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Digestibility, Milk Yields, and Milk Quality of Ettawa Crossbred Goats Fed *Coleus amboinicus* L. Leaf Extract

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ABSTRACT

Coleus amboinicus **L. leaf (CAL) could reduce the rate of amino acid deamination and protein degradation within the rumen. This phenomenon would hopefully increase the amount of protein that passes through the rumen to the abomasum. Therefore, it might improve the digestibility of the ration and then influence the milk yield and quality. The objective of this experiment was to evaluate the effect of extracted CAL within the ration on the ration digestibility, milk yield, and milk quality of Ettawa crossbred (EC) goats. This study used sixteen EC with an average weight of 32.25 ± 3.31 kg and aged 1.5–2.5 years. Experimental goats were fed diets with different CAL extracts: P_0, 0% (control); P1, 2% powdered CAL; P2, 2% ethanol-extracted CAL; and P_3, 2% water-extracted CAL. The experiment was conducted in a randomized block design with four replication blocks. All variables were statistically analyzed with ANOVA and significances were followed by Duncan's test. P_3 treatment significantly ($p<0.05$) increased milk yield and milk quality in comparison with control. Unlike the other treatments, P_3 treatment was water-extracted CAL that could affect these variables. P_3 treatment showed the best result among the four treatments. In conclusion, P_3 treatment, the supplementation of 2% water-extracted CAL within the ration, could improve the milk yield up to 30.24% in comparison with the control ration and also improve milk composition, such as milk protein (4.47%), milk casein (3.99%), milk fat (3.85%), and solid nonfat (SNF) (6.53%). The supplementation of water-extracted CAL within ration could improve the milk yield and milk quality of EC.**

Keywords: Coleus amboinicus; digestibility; Ettawa crossbred; milk yields

INTRODUCTION

There have been some efforts to improve the milk yields of Ettawa crossbred (EC) goats. Acharya *et al*. (2015) and Dharmawan *et al*. (2019) have modified the ration composition to increase milk yields. Djoković *et al*. (2015) tried to use hormone therapy to accelerate the growth of the mammary gland during pregnancy and Resthu *et al*. (2019) studied the effect of oxytocin induction on the milk chemical composition of EC milk in Aceh, Indonesia. Other studies have aimed to improve nutrient intake (Marwah *et al*., 2010) and nutrient digestibility (Arief *et al*., 2016) during pregnancy and lactation. However, the optimal method to improve the quantity and quality of milk is still uncertain, especially during early lactation in EC goats. For example, Arief *et al*. (2020) reported that the milk yield of EC goats was 0.84–1.23 kg/head/d. Monica *et al*. (2014) reported a milk yield of local African goats from 1.01 to 2.71 kg/head/d in Kenya. In addition, goats are prolific animals and must provide sufficient quantities of breast milk for kids. Therefore, it is a good challenge to improve the productivity of dairy goats by manipulating feed intake, especially by utilizing local feed sources. Using extracts from *Coleus amboinicus* L leaf (CAL) within the ration fed to goats is one way to improve the milk yield.

In North Sumatra, mothers usually consume this leaf to increase the breast milk for their babies. This leaf contains lactogogue having an activity to stimulates the transports and absorptions of nutrients from the blood circulation into the secretory cells of the udder gland, a phenomenon that increases milk yield (Kent *et al*., 2012). In addition, CAL also contains antioxidant compounds that could serve to inhibit cancer growth (Laila *et al*., 2020). This leaf also contains high levels of potassium, which functions as a calming agent, a blood cleaner, and a pain reliever. Damanik (2009) reported that CAL leaf could enhance breast milk yields of mothers in North Sumatra while Adriani *et al*. (2019) added that CAL could improve the *in vitro* digestibility of dry matter (DM) and organic matter (OM). This outcome might be due to the effect of the active compound carvacrol, which can reduce the rate of amino acid deamination and protein degradation. Zamiri *et al*. (2015) stated that carvacrol mainly increased the ammonia concentration in the rumen and the nitrogen balance. Haro *et al*.

(2018) stated that inhibition or reduction of the rate of amino acid deamination and protein degradation would reduce protein degradation in the rumen. Therefore, more protein could pass through the rumen and into the abomasum, from where it is absorbed. For example, the addition of 250 and 500 mg/L carvacrol within the ration could reduce protein degradation by 51.5% and 72.8%, respectively (Garcia, 2007). Thus, the amount of undegradable protein increases in the rumen and also increases the absorption from the hindgut, a phenomenon that also increases the OM digestibility. Furthermore, Turkyılmaz *et al*. (2011) expected that galactogogue effect of CAL on phytoestrogen and estradiol 17ß hormone (E2), an endogenous estrogen, stimulated the proliferation of the mammary gland cells. This stimulation would increase the production of casein and the activity of enzyme lactose synthetase in the epithelial cells of the mammary gland. Overall, this would have an impact on DM digestibility and, subsequently, the milk yield, quality, and quantity.

The supplementation of CAL in ration could be applied in this study. There were many methods to be applied, such as feeding the animal in the form of CAL simplicial, ethanol extracted of CAL or water extracted of CAL. The preliminary study was done using 2% ethanol or water extracted from CAL. Based on the above information, this experiment examined the effect of supplementation of CAL extract in the ration fed to EC goats on their milk yields. It was expected that this study would provide the best treatments of CAL extract in the ration of EC on the ration digestibility, milk yield, and milk quality. The objective of this experiment was to evaluate the effect of extracted CAL within the ration on the ration digestibility, milk yield, and milk quality in EC goats.

MATERIALS AND METHODS

This experiment was conducted at Fostered Farmer Field, the University of Jambi for two months and chemical analysis was done at the Ruminant Laboratory of University of Jambi and Livestock Research Centre, Ciawi, Bogor, Indonesia. This experiment followed animal ethics and welfare compliance according to the general animal welfare provisions from Laws of The Republic Indonesia Number 18 of 2009 about Livestock and Animal Health. The ethical clearance for health, Faculty of Medicine and Health Science, University of Jambi was issued a Letter of Ethical Clearance to run this project with the approved number of 284/UN21.8/PT/2020.

Sample Preparation

Fresh CAL was ordered online through Tokopedia and imported from Bekasi, West Java, Indonesia. The sample of CAL was cleaned by using tap water to remove odd dirty material and drained. The samples were chopped into 2-cm-long pieces and indirectly sundried in a chamber covered with a black cloth until dry. The following day, the dry sample was ground through a 1.5 mm sieve to obtain a powder. The powder was then extracted with 96% ethanol and water following the procedure reported by Ahirwar & Tembhre (2016) with slight modifications. For water extraction, the sample was put into a container with a 1:5 (CAL powder: distilled water) ratio and then boiled at 90°C for 20 minutes. The sample was soaked in 96% ethanol at room temperature for 3 days and then filtered for ethanol extraction. The solvent was then evaporated using a rotary evaporator. All extracts were stored at 4°C for further use.

Animals and Feed

Sixteen female EC goats, aged 1.5-2.5 years and 32.25 ± 3.31 kg, were used in this study after the second delivery and for around 1-1.5 months lactation. For medical care, each goat was treated with a worm tablet and vitamin B before the adaptation period. Each goat was grouped into four blocks based on weight for replication and randomly allotted into an individual pen previously sterilized with disinfectant.

Experimental goats were fed with the same ration containing fresh *kumpe* grass (*Hymenachne amplexicaulis*), rice bran, soya bean meal, coconut cake, and tofu waste (Table 1). Ration used in this study was estimated to have 17.64% crude protein (CP) and 3,352.5 kcal/kg ME and it was higher than the theoretical requirement of goat (15% CP and 2,820 kcal/kg ME) (NRC, 1981).

Table 1. Ration composition of experimental goats

Feed	Percentage	Chemical component				
		ME (kcal/kg)	DM (%)	CF (%)	EE (%)	CP (%)
Kumpe grass	70	3,861	20.29	29.32	1.85	14.10
Rice bran	10	2,980	91	11.4	13	12.9
Soya bean meal	12	2,290	90	0.41	0.51	45
Coconut cake	5	1,540	100	12.1	10.2	21.6
Tofu waste	3	414	89.5	16.53	5.54	19.55
	100	3,352.5	39.103	22.3182	3.1662	17.64

	Chemical component (%)						
	DM (%)	Ash (%)	WSE (%)	ASE (%)	Saponin (%)	Tannin (%)	Flavonoid (%)
CAL	92.2	12.5	20.53	6.53	1.35	3.28	0.72

Note: WSE= Water soluble extract; ASE= Alcohol soluble extract; ME= metabolizable energy; DM= dry matter; CF= crude fiber; EE= ether extract; CP= crude protein; CAL= *Coleus amboinicus* L. leaf.

The amount of ration fed to each experimental goat was manually prepared as 100% of full consumption per goat per day. Each treatment of experimental ration was prepared by calculating the amount of feed provided and subtracted by the amount of feed refused from each experimental goat within 24 h during the pre-adaptation period. The treatment of 0% CAL (P_0), 2% powdered CAL (P_1), 2% ethanol-extracted CAL (P_2), or 2% water-extracted CAL (P_3) was prepared proportionally based on the initial weight of each experimental goat and mixed with concentrate to allow the goats to consume all CAL during the morning feeding. The composition of powder CAL can be seen in Table 1. The goats were fed with grass at 09.00 and 15.00. Moreover, there were 7 days of adaptation prior to 5 days of data collection.

Digestibility

The nutrient digestibility was assessed during 5 days of data collection. The conventional method of digestibility was applied by calculating the amount of ration input minus fecal output. Ration input was calculated as feed provided minus feed refused. The amount of ration provided and refused and fecal output was recorded at 08.00 in the morning during the data collection period. Ten percent of feed and fecal samples were sundried, oven-dried at 60°C overnight, ground in a 1 mm sieve, and stored at 4°C until further chemical analysis. These chemical analyses were used to calculate intake, the digestibility of DM, CP, crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF), and hemicellulose.

Milk Collection

For milk collection, each goat was manually milked by hand at 07.00 and 16.00 for 5 days of sampling. The nipple was cleaned with 70% alcohol before milking to prevent microbial contamination. The milk was weighed and milk pH was measured soon after milking. Then, a 150 mL milk sample was separately poured into a cool container and transported to the Laboratory of the Faculty of Animal Husbandry, Jambi University, for further analysis. The specific gravity (SG) was measured by using a lactometer; milk fat was analyzed by following the technique reported by Gerber (1960). For SG, around 100 mL of fresh milk sample of every single treatment was prepared, filtered, and poured into a beaker glass at a temperature between 20-30 °C. Then by holding the lactometer tip, it was inserted into the milk. The lactometer was allowed to float freely until it was at equilibrium. Immediately, a thermometer was put into the milk sample, and the milk's temperature was recorded (Tesfay *et al*., 2015). The SG of the milk was calculated by the following formula:

SG = (L/1000) + 1

where, L was the correction factor for recording lactometer at a given temperature, i.e., for every degree more than 60 °F, 0.2 was added to the lactometer reading, but for every degree less than 60 °F, 0.2 was subtracted from the lactometer reading.

The milk fat content was determined following the Gerber method (Tesfay *et al*., 2015). Ten milliliters of concentrated sulfuric acid (91%-92%) was pipetted into a butyrometer, and this acid was added with 11 mL of milk sample and mixed. Next, 1 mL of amyl alcohol was added to the butyrometer, closed with a lock stopper. Then the mixture was shaken and upturned several times until the acid completely digested the milk. Finally, the butyrometer was kept left in a water bath for 5 minutes at 65 °C and centrifuged in a Gerber centrifuge for 5 minutes. The butyrometer was placed in a water bath again at 65 °C for 5 minutes. In the end, the butyrometer reading was recorded.

Milk protein was analyzed by following the technique described by Rowland (1938). Ten milliliters of milk sample were poured into an Erlenmeyer Flask, then it was added with 20 mL of distilled water, 0.4 mL of concentrated Potassium Oxalate, and 1 mL of phenolphthalein indicator. Finally, the solution was left for around two minutes. The solution was titrated with NaOH 0.1 N until it was indicated with pink color. Next, 2 mL of formaldehyde (40%) was added and re-titrated until it showed the pink color. The same procedure was done for the blank. The percentage of protein was calculated by using the following formula:

% Protein = (p – q) mL x 1.77 (formol factor)

where p is the volume of sample titration and q is the volume of blank titration.

Chemical Analysis

Around 25 g of dry sample of feed, refusal, and feces collected during data collection was prepared to analyze DM, OM, CP, ether extract (EE), and CF by following the established analytical procedures (AOAC, 1990). For DM analysis, around one gram of sample was oven-dried at the temperature of 105 °C overnight and reweighted. The content of DM was calculated with the following formula:

DM = [Weight after oven-dried (g) / Weight before oven-dried (g)] x 100%

For OM analysis, the dry sample was then furnaced at 600 °C for 5 h and reweighted. The content of OM was calculated as 100% minus ash content in which ash with the formula below:

OM= {[Weight before oven-dried (g) – Weight after furnaced (g)] / Weight before oven-dried (g)} x 100%

For CP analysis, the sample was digested in a Kjeldahl tube with the following steps. Around one gram of sample was digested in boiling concentrated H_2SO_4 in a Kjeldahl tube to alter protein nitrogen to ammonia. Caustic was added to evaporate ammonia and ammonia produced was collected by distillation. The amount of ammonia collected by titration was measured. The percentage of nitrogen in the samples was calculated using the formula provided (AOAC, 1990).

For EE analysis, around ten grams of sample was extracted with hexane, fat solution, for 6 hours. Then

the extract solution was distilled and the fat extract was oven-dried at temperature of 105 °C, cooled, and weighted. The fat content was calculated by using the following formula:
Fat content = [(A - B) / C] x 100%

where C was sample weight (g), A was container weight before extraction (g), and B was container weight after extraction (g).

There are two steps for CF analysis. In the first step, the sample was extracted using H_2SO_4 on the boiling point for 30 minutes and filtered. Then the residue was added with KOH solvent and washed using acetone until completely neutral. The residue was oven-dried at 105 °C and burned by the furnace at a temperature of 550 °C for three hours. The CF was calculated by using the following formula:
CF (%) = [(A - B) / C] x 100

where A was weight before drying, B was weight after drying, and C was sample weight.

NDF and ADF were analyzed by following the procedure of Van Soest (1963). For NDF analysis, half gram of sample (A) was put into a weighted crucible dish (B), added with 50 mL of neutral solvent and boiled for extraction for around one hour. After extraction, the mixture was vacuum-filtered and rinsed with hot water and finally rinsed with acetone (96%). After that, the dish and sample were oven-dried at a temperature of 135°C for two hours, cooled into a desiccator, and weighted (C). The content of NDF was calculated by using the following formula:
NDF (100%) = [(C - B) / A] x 100%

For ADF analysis, half gram of sample (A) was put into a weighted crucible dish (B), added with 50 mL of acid solvent, and boiled for extraction for around one hour. After extraction, the mixture was vacuum-filtered and rinsed with acetone (96%). After that, the dish and sample were oven-dried at a temperature of 135 °C for two hours, cooled into a desiccator, and weighted (C). The content of NDF was calculated by using the following formula:
%ADF (100%) = [(C - B) / A] x 100%

For Ca analysis, the ash sample was added with five milliliters of concentrated HCl, diluted with distilled water, evaporated until 10 mL and poured into 100 mL beaker glass, rinsed with distilled water and shaken. Then around 20 mL of this mixture was put into Erlenmeyer glass and added with few drops of methylene red. Next, NH_4OH was added until yellow color appeared and added with two drops of HCl until red color appeared. The mixture was boiled and added with 15 mL of ammonium oxalate, re-boiled until residue was formed. This residue was filtered with filter paper and rinsed with hot water until free acid and dried. Filter paper, including residue, was put into Erlenmeyer Flask containing 100 mL of distilled water and 5 mL of concentrated H_2SO_4 and boiled at a temperature of 70–80 °C. Finally, it was titrated with $KMnO_4$. The Ca content was calculated by using the following formula:
Ca content (%)= [(mL titration x N $KMnO_4$ x ½ AWCa) / Sample weight (mg)] x 100%

where AW was atomic weight.

For P analysis, the ash sample was added with 5 mL of concentrated HCl and kept leave for one hour and poured into a 10 mL volumetric flask and rinsed and shaken homogenously. One mL of this solution was transferred into 50-mL-cuvet and added with 3 cc ammonium molybdate, 2.5 vitamin C solution, and distilled water, then shaken homogenously and wait for 30 minutes. Finally, the solution was read by a spectrophotometer at a wave length of 570. The P content was calculated by using the following formula:
P content (%)= [(Spectrophotometer read (absorbance) x 11.293 + 0.087) / Sample weight (mg)] x100%

Measured Variables

The measured variables were 1) intakes of DM, OM, CP, EE, CF, energy, Ca, and P; 2) digestibility of DM, CP, CF, ADF, NDF, and hemicellulose; and 3) milk yield and milk quality factors including DM, protein, casein, fat, solids non fat (SNF), and SG.

Experimental Design and Statistical Analysis

This study was designed in a 4 × 4 randomized block design with four treatments (P_0, P_1, P_2, and P_3) and four blocks for replication. All measured variables were statistically analyzed with ANOVA followed by Duncan's test for multiple comparisons (SAS, 2002).

RESULTS

Nutrient Intake

Table 2 presents nutrient intake in the experimental goats. The supplementation of extracted CAL in the ration did not affect the intakes of DM, OM, CP, EE, CF, Ca, P, and energy in the experimental goats.

Nutrient Digestibility

Table 3 presents the digestibility of DM, CF, CP, ADF, NDF, and hemicellulose in the experimental goats. The supplementation of extracted CAL in the ration did not affect nutrient digestibility.

Milk Yield and Quality

Table 4 presents the milk yield and quality parameters. There were significant differences ($p<0.05$) in milk yield, milk protein, and milk casein and highly significant differences ($p<0.01$) in milk DM and SNF among the treatment groups. There were no differences in milk fat and SG among the groups.

Table 2. The nutrient intake of Ettawa crossbreed goat fed with the addition of extracted *Coleus amboinicus* L. leaf in diet

Intake	Treatments			
	P_0	P_1	P_2	P_3
DM (g/d)	1,192.00± 49.62	1,245.72±6.34	1,227.26± 9.88	1,232.30± 62.49
OM (g/d)	1,071.22±44.51	1,118.59±5.72	1,101.98±17.87	1,106.58±56.19
CP (g/d)	201.27± 8.16	210.27±1.18	207.03± 3.45	208.09± 10.87
EE (g/d)	43.25±2.26	45.08±0.06	44.69±0.50	44.42±1.62
CF (g/d)	327.69±13.14	342.27±1.98	336.90±5.69	338.77±17.94
Ca (g/d)	4.99±0.24	5.21±0.01	5.16±0.07	5.15±0.22
P (g/d)	15.81±0.95	16.45±0.06	16.37±0.14	16.17±0.51
Energy (kcal/kg DM)	3,208.42±88.76	3,365.31±11.45	3,300.31±56.34	3,263.42±61.91

Note: DM= dry matter; OM= organic matter; CF= crude fiber; EE= ether extract; CP= crude protein; P_0= treatment of 0% *Coleus amboinicus* L. leaf (CAL); P_1= treatment of 2% powdered CAL; P_2= treatment of 2% ethanol-extracted CAL; P_3= treatment of 2% water-extracted CAL.

Table 3. Digestibility of some nutrients within ration on Ettawa crossbreed goats (%)

Digestibility (%)	Treatments			
	P_0	P_1	P_2	P_3
DM	67.57±2.43	69.10±1.89	67.85±2.50	67.83±2.51
CP	53.68±3.53	55.81±2.59	56.50±2.50	56.91±1.80
CF	30.73±7.45	30.93±6.47	30.42±6.46	32.00±5.06
ADF	72.06±1.32	72.21±1.12	72.00±1.23	72.66±0.54
NDF	74.02±1.10	74.11±1.02	73.72±1.11	73.87±0.75
Hemicellulose	83.41±0.29	83.07±0.36	83.29±o.13	83.05±0.15

Note: DM= dry matter; CF= crude fiber; EE= ether extract; CP= crude protein; ADF= acid detergent fiber; NDF= neutral detergent fiber; P_0= treatment of 0% *Coleus amboinicus* L. leaf (CAL); P_1= treatment of 2% powdered CAL; P_2= treatment of 2% ethanol-extracted CAL; P_3= treatment of 2% water-extracted CAL.

Table 4. The milk yields, chemical composition, and specific gravity of Ettawa crossbreed goats fed with extracted *Coleus amboinicus* L. leaf diet in ration

Variables	Treatments			
	P_0	P_1	P_2	P_3
Milk yields (g/d)	$308.57±57.30^{a}$	$401.87±79.52^{b}$	$347.23±66.83^{c}$	$312.90±73.36^{a}$
Milk protein (%)	$3.86±0.35^{a}$	$3.71±0.31^{a}$	$3.71±0.27^{a}$	$4.47±0.34^{b}$
Milk casein (%)	$3.44±0.31^{a}$	$3.30±0.28^{a}$	$3.29±1.25^{a}$	$3.99±0.30^{b}$
Milk fat (%)	3.70±0.16	3.68±0.19	3.50±0.08	3.85±0.51
Milk DM (%)	$8.50±1.00^{A}$	$8.10±0.01^{A}$	$10.25±1.04^{B}$	$10.37±0.48^{B}$
SNF (%)	$4.809±1.01^{A}$	$4.33±1.19^{A}$	$6.75±1.03^{B}$	$6.53±0.55^{B}$
Specific gravity	1.0283±0.01	1.0290±0.01	1.0287±0.01	1.0294±0.01

Note: Means in the same row with different superscripts differ significantly ($p<0.05$); Means in the same row with different capital superscripts differ significantly ($p<0.01$); DM= dry matter; SNF= solids non fat; P_0= treatment of 0% *Coleus amboinicus* L. leaf (CAL); P_1= treatment of 2% powdered CAL; P_2= treatment of 2% ethanol-extracted CAL; P_3= treatment of 2% water-extracted CAL.

DISCUSSION

Intake

As shown in Table 2, the intakes of DM, OM, CP, EE, CF, Ca, P, and energy of experimental goats in the four treatments were not different. These results might be because each experimental goat was fed with the same ration with the same characteristics and composition. The experimental goats were maintained in the same environmental conditions. Indeed, McDonald *et al*. (1995) reported that factors affecting feed intake are feed characteristics and environmental conditions. Sebata & Ndlovu (2010) added that morphology and phenology of browsing usually influence herbivores' forage selection and intake. Apart from this condition, each experimental goat was fed with 80% full daily intake with the same ration except for the content of CAL extract. The concentrate, tofu waste, and extracted CAL levels were completely given during the morning feeding to reduce the remaining feed. After concentrate feeding, forage was chopped into 5-cm-long pieces and then fed to goats. Therefore, the remaining feed was much less than the morning feed. In addition, all experimental goats were located in the same environmental condition. Consequently, the intake of each nutrient was not different among the four treatment groups.

The DM intake from this experiment is higher than the results reported previously. Badarina *et al.* (2015) reported that the range of DM intake was 1.44–1.56 kg/d.

The ration provided in this experiment was over-sufficiency of goat requirement according to the standard of NRC (1981). In addition, the experimental goats were younger than the goats used by Badarina *et al.* (2015). The effect of treatment groups is not significant on the DM intake of the experimental goats because they were fed with the same DM composition and possibly because they are all in the same breed.

The OM intake in this experiment is lower than the results reported by Suryanindyah & Astuti (2012); the OM intakes for that study were 1561.8 ± 17.8 and 1496.7 ± 25.6 g. This difference might be due to the goats fed different rations. The lack of difference in OM intake among the groups in this study might be related to the same ration fed to the experimental goats, except for the amount of CAL, which comprises a small portion of the feed. Therefore, the ration likely also has the same nutrient composition, appearance, taste, and palatability. Decruyenaere *et al*. (2009) report that feed intake in the animal is affected by nutrient composition, physical appearance, flavor (taste and smell), and texture.

The CP intake in this experiment is relatively higher than those reported by Supriyati *et al*. (2016) (148–157 g/d) and by Astuti *et al*. (2019) (129.33–132.08 g/d) for post-weaning EC goats. In this study, the different intakes of CP might be due to the different rations consumed, weight, and age of goats, although the breed was the same with the two previous studies. The age of goat might physiologically affect the intake of CP. This difference might be due to the carvacrol content of the ration due to the CAL treatment. Carvacrol might reduce the rate of amino acid deamination and CP degradation in the rumen. Foskolos *et al.* (2016) reported that carvacrol inhibited deamination. These phenomena likely increase post-rumen CP absorption and could also increase DM and OM digestibility (Salem *et al*., 2015). Moreover, previous studies have shown that feed intake increases when there is more undegradable protein in the ration (Kholif *et al*., 2014, 2016).

The EE intake parameters were almost the same among the four treatments and relatively higher than the previous results reported by Astuti *et al*. (2019) (20.34–57.35 g/d) and by Supriyati *et al*. (2016) (34.83–35.47 g/d). The effect of CAL treatments in the ration is not clear on the intake of EE because the goats were exactly fed the same content of EE in the ration. In the present study, there was no effect of treatment of CAL in the ration. However, compared with the previously mentioned studies, CAL increased the EE intake due to the elevated ruminal degradation. In addition, the differences in EE intake might be due to the different types of feed consumed by the experimental goats in these experiments.

The CF intake in the present study was higher than those previously reported by Astuti *et al*. (2019), i.e., 78.20–88.82 g/d for post-weaning EC goats. This difference might be due to the age and body weight difference in the experimental goats in this study compared with those in the study of Astuti *et al*. (2019). The fiber in the ration would influence the composition of milk fat and, therefore, the composition of CF in the ration also influences other nutrients.

The Ca intake in the present study was lower compared with those reported by previous studies due to the low Ca concentration in this leaf. This might be possibly due to the low content of Ca in this leaf. Wadikar & Patki (2016) mentioned that the content of Ca within CAL was relatively low, around 2.08 g/kg. Ganai *et al*. (2017) reported that the Ca intake in EC goat was between 14.86 and 22.38 g/d, while Muscher *et al*. (2011) mentioned that Ca intake in male White Saanen goats was between 7.4 and 7.7 g/d. Ca is a crucial element during lactation, and Ca deficiency in the ration could cause paralysis in a goat mother. The P intake was higher than that reported by Ganai *et al*. (2017), i.e., 7.92–8.20 g/d. Muscher *et al*. (2011) reported that the P intake in female White Saanen goats was between 3.25 and 3.33 g/d. P intake is affected by the composition of the ration.

Digestibility

The nutrient digestibility was also similar among all treatment groups (Table 3). This outcome might be caused by the reality that all experimental goats received the same nutrient components in their diets and are the same breed. The effect of CAL extract was not significant in this study because it comprised only a small portion of the ration. This leaf extract could influence or inhibit the deamination process within the rumen. Consequently, degradation or fermentation of nutrients by microbes in the rumen were likely similar among all groups. Hasmawati & Hasnaeni (2016) state that nutrient digestibility depends on the fiber composition and anti-nutritional substances in the whole ration fed. These factors would also influence the microbial activity within the rumen and nutrient degradation. On the other hand, Suksombat *et al*. (2017) mentioned that the use of essential oil in the ration could increase the degradability of DM and NDF but not ADF and CP.

The effect of CAL extract in the ration might influence protein ammonization in the rumen. This could increase the amount of undegradable protein absorbed, increase the OM digestibility, and impact the DM digestibility. CAL extract contains carvacrol, which delays protein deamination in the rumen (Mbiriri *et al*., 2015) and has antimicrobial activity (Fang *et al*., 2019). These effects allow more OM to pass through the rumen that will be digested and absorbed in the hind gut. Garcia (2007) stated that using 250 mg/L and 500 mg/L carvacrol in the ration could reduce protein degradation by 51.5% and 72.8%. The effect of carvacrol could be seen from days 3 to 9 with the indicator of increasing methane production (Mbiriri *et al*., 2015). However, Castañeda-Correa (2018) reported that the combination of carvacrol and thymol decreased methane production.

The DM digestibility of the experimental ration was not different among the four treatment groups. These values are higher than those reported by Arief *et al*. (2016); those authors reported DM digestibility of about 40%. The differences in DM digestibility might be due to the differences in the ration compositions and the effect of different active compounds contents (that reduced amino acid deamination and protein degradation) within CAL in the whole ration, as was hypothesized by

Khamisabadi *et al*. (2016). Salem *et al*. (2015) mentioned that these changes would allow the increased absorption of protein and also directly increase DM digestibility.

The ADF, NDF, and hemicellulose digestibility did not differ among the four treatments. However, Kim *et al*. (2019) state that the use of CAL essential oil tends to increase the digestibility of fiber without affecting VFA production. This outcome might be caused by the reality that all goats received the same amount of fiber in their rations, so the effect of CAL on fiber digestibility was not significant. It is also possible that carvacrol within CAL only influences the degradation of protein rather than fiber, as was postulated by Khamisabadi *et al*. (2016).

Milk Yield and Quality

The treatments significantly influenced the milk yield ($p<0.05$). P_1 treatment provided the highest milk yield among the treatments. P_2 treatment had a higher milk yield than treatments P_0 and P_3 ($p<0.05$), and treatment P_0 was not different from treatment P_3 ($p>0.05$) in milk yield. It might be possible that carvacrol in the CAL could indirectly increase the milk yield and stimulate the udder to produce more milk. Benchaar (2020) states that carvacrol functions as rumen fermentation modifier by its antimicrobial properties. This condition would reduce protein degradation and ammonia production in the rumen (Benchaar, 2020); therefore, it increases undegradable protein to pass through the hindgut. Carvacrol in ration also improves reproductive performance in dairy cows (Pinedo *et al*., 2015). However, to the best of our knowledge, there is no information concerning the transfer of carvacrol into the milk. Silva *et al.* (2020) concluded in their study that the supplementation of capsaicin, carvacrol, cinnamaldehyde, and eugenol could reduce DMI and increase milk yield. Xu-dong *et al*. (2012) state that CAL contains phytochemical components, such as polyphenols, tannins, and alkaloids, having capacities to increase milk yields. Moreover, the regular addition of flavonoids to the ration could increase prolactin secretion, hormone stimulating the mammary gland cells to synthesize and produce milk. Fati *et al*. (2014) reported that CAL could increase milk yields up to 10%. Hutajulu & Junaidi (2013) add that CAL contains thymol, forskolin, and carvacrol, which may stimulate milk synthesis that eventually increases milk yield. Yanza *et al*. (2018) also report that bioactive components can modify the ruminal microbial fermentation and modulate methanogenesis and biohydrogenation of fatty acids. For example, some active compounds such as phenolics, saponins, tannins, flavonoids, carvacrol, and other essential oils inhibit methanogenesis within the rumen. This condition could reduce methane emission from rumen and simultaneously improve livestock productivity (Patra & Saxena, 2010).

Related to this experiment, P_1 and P_2 treatments produce higher milk yields than P_3 treatment. This effect might be possibly due to the treatment's effects on the supply of carvacrol and other active compounds as rumen modifiers in the rumen. P_1 treatment could possibly supply the highest level of carvacrol among four treatments as it was CAL powder that fully contains carvacrol and other active compounds. The supply of carvacrol in the rumen was then followed by P_2 and P_3 treatments. We assume that ethanol extract, as in P_2 treatment, contain higher level of carvacrol than water extract, as in P_3 treatment, while P_0 treatment as a control does not have carvacrol. Therefore, these treatments would possibly influence the milk yield. Apart from this condition, other factors such as parity and stage of lactation (DeVries *et al*., 2011) also influence milk yield. Unfortunately, this information was not available in this study.

The addition of CAL extracted in the ration significantly ($p<0.05$) increased the milk protein content. P_3 treatment showed the highest milk protein content of 4.47%. This highest value is also higher than those reported by Dharmawan *et al*. (2019), with milk protein contents between 3.09% and 3.58%. However, the digestibility of nutrients (Table 2) is relatively the same. Theoretically, milk protein was synthesized by using amino acid from the blood (Rhoads & Nogalska, 2007). Supplementation of CAL water extract in the ration (P_3) might possibly influence the transport of nutrients from the blood into the cells of the mammary glands for the synthesis of milk protein. The different levels of CAL supplementation also significantly ($p<0.05$) affected the milk casein contents. This result might be related to the fact that milk casein content is related to the milk protein content; total milk protein in this study is 2.7%.

P_3 treatment with CAL water extracted was the best result in terms of milk protein content compared to the other treatments. This highest milk protein content might be because P3 treatment was supplementation of CAL water extract that provide carvacrol an antibacterial agent that could protect the degradation of protein in the rumen and pass it into abomasum intestine. Widyawati *et al* (2014) reported that water was the best solvent in extracting *Pluchea indicia Less* leaves. This undegradable protein was enzymatically digested to be amino acid and absorbed into the blood. This condition will increase the amino acid concentration in the blood. These amino acids would be absorbed by the mammary gland cells and will be used to synthesize milk protein such as casein, lactalbumin, and lactoglobulin.

There was no difference ($p>0.05$) in the milk fat contents among the treatments. The average fat milk content was 3.50%–3.85%. This range of milk fat content is similar to that reported by Rojo *et al*. (2015) that milk fat content ranges between 3.4% and 4.0%. The milk fat content is easily changeable and depends on the fiber content of the consumed feed. Khan *et al*. (2015) state that there is a correlation between nutritive values of feed consumed and milk yield and quality. Low fiber content in the ration would cause low production of acetic acid in the rumen and thus reduces milk fat because acetic acid is the main component used to synthesize milk fat.

Some articles reported that the milk fat content is influenced by the nutrient content of feed fed by the animal (Frelich *et al*., 2012; Adler *et al*., 2013; Hanuš *et al*., 2016). The milk fat is especially affected by fiber

content in the ration. All experimental goats were fed with the same ration with the same composition of nutrients (Table 1), with the same intake of CF (Table 2) and digestibility (Table 3). Therefore, it could possibly provide the same effect on the milk fat content of each experimental goat in which the milk fat content of the experimental goat was not different among the four treatments.

The method of CAL extraction significantly affected the composition of milk DM ($p<0.01$). These results are lower than those reported by Rojo *et al*. (2015), with a range of DM content between 10.4%–11.4%.

The solvent used for CAL extraction did not significantly affect ($p>0.05$) SG of the milk. The average SG of the milk samples was 1.0288, which is a little bit lower than the value reported by Harjanti *et al*. (2017) at one day post-partum (1.041) and two days post-partum (1.034) for EC goats. While Tesfay *et al*. (2015) reported that the SG of pasteurized milk was 1.031 and SG of milk taken from a vendor was 1.025. The SG of milk found in the present experiment was also lower than those found by Basitan & Jarcia (2013), who conducted the study of the SG of milk goat fed with *Malunggay* (*Moringa oleifera*).

Milk SG in this experiment was somewhat difficult to explain. In fact, SG was relatively similar with the milk fat content, while DM, casein protein, and SNF composition were significantly different among four treatments. Generally, SG is influenced by the milk composition of DM, CP, fat, SNF, and CF. Partially, SG in this study could be affected by the same chemical composition of ration, the similar digestibility of ration, and the relatively similar content of milk fat for all treatments. This statement could be related to the result of Şahan *et al.* (2005) that the variations influenced SG of milk in Awasi ewes in Turkey in fat content. On the other hand, contrary to the results of other parameters in which nutrient composition of milk such as DM, protein, and casein were different among four treatments. Other possibilities, SG could also be affected by the parity and the stage of lactation of experimental goats, the environment temperature, and the gas evaporation from milk. William *et al.* (2012) stated that SG and milk composition such as milk protein, lactose, fat, solid non-fat, and total solid except ash was affected by the stage of lactation and parity in the case of West African Dwarf sheep. Unfortunately, there was no data on parity, the stage of lactation, and environmental temperature in this study. Apart from this, evaporation of CO_2 and N_2 gases also influences SG during the milking process (Rosatio *et al.*, 2015).

CONCLUSION

Supplementation of 2% CAL powder in the ration for EC goats improved milk yield by 30.24% compared with the control ration. The supplementation of CAL 2% water extract in the ration improved the quality of milk in terms of DM, protein, casein, fat, and SNF.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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