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Original Article

Supplementation of natural growth enhancer on Ongole grade cattle: effects on nutrient utilization and growth performance

Wara Pratitis Sabar Suprayogi[1,*], Susi Dwi Widyawati[1], Adi Ratriyanto[1], Agung Irawan[2,3]

[1]Department of Animal Science, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, 57126, Indonesia
[2]Vocational School, Universitas Sebelas Maret, Surakarta 57126, Indonesia
[3]Department of Animal and Rangeland Sciences, Oregon State University, Corvallis 97331, OR, USA
*Correspondence: warapratitis@staff.uns.ac.id

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Abstrak

Tujuan: Penelitian ini dilakukan untuk mengevaluasi pengaruh suplementasi stimulan pertumbuhan terhadap metabolisme nutrien dan performa sapi Peranakan Ongole (PO).
Metode: Stimulan alami berupa growth enhancer (GE) diformulasikan dalam bentuk padat, mengandung molasses, urea, tepung daun lamtoro, bungkil kedelai, minyak ikan, daun ketapang dan mineral premix. Sebanyak 25 sapi PO (umur 1-1,5 tahun) dengan bobot badan (BB) awal 150 ± 12 kg dipelihara pada kandang individu dan dibagi secara acak pada lima (5) perlakuan: P0 = ransum basal; P1 = P0 + 5 g/kg $BB^{0.75}$ GE; P2 = P0 + 10 g/kg $BB^{0.75}$ GE; P3 = P0 diet + 20 g/kg $BB^{0.75}$ GE; dan P4 = P0 + 30 g/kg $BW^{0.75}$ GE, masing-masing menggunakan lima (5) ekor sapi sebagai ulangan. Penelitian dilakukan selama 10 bulan.
Hasil: Suplementasi GE pada level 5g/kg $BB^{0.75}$ (P1) meningkatkan pertambahan BB harian dan BB akhir secara signifikan dibandingkan P0 ($P<0,05$). Konsumsi bahan kering (BK), protein kasar (PK), dan *total digestible nutrient* (TDN) lebih tinggi dengan suplementasi GE dibandingkan P0 sebagai kontrol ($P<0,05$), namun tidak terdapat perbedaan antar level suplementasi ($P>0,05$). Kecernaan BK dan serat kasar (SK) pada P1 menunjukkan nilai tertinggi sedangkan retensi N tertinggi terdapat pada P1 dan P2 dan retensi N terendah terlihat pada P0 ($P<0,05$). Sapi pada kelompok P1 dan P2 menghasilkan ekskresi alantoin, asam urat, dan estimasi sintesis protein mikroba lebih tinggi dibandingkan kelompok lain ($P<0,05$).
Kesimpulan: Disimpulkan, suplementasi GE meningkatkan densitas nutrien ransum dan pemberian setara dengan 5g/kg $BB^{0.75}$ pada ransum dapat meningkatkan konsumsi dan penggunaan nutrien sehingga dapat meningkatkan performa sapi PO.

Kata Kunci: Protein terproteksi; Suplemen pakan; Sapi Peranakan Ongol

Abstract

Objective: This experiment aimed to assess natural growth enhancers supplementation on nutrient metabolism and production performance of Ongole grade cattle.
Methods: The solid growth enhancer (GE) was formulated to contain highly soluble energy sources (molasses), nitrogen (urea), bypass protein (dried-Leucaena leaves and soybean meal), polyunsaturated fatty acids (fish oil), phytonutrient-rich source (*T. catappa*), and mineral premix. In

total, 25 Ongole grade cattle at 1-1.5 years in age averaged 150 ± 12 kg of initial body weight were distributed to receive five dietary treatments as follow: P0 = basal diet; P1 = P0 + 5 g/kg $BW^{0.75}$ GE; P2 = P0 + 10 g/kg $BW^{0.75}$ GE; P3 = P0 + 20 g/kg $BW^{0.75}$ GE; P4 = PO + 30 g/kg $BW^{0.75}$ GE, respectively, (5 replicates per treatment). The experiment lasted 10 months.

Results: GE supplementation at 5g/kg $BW^{0.75}$ (P1) significantly increased daily gain and final BW compared to P0 ($P<0.05$). Voluntary intake for dry matter, crude protein, and total digestible nutrient increased for cattle fed diets containing GE ($P<0.05$) compared to P0 but no difference among supplementary levels ($P>0.05$). Digestibility of DM and CF were significantly higher at P1 t ($P<0.05$) while N retention was highest on P1 and P2 and P0 was the lowest ($P<0.05$). Cattle fed with P1 and P2 treatments excreted higher allantoin and uric acid thus produced higher microbial protein synthesis than other treatments ($P<0.05$).

Conclusions: In conclusion, dietary GE increased nutrient density of the ration and supplementation at 5g/kg $BW^{0.75}$ could increase voluntary intake and nutrient utilization, and thus increased performance parameters of Ongole grade cattle.

Keywords: Bypass protein; Feed supplement; Ongole grade cattle

INTRODUCTION

Complex microbial communities that reside in the rumen are well-recognized to play a central role in supporting ruminant productivity and health. Among their biological roles, rumen microbes contribute to maintaining homeostasis of the intestine, activate host immunity, and developing mucosal and lymphoid organ structures through rumen fermentation activity [1]. Given the fact that bacteria and other rumen communities have the first opportunity to utilize substrate consumed by ruminants, providing them with nutrient-balanced diets is essential to produce energy for the optimum growth of ruminants. Therefore, there have been ongoing efforts to feed cattle in tropical countries with a variety of feed supplements to modulate rumen fermentation [2,3].

In tropical regions, on the other hand, forages and agricultural byproducts are the main sources of the feedstock of ruminants. These are typically high in fiber and low in crude protein (CP) contents. Feeding such diets for cattle is particularly a common situation in Indonesia whereas often resulted in low productivity due to insufficient supply of the need of nutrients. However, incorporating a commercial concentrate requires a high cost which is not affordable for smallholder farmers or farmer groups. Therefore, searching available local alternatives as a protein source to compensate for protein deficiency has attracted researchers to evaluate their potential use to improve livestock operation efficiency. *Leucaena leucocephala* (Leucaena), a legume tree rich in protein, and *Terminalia catappa* (*T. catappa*), a medicinal plant, contain medium to high concentration of phytonutrients contents such as condensed tannins, flavonoids, and polyphenols [4,5]. A number of empirical works have suggested that supplementary Leucaena in a particular portion to cattle could positively modulate rumen fermentation, nutrient digestibility, as well as reduce methane production and ruminal biohydrogenation [6,7]. In addition, *T. catappa* has also been reported to have antioxidant and anti-inflammatory properties [8]. Other studies evaluating the use of fodder trees have shown to improve ruminant productivity [3,9].

Nevertheless, lack of highly digestible energy and protein supplies should be considered as the major constrain in the low productivity of cattle. Therefore, supplementing diets containing complete necessary and functional nutrients with high solubility such as molasses, urea, fish oil, and soybean meal is a way to promote a more efficient production system. The effects of dietary supplementation using molasses, urea, fish oil, and soybean meal have been previously assessed [10-13] and have been suggested as promising supplementation strategy to boost production of ruminant animals. The use of solid feed supplements containing the aforementioned ingredients

was reported to enhance volatile fatty acids (VFA) production, increase nutrient digestibility and subsequently improve milk production as well as feed efficiency [2]. However, evaluation on the mixtures of highly soluble energy and protein sources in combination with phytonutrients sources has never been reported. Thus, we formulated a solid feed-supplement containing highly digestible energy (molasses), nitrogen (urea), bypass protein (soybean meal and Leucaena leaves), and leaves rich in phytonutrient content (*T. catappa*) as a promising growth-promoting supplement for cattle. In this study, supplementary effects of growth enhancers on voluntary nutrient intake, digestibility, performance, and microbial protein synthesis of Ongole grade cattle were evaluated.

MATERIALS AND METHODS

Animals, diets, and experimental design

The use of animals in this present research followed the Institutional Animal Care and Use Committees which in accordance with the national and global animal welfare standard. No clinical disorders were observed during the experimental period.

This study was performed at mini farm of the Department of Animal Science, Faculty of Agriculture, Universitas Sebelas Maret, located at Jatikuwung, Gondangrejo district, Karanganyar regency (-7°51S, 110°84E). A total of 25 Ongole Grade cattle at 1-1.5 years averaged 150±12 kg body weight (BW) were distributed to receive five dietary treatments including control. The treatments were based on increasing dietary natural growth enhancer (NGE) that were calculated based on metabolic body weight from 0 to 25 g/kg $BW^{0.75}$ as follows: P0 = basal diet; P1 = basal diet + 5 g/kg $BW^{0.75}$ GE (equal to 8.7% DM); P2 = basal diet + 10 g/kg $BW^{0.75}$ GE (equal to 13% DM); P3 = basal diet + 20 g/kg $BW^{0.75}$ GE (equal to 17.3% DM); and P4 = basal diet + 30 g/kg $BW^{0.75}$ GE (equal to 21.6% DM), respectively. Each treatment group consisted of five animals as replicates. The GE was a solid form of feed supplement formulated to contain highly soluble energy source (5% molasses), nitrogen (5% urea), bypass protein (30% dried-Leucaena leaves and 30% soybean meal), polyunsaturated fatty acids source (5% fish oil), phytonutrient-rich content source (10% *T. catappa*), and mineral premix (5%), on dry matter basis, to continuously supply necessary nutrients for rumen microbes as well as to provide functionally compounds that bring benefit to the animals.

The basal diet was formulated based on 50% fresh rice straw and 50% king grass while the supplement was given to replace the rice straw according to their levels. The rice straw was purchased from local farmers and the king grass was grown and available in the site of study. The king grass was chopped and fed to the animals together with the rice straw in a 50:50 proportion based on known dry matter (DM). The dietary supplement (GE) was in a premixed batch and hand-mixed with the diets and offered to the animals in a daily basis. Samples of each ingredient was collected prior to the commencement of the study for proximate analysis. The chemical composition of feed ingredients and nutritional profile of dietary treatments are presented in Table 1 and Table 2, respectively.

Animals were maintained in 2×3 m individual stalls, equipped with individual

Table 1. Chemical composition of feed ingredients

Nutrient composition	NGE	KG	RS
Dry matter (%)	87.22	17.20	32.79
Ether extract (%)	6.37	2.46	2.39
Crude protein (%)	28.08	9.56	2.19
Crude fiber (%)	5.61	29.92	31.39
Ash (%)	14.07	18.50	6.51
Nitrogen free extract (%)	42.48	54.13	60.44
Total digestible nutrient[1] (%)	80.84	46.94	44.54

NGE = Natural growth enhancer; KG= King grass; RS= Rice straw

[1]Estimated according to Hartadi *et al.* [14].

Table 2. Diet composition and chemical composition of dietary treatments

Ingredients	Proportions, % DM				
	P0	P1	P2	P3	P4
Growth enhancer[1]	0.0	8.7	13.0	17.3	21.6
Fresh rice straw	50.0	41.3	37.0	32.7	28.4
Fresh king grass	50.0	50.0	50.0	50.0	50.0
Nutrient composition	Chemical composition of dietary treatments				
Dry matter (%)	25.00	31.06	34.09	37.12	40.15
Ether extract (% DM)	2.43	2.76	2.93	3.10	3.27
Crude protein (% DM)	6.38	7.98	8.78	9.58	10.38
Crude fiber (% DM)	30.66	28.55	27.50	26.45	25.39
Ash (% DM)	12.51	12.12	11.93	11.74	11.55
Nitrogen free extract (% DM)	43.17	46.56	48.25	49.95	51.64
Total digestible nutrient[2] (%)	42.85	46.30	48.02	49.74	51.47

[1] Growth enhancer was provided in solid form, composed of urea, molasses, *Cassia alata* leaves, dried Leucaena foliage, fish oil, and soybean meal.

[2] Calculated according to Hartadi *et al.* [14].

P0 = basal diet; P1 = P0 + 5 g/kg $BW^{0.75}$ GE; P2 = P0 + 10 g/kg $BW^{0.75}$ GE; P3 = P0 + 20 g/kg $BW^{0.75}$ GE; P4 = PO + 30 g/kg $BW^{0.75}$ GE

feed bunk and cement well, and were fed ad libitum twice a day at 07.00 am and 03.00 pm with expected 10% feed refusal. They had free access to fresh and clean water. The house was cleaned daily and the water was replenished regularly. The experiment lasted for 10 months, comprising 14 d for the adaptation period and the subsequent period up to 10 months (298 d) for the experimental period. At starting period, the end of the adaptation period, and bi-weekly during the experimental period, animals were weighed. Body weight gain was determined by subtracting the final BW to the initial BW.

Sample collection and analyses

During the experimental period, daily feed intake for individual animals was determined by weighting the refusal feed before morning feeding and was subtracted with the offered feed. For digestibility and purine derivatives measurement, fecal and urine total collection were conducted during the last 7 d of the experimental period. Briefly, the amount of fecal excreted was recorded daily and a proportion of ±5% of the fresh weight of feces was collected into a small container during the collection period and they oven-dried at were composited for eventual DM, CP, and CF determination. The feces samples were oven-dried at 60°C for 48 to 72 h and were finely ground. The feces samples were composited for each animal considering each composite consisted of equal DM from daily samples and were analyzed for their chemical analysis. Analysis for DM, OM, EE, CP, CF, and ash was according to the AOAC [15] procedure.

The urine volume excreted daily was measured by collecting the urine using a harnessed-bag urine collector. Samples of 50 mL urine were collected and homogenized using 0.1 M solution of H_2SO_4 (5 mL) at pH<3 to prevent microbial activity. The collected urine samples were kept in a 20°C freezer for purine derivatives (PD) determination. The procedure of PD analysis followed the method of Chen and Gomes [16]. Following this, microbial protein (N) synthesis (g/d) was calculated from the urinary purine derivatives [16] as follows:

Total purine derivatives excreted = 0.85X + 0.385 $BW^{0.75}$ (1)

Microbial N synthesis = X (mmol/d) x 70/0.116 x 0.83 x 1000 (2)

Where X in the (1) equation is purine derivative absorption per day and it was used for the (2) equation to estimate microbial N synthesis.

Statistical analysis

All data obtained from the experiment were subjected to one way ANOVA according to completely randomized block design and were analyzed using the MIXED PROC of SAS (SAS Studio 3.8, University Edition)

Table 3. Daily nutrient intake and performance of treatment groups

Variables	Levels of dietary growth supplement					SEM	p-value
	P0	P1	P2	P3	P4		
Daily intake, kg/d							
DM intake	3.65^{b}	4.93^{a}	5.20^{a}	4.95^{a}	4.69^{a}	0.426	< 0.05
CP intake	0.23^{b}	0.39^{a}	0.46^{a}	0.47^{a}	0.49^{a}	0.003	< 0.05
CF intake	1.12	1.41	1.43	1.31	1.19	0.008	> 0.05
TDN intake	1.56^{b}	2.28^{a}	2.50^{a}	2.46^{a}	2.41^{a}	0.027	< 0.05
Performance							
Final BW, kg	199^{c}	286^{a}	274^{a}	211^{bc}	232^{b}	9.174	< 0.05
Daily gain, kg/d	0.16^{b}	0.45^{a}	0.41^{a}	0.20^{ab}	0.27^{ab}	0.002	< 0.05
Feed/ gain	22.81^{a}	10.96^{b}	12.68^{b}	24.75^{a}	17.37^{ab}	1.314	< 0.05

$^{a, b, c}$ Means in the same row with different superscript significantly difference at P < 0.05

P0 = basal diet; P1 = P0 + 5 g/kg $BW^{0.75}$ GE; P2 = P0 + 10 g/kg $BW^{0.75}$ GE; P3 = P0 + 20 g/kg $BW^{0.75}$ GE; P4 = PO + 30 g/kg $BW^{0.75}$ GE

classifying the treatment groups as fixed effect and animal as block or random effect according to the following mathematical model:

$$yij=\mu+\tau i+\beta j+\epsilon ij$$

Where Yij is the observation, μ is the overall mean, τi is the effect of GE supplementation, βj is the blocking effect, and ϵij is the residual effect. The repeated measure was declared in the model to count the effect of sampling periods during the experiment. The means between dietary treatments were compared using Tukey's HSD test when P < 0.05.

RESULTS

Voluntary intake and growth performance

Data on the nutrient composition of ingredients and dietary treatments are presented in Table 1 and Table 2, respectively. Dietary treatments were based on increasing proportions of growth enhancers (GE) composed of locally available ingredients whereas some of the ingredients are rich in functional properties. The GE was rich in protein (28.08% DM), contributing to proportionally increase the CP contents of dietary treatments. Thus, an increasing proportion of GE resulted in linear increased in CP and TDN contents while decreased CF content (Table 2). Results of DM, CP, CF, and TDN intakes as well as N retention are shown in Table 3.

Attractive results were found on these nutrients intake and N retention whereas cows supplemented with 10-30 g/kg $BW^{0.75}$ had significantly greater DM, CP, and TDN and N retention, when compared with cows, received control diet (P<0.05). However, there was no difference in CF intake among dietary treatments. Intake of DM, CP, and TDN was also similar for P1 to P4. Final body weight (BW) and average daily gain (ADG) among treatment groups were significantly different (P<0.05) where cows fed P1 had the highest final BW (286 kg) and ADG (0.45 kg/d) especially when compared with the control group (final BW = 199 kg and ADG = 0.16

Table 4. Nutrient digestibility and nitrogen (N) utilization of the treatment groups

Variables	Levels of dietary growth supplement					SEM	p-value
	P0	P1	P2	P3	P4		
Dry Matter, %	44.64^{c}	55.09^{a}	51.76^{b}	49.50^{ab}	53.20^{b}	6.241	< 0.05
Crude fiber, %	55.01^{c}	67.57^{a}	62.24^{ab}	62.53^{b}	62.98^{b}	6.927	< 0.05
N intake, g/d	36.80^{c}	62.40^{b}	73.60^{ab}	75.20^{a}	78.40^{a}	6.809	< 0.05
Fecal N output, g/d	10.75	15.44	14.79	11.39	12.53	0.827	> 0.05
Urine N output, g/d	5.73^{c}	29.41^{b}	36.72^{a}	34.73^{a}	39.18^{a}	5.432	< 0.05
N retention, % N	44.78^{c}	71.88^{a}	70.01^{a}	61.33^{b}	65.96^{ab}	4.341	< 0.05

$^{a, b, c}$ Means in the same row with different superscript significantly difference at P < 0.05

P0 = basal diet; P1 = P0 + 5 g/kg $BW^{0.75}$ GE; P2 = P0 + 10 g/kg $BW^{0.75}$ GE; P3 = P0 + 20 g/kg $BW^{0.75}$ GE; P4 = PO + 30 g/kg $BW^{0.75}$ GE

Table 5. Concentration of purine derivatives and microbial protein synthesis of treatment groups

Urinary purine derivatives	Levels of dietary growth supplement					SEM	p-value
	P0	P1	P2	P3	P4		
Allantoin, mmol/d	23.98^{c}	35.73^{b}	48.67^{a}	32.51^{b}	30.07^{ab}	4.163	< 0.05
Uric acid, mmol/d	1.77^{b}	2.76^{a}	2.87^{a}	2.41^{ab}	2.64^{ab}	0.342	< 0.05
Microbial N synthesis, g/d	5.19^{c}	14.31^{b}	24.87^{a}	9.50^{ab}	8.61^{ab}	1.284	< 0.05

$^{a, b, c}$ Means in the same row with different superscript significantly difference at P < 0.05

P0 = basal diet; P1 = P0 + 5 g/kg $BW^{0.75}$ GE; P2 = P0 + 10 g/kg $BW^{0.75}$ GE; P3 = P0 + 20 g/kg $BW^{0.75}$ GE; P4 = PO + 30 g/kg $BW^{0.75}$ GE

kg/d). The present experiment also found that P1 had the lowest feed/gain in comparison to all dietary treatments (P<0.05), except to P2 which the value for feed/gain was comparable (P>0.05).

Nutrient digestibility and N utilization

Table 4 reports the digestibility of DM and CF as well as N retention of experimental groups with the increasing proportion of GE in the diets. The DM and CF digestibility were substantially increased as a result of GE inclusion (P<0.05). For DM digestibility, P1 treatment resulted in a 23.41% increased than the control group whereas this treatment also elevated the CF digestibility by 60.52% and 22.83%, respectively, which being the highest increase among other treatments. Although increasing dietary GE increased N intakes as a consequence of higher CP contents, they also excreted significantly higher urinary N (P<0.05) when compared to control. However, N retention was found to be significantly higher for those animal groups receiving elevated GE treatments (P<0.05), where P1 and P2 treatments being the highest and control group was the lowest. In this case, increasing GE supplement at >13% DM was no longer effective to increase N utilization.

Purine derivatives and microbial protein synthesis

Table 5 provides the data of purine derivatives excretion and microbial protein (N) synthesis (MPS). The data showed that allantoin and uric acid excretions were significantly affected by the dietary treatments (P<0.05). Moreover, MPS was also significantly enhanced by increasing dietary GE (P<0.05). In this study, P2 resulted in the highest excretion of purine derivatives and MPS than those other dietary treatments, which is doubled in the allantoin excretion and more than tripled increased on MPS than control.

DISCUSSION

In the present study, the control diet (P0) reflects if not most, many actual conditions of beef cattle farming in Indonesia, especially those managed by smallholder farmers which only fed their cattle with grass and low-quality roughages such as rice straw. The lack of obtaining optimum growth performance by using such ingredients as the main feed sources have been reported extensively. This is plausible because relying on only grass and rice straw diets containing low CP (6.34%) and high fiber (30.66%) contents might result in low fermentation characteristics in the rumen.

The results of nutrient intakes recorded in this study indicated that the experimental animals consumed less than expected DM intake per BW, which were ranged between 1.73 to 2.35% BW. Previous study have reported relatively similar DM intake for PO cattle (2.01% BW) and for PO crossbreed (2.17% BW) [19]. This low feed intake could be related to the basal diet which mainly composed of rice straw and king grass without sufficient energy source added to diets such as concentrate. It was suggested that concentrate supplementation is important factor to achieve sufficient DM and nutrient intake [18]. This resulted also indicated that the growth enhancers supplementation boosted voluntary intake for DM, CP, and TDN compared to grass and rice straw alone. This could be attributed to the composition of GE which is highly palatable such as molasses, soybean meal, Leucaena, and *T. catappa.* Molasses is known for their superior palatability and have been extensively used as a feed supplement particularly in straw-based diets to compensate for energy deficit in dairy

and beef cattle. Numerous studies have demonstrated that supplementing molasses could promote higher voluntary intake, rumen fermentation, and feed efficiency as recently reviewed recently [19]. A recent meta-analysis has shown that molasses supplementation increased DMI up to 7.1% and increased NDF digestibility by 2-3% [20], which corroborated the results of this study. An improvement in daily intake by 14.2% and milk production by 3.7% of early lactating dairy cows was also reported by supplementing diets with molasses at 1 kg per animal/d [21]. Other positive results were reported on beef cattle [2,22]. In this study, the increase of DMI was higher than other previous reports and it might be related to the higher nutrient improvement on our dietary treatments in term of CP while the other studies mostly used identical dietary CP.

Moreover, it is important to note that molasses alone is not sufficient to optimize rumen fermentation because molasses has a marginal concentration of nitrogen and was reported to depress fiber digestion on low CP diets [12]. Therefore, providing nitrogen source with high solubility was also important to balance energy-protein supply. In this study, increasing voluntary intake was a result of a synergistic effect among ingredients including Leucaena, soybean meal, fish oil, urea, and *T. catappa* rather than molasses alone. It was supported by previous study revealed that beef cattle raised in Leucaena based pasture was reported to have a greater daily weight gain when supplemented with moderate level of molasses ($BW^{0.75}$) while higher supplementary levels depressed the ruminal fermentation [23]. When supplemented at low to moderate levels, Leucaena could effectively boost rumen fermentation by increasing microbial protein synthesis and nitrogen use efficiency, depressing protozoal population and methane formation [7,24] because it has protective effect toward ruminal protein degradability due to its condensed tannins content [25,26]. These led to improve overall ruminant metabolism thus resulted in higher average daily gain especially in beef cattle [3,22,27].

Additionally, fish oil and soybean meal in the GE also served as high energy and protein densities, respectively, that provide higher nutrient supply. These theoretical backgrounds were in agreement with the results recorded in the present study whereas higher final BW and ADG was obtained from cattle fed natural growth enhancer. This is especially correct in P1 and P2 treatments that resulted in greater final BW and ADG while higher supplementary NGE levels were showed to decrease the animal performance (P3 and P4, Table 3). The levels-dependent effect might be associated with the toxicity effect from the ingredients used such as urea and Leucaena especially with excessive supplementation levels.

Leucaena is a perennial tropical legume characterized by a highly digestible and palatable and is a valuable protein source for ruminants. Feeding Leucaena was suggested to be better accepted by ruminants than grass-based diet and often resulted in higher daily gain performance [24,27]. When combined with other highly digestible feedstuff such as molasses, urea, and soybean meal, it was expected to significantly increase nutrient utilization thus improve animal performance. Previous experiments have reported synergistic effects on improvement of nutrient digestibility and animal performance supplemented with molasses and urea blocks [12,22,28]. In this experiment, replacing rice straw with 8.7% NGE increased CP and CF digestibility by 60.52% and 22.83%, respectively. Although higher levels of NGE also increased nutrient digestibility compared with control, however, they were lower than that of P1. This might be related to the excessive amount of molasses, urea and Leucaena which was reported to negatively affect nutrient digestibility due to their toxic properties on rumen microbes. A previous study reported that increasing levels of Leucaena in a king grass-based diet above 40% decreased CP and NDF digestibility [8]. Negative effect of molasses supplementation was also found on grazing beef cattle at above 9 g/kg metabolic weight [23].

The inclusion of GE in this study has shown a markedly increase in allantoin, uric acid, and microbial protein synthesis (MPS) (Table 5). Several studies have reported that soluble materials such as molasses, urea, and

highly digestible protein sources such as Leucaena, *T. catappa*, and soybean meal are beneficial for ruminants when provide at moderate levels [21,22]. These ingredients could optimize ruminal fermentation by enhancing volatile fatty acids production, improving rumen microbial activity and protein synthesis [5,13,21]. Bioactive compound containing in the *T. catappa* leaves such as flavonoids, essential oils and other phenolic compounds might contribute to enhance rumen fermentation efficiency [5]. Recently, metabolites profiling has shown that flavonoids including their glycosides are the most abundant compounds presented in *T. catappa* leaves, followed by metabolites belong to phenolic acids and alkaloids [29]. In addition to that, a recent review also highlighted the favorable effects of flavonoids on rumen fermentation, inflammation, and liver health, and production performance of ruminant [30]. Rumen compartment plays a central role to produce energy and protein for ruminant metabolism. Therefore, balancing energy-nitrogen supplies is a key factor to enhance MPS as the main protein source for a ruminant. The results from this study were in agreement with most of the previous reports that optimum VFA and N concentration are required to enhance microbial growth thus increase microbial protein synthesis [2,13].

However, it is important to note that the proper proportion of molasses and urea should be considered and provided in a correct equilibrium with other supplementary materials to obtain optimum stimulation in the rumen protein synthesis [19]. If not, detrimental effects on nutrient digestibility and ruminal fermentation might occur and this might be related to the present finding whereas higher supplementary levels tend to decrease nutrient digestibility. It is because rumen has a limiting capacity for NH_3-N production, i.e., when NH_3-N production exceeds the maximum threshold, it would impair microbial growth [27]. Moreover, secondary compounds especially condensed tannin present in Leucaena and essential oils (terpenoids) present in *T. catappa* also contributed positively to enhance rumen fermentation. Large number of evidences is available regarding these bioactive components in improving animal performance [2,11]. Above all, improving voluntary intake, nutrient digestibility, feed efficiency, and microbial protein synthesis are the reason for higher daily gain achieved in this experiment.

CONCLUSION

The present study highlighted that supplementary feeding with 8.7% growth enhancer to partially replace rice straw could improve the growth performance of Ongole grade cattle. The improvement on average daily gain and final body weight could be the results of increasing voluntary intake for DM, CP, TDN, and improving nutrient utilization of the ration as reflected from the increased of nutrient digestibility and N retention as well as microbial protein synthesis. GE supplementation at 8.7% could also improve feed efficiency.

CONFLICT OF INTEREST

The authors declare no competing interests exist.

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