu leaf extract. Values followed by same superscripts in the same column was not significantly different (P>0.05)

The Protein content of milk from bali cows given katu leaves extract were not significantly different compared to control (P>0.05). Milk protein was synthesized from amino acid in blood. Blood amino acid content of ruminant revealed as the result of feed protein degradation in the rumen and undegradable protein digestion in the intestine. Protein degradation in the rumen will yield amino acids. Vasconcelos *et al.* (2006) stated that there is correlation between crude protein intake and blood urea concentration. Increased crude protein intake will followed by the increase in blood urea concentration. Furthermore they stated that feed's protein that reach the intestine will hydrolyze to NH_3 which absorb into blood, then metabolized to blood urea in the liver. Tillman *et al.*(1991) stated that intake protein entered the rumen were hydrolyzed to amino acids, organic acid, ammonia and CO_2 by rumen microbes. The ammonia was then circulate in blood and hydrolyzed to blood urea in the liver. Therefore the blood urea of ruminant was also depict the amino acid content in the blood. It can be explained that the statistically not significantly different in milk protein of bali cows given treatments A, B and C were due to a not significantly different in protein intake, and blood urea concentration (Table 1 and Table 4)

Table 4. Average blood glucose and urea of bali cow given katu leaves extract supplementation.

Blood milk precursor	Treatments		
	A	B	C
Glucose (mg/dl)	48.67 ± 1.78[a]	52.03 ± 2.08[a]	52.66 ± 2.44[a]
Urea (mg/dl)	29.87 ± 2.33[a]	30.97 ± 0.83[a]	31.03 ± 1.66[a]

Values followed by same superscripts in the same row was not significantly different (P>0.05)

Lactose percentage of milk obtained from bali cows given katu leaf extract were not significantly different (P>0.05). Lactose percentage of milk from bali cows in treatments B and C were respectively 6.9% and 8.1% higher than that of milk from bali cows in treatment A. Milk lactose was synthesized from blood precursor glucose. Blood glucose of bali cows in treatment A, B and C were not significantly different (P>0.05) (Table. 4). Therefore the lactose percentage of bali cows milk in treatments A, B and C were not significantly different (P> 0.05).

Total solids of milk obtained from bali cow given treatments A, B and C were significantly not different (P>0.05). Total solid of milk was composed of lactose, protein, fat, mineral and vitamin of the milk. Since the lactose, protein and fat percentage of the milk was not significantly different, the total solid of the milk was also not significantly different (P>0.05).

4. Conclusion

Supplementation of 0.05% (w/BW) katu leaves extract increased milk yield of bali cows of about 43,6% compared to control. Milk composition of bali cows, such as protein, fat and lactose percentage did not change by the katu leaf extract supplement.

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