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Original Article

Blood metabolites profile and Growth Hormone mRNA expression of Peranakan Ongole cattle fed with finishing ration containing Vinasse-molasses

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Abstract

Objective: This study aimed to investigate effects of Molasses replacement with Vinasse-molasses in ration on the blood metabolite profiles and mRNA expression of *Growth Hormone 1* (*GH1)* of finishing Peranakan Ongole (PO) Cattle.
Methods: Ten male PO cattle in averaged 209 ± 21 kg of body weight were equally allocated to receive either molasses or Vinasse-molasses dietary treatments. The concentrate diet was contained of either 15% of molasses (control) or Vinasse-molasses. This study observed the nutrient intake, percentage of weight gain, blood metabolite profiles and *GH1* mRNA expression of PO cattle.
Results: The result showed that replacing molasses with vinasse-molasses reduced (P<0.05) dry matter intake (DMI) and intakes of crude protein (CP), ether extract (EE), crude fiber (CF), and total digestible nutrient (TDN). Nevertheless, Vinasse-molasses inclusion did not change (P>0.05) percentage of weight gain. Vinasse-molasses increased (P<0.05) the blood urea nitrogen level, while it did not affect (P>0.05) the *GH1* mRNA levels.
Conclusions: It can be concluded that replacing molasses with vinasse-molasses does not change blood metabolite profile, *GH1* mRNA expression and performance of PO cattle. Vinasse-molasses could be applied as energy sources ingredient to replace molasses in finishing cattle feed.

Keywords: Blood metabolites; Cattle; Growth hormone; Vinasse-molasses

INTRODUCTION

Recently, utilization of by-product from food processing industry has been attracting attention as alternative energy source ingredients for ruminants. Molasses, a residue of sugar extraction process contained of high energy and rich in mineral salts [1]. Vinasse, a residue of condensed molasses is a co-product in production of alcohol, sugar, and citric acid yeasts, which is not reprocessed and become polluted in the manufacture**.** Parsaee *et al*. [2] reported that vinasse contained a wide range of organic compounds including sugar and carbohydrates, organic acids, and some minerals (potassium, calcium and sulfate) which makes it potentially used as feed ingredient in animal feed. Another study reported that Vinasse-molasses contained of 20% crude protein and mineral, such as potassium (64 g/kg), sodium (19.2 g/kg), and calcium (17.5 g/kg) [3]. Thus, vinasse has a high potency to be used as an alternative to molasses.

A number of hormones affect growth and nutrient metabolism, i.e., gluconeogenesis, lipogenesis, lipolysis, and protein synthesis. One of genes affecting meat production is Growth hormone (GH). Growth hormone (GH) released from anterior pituitary including on somatotropic axis in the ruminant. The presence of GH recognized as one of the essential hormones for normal animal growth [4]. Glucose serves as primary metabolic fuel in mammals and biological synthesis pathways in animals [5]. Growth hormone plays a role to maintain glucose homeostasis due to its effects on stimulating uptake of amino acids and its production into protein, increasing fatty acids oxidation and its release from adipose tissue, and antagonizing the effects of insulin in increasing the plasma glucose concentration [6]. Therefore, GH may regulate the glucose availability to support cattle growth.

Utilization of vinasse in ruminant diets has been studied related to its effects on performance, digestibility and carcass quality [7,8]. López-Campos *et al*. [9] reported vinasse 200 g/kg concentrate increase urea nitrogen in blood of sheep. Limited study was available related to effect of vinasse-molasses on blood metabolite profile and mRNA expression of *GH1* in ruminants. It is hypothesized that 15% vinasse-molasses in concentrate diet can replace molasses as energy source ingredient of finishing cattle without any negative effects on blood metabolites profile and *GH1* mRNA expression. This study aimed to investigate the replacement of Molasses with Vinasse-molasses as alternative energy source ingredient on finishing ration of PO Cattle on blood metabolites profile and mRNA expression of *GH1*.

MATERIALS AND METHODS

Animals and diet

This study was conducted at Field laboratory of Division of Meat and Draught Animal Nutrition, Faculty of Animal Science, IPB University, Bogor, Indonesia. A total of 10 male Peranakan Ongole cattle were used in this study. All bulls were 18 months old with an average body weight of 209 ± 21 kg equallly allocated either to molasses or Vinasse-molasses dietary treatment group. Cattle were adapted for 14 days followed by 68 days experimental period. Cattle fed an experimental diet (20% forage and 80% concentrate) formulated to meet nutrient requirement according to Beef NRC model [10]. The concentrate contained of either 15% molasses or Vinasse-molasses. The chemical composition of vinasse-molasses is starch 74.98%, crude protein 7.70%, crude fat 7.66%, crude fiber 0.87%, and ash 8.79%, which was analysed at Biotech Center, IPB University. Feed formulation and chemical composition, as well as the nutrient composition of experimental diets were shown on Table 1 and Table 2. Amount of concentrate and forage offered and refused were recorded daily to determine the nutrient intake. Following the 68 days of experimental period, each cattle were weighed and percentage of weight gain were calculated.

Blood sampling

Following 68 days of experimental period, the blood was collected by jugular venipuncture after a 3 h of feeding. Blood samples were immediately divided into non-heparinized vacutainer (20 mL) for serum, and EDTA-treated vacutainer (20 mL) for plasma. The non-heparinized vacutainers were cooled at 5 ^{o}C, whereas the EDTA-treated vacutainers were frozen at liquid nitrogen. Blood samples on non-heparinized vacutainers were centrifuged at 3000 rpm for 15 minutes to separate the plasma for the determination of metabolites then stored in -20 ^{o}C until analyzed. The blood samples on EDTA-treated vacutainers were immediately stored on -80 ^{o}C until mRNA expression analysis.

Blood metabolites measurements

Blood metabolites were determined using blood serum samples. Glucose and cholesterol concentrations were determined using the commercial colorimetric kit number 139204 and 118001, from Greiner Diagnostic GmbH (Bahlingen, Germany), whereas triglyceride (kit number ETI11630200-2) and total protein (kit number ETI15700400-5) concentrations were determined calorimetrically using commercial kits from Human Diagnostic Worldwide (Weisbaden, Germany).

Table 1. Ingredients and nutrient content of feed fed to Peranakan Ongole cattle

Ingredient, % DM	Concentrate		*Pennisetum purpureum*
	Molasses	Vinasse-molases	
Cassava waste	15.0	15.0	-
Corn	10.0	10.0	-
Cassava cake	9.3	9.3	-
Pollard	35.0	35.0	-
Soy bean meal	14.0	14.0	-
Molasses	15.0	-	-
Vinasse-molasses	-	15.0	-
$CaCO_3$	0.5	0.5	-
Urea	0.5	0.5	-
Premix	0.5	0.5	-
Salt	0.2	0.2	-
Nutrient content[1], %			
Dry matter (%)	85.47	84.11	16.36
Crude protein (% DM)	18.54	16.54	9.38
Ether extract (% DM)	2.44	2.17	1.96
Ash (% DM)	9.03	8.58	13.94
NFE (% DM)	50.05	51.42	43.77
Crude fiber (% DM)	5.41	5.40	30.95
TDN (% DM)	86.52*	86.27*	54.09**

[1]Analysis result of Biotech Center, IPB University (2021)

* TDN = 40,2625 – 0,1379 CF + 1,1903 EE + 0,4228 Starch + 0,1969 CP [28]

** TDN = -21,7656 + 1,4284 CP + 1,0277 Starch + 0,4867 CF + 1,2321 EE [28]

DM = Dry matter, NFE = Nitrogen free extract; TDN = Total digestible nutrient

Table 2. Nutrient composition of experimental diets fed to Peranakan Ongole cattle

Nutrients	Experimental diets	
	Molasses	Vinasse-molasses
Dry matter (%)	71.65	70.56
Crude protein (% DM)	16.71	15.11
Ether extract (% DM)	2.34	2.13
Ash (% DM)	10.01	9.65
NFE (% DM)	48.79	49.89
Crude fiber (% DM)	10.52	10.51
TDN (% DM)	80.03	79.83

NFE = Nitrogen free extract; TDN = Total digestible nutrient

Gene expression

Total RNA from blood was isolated using RNEasy Mini Kit according to the supplier's protocol. Total RNA was treated using one-coloumn RNase-Free DNase set (Promega) and quantified using a spectrophotometer (NanoDrop, ND8000, Thermo Scientific). RNA yield and purity were assessed using an Agilent 2100 Bioanalyser and RNA Nano 6000 Labchip kit (Agilent Technology). Total RNA was reverse-transcribed into cDNA using the ReverTra Ace qPCR RT Master Mix with gDNA Remover Toyobo kit according to supplier's protocol.

Real-time polymerase chain reaction (rt-PCR) analysis was performed to analyze mRNA expression levels (Analytic Jena, AG qTower 4 Kanal, Germany). The rt-PCR was carried out following cycle program as follows: 95 ^{o}C for 1 min, and 40 cycles: 95 ^{o}C for 15 s and 60 ^{o}C for 1 min on the StepOne Plus qPCR system (Applied Biosystem). Each rt-PCR performed in 10 µL final reaction volume containing 5 µL THUNDERBIRD Sybr qPCR Mix Toyobo, 1 µL cDNA (50ng/ µL) and 1 µL of primers and nuclease free water. The levels of mRNA expression were analyzed using the ΔΔCT method to determine relative

Table 3. Primer sequences for real-time PCR analysis

Gene name (symbol)	Gene bank accession no.	Primer	Sequence (5'-3')	Length (bp)
Growth hormone 1 (*GH1*)	NM_180996.1	Forward Reverse	CTG CTT CTC TGA AAC CAT CC TGT TGG TGA AGA CTC TGC TG	143 bp
Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)[1]	NM_001034034.2	Forward Reverse	CTG GAG AAA CCT GCC AAG TA TGA CAA AGT GGT CGT TGA GG	181 bp

[1]Housekeeping gene

fold changes [11], and normalized with *GAPDH* as the housekeeping gene. The delta Ct (ΔCT) values were calculated as the difference between target gene (*GH1*) and the housekeeping gene (*GAPDH*). The primer sequences were designed based on published sequences from the National Center for Biotechnology Information (Table 3).

Statistical analysis

Data were analyzed using independent sample t-test procedure performed in SPSS 26.0 (SPSS, Chicago, IL). All data are presented as the mean ± standard error of the mean.

RESULTS

Growth performance

The effect of replacing molasses with vinasse-molasses on nutrient intake and body weight change of Peranakan Ongole cattle were shown on Table 4. Vinasse-molasses reduced (P<0.05) total DMI, CP intake, EE intake, and TDN intake, while it did not affect (P>0.05) CF and NFE intake. Diet containing vinasse-molasses did not change (P>0.05) final body weight, weight gain and weight gain percentage of PO cattle.

Blood metabolite profile and gene expression

Table 5 showed the blood metabolite profile of PO cattle fed with finishing ration containing vinasse-molasses or molasses. Vinasse-molasses increased (P<0.05) blood urea nitrogen in PO cattle, whereas it did not change (P>0.05) the blood glucose, triglyceride, and cholesterol levels (Table 5). Vinasse-molasses utilization in the finishing diet did not change the mRNA levels of *Growth hormone 1* (Figure 1).

DISCUSSION

In this study, 15% Vinasse-molasses in the ration to substitute molasses reduced total dry matter intake by 4.71% followed by reduction in CP, EE and CF intakes. The intake of nutrient is positively correlated with DMI. Previously reported, DMI is the primary constituent for calculating the animal nutrient requirement which makes it has the most effect to the cattle performance in feedlot [12]. In this study the nutrient composition in both diets were formulated in iso protein and energy. Thus, the differences in CP, EE, CF and TDN intakes in this study were mainly due to the difference in total dry matter intake.

Table 4. Nutrient intake and body weight change of Peranakan Ongole cattle fed with finishing ration containing molasses or vinasse-molasses

Parameter	Molasses	Vinasse-molasses	SEM	P-value
Initial body weight (kg)	221	197	6.61	0.06
Final body weight (kg)	314	296	8.78	0.33
Percentage of weight gain (%)	42.5	50.9	3.73	0.27
Total dry matter intake (kg/d)	6.80	6.48	0.07	0.01
CP intake (kg/d)	1.13	0.98	0.03	<0.001
EE intake (kg/d)	0.16	0.14	0.01	<0.001
CF intake (kg/d)	0.72	0.68	0.01	0.01
NFE intake (kg/d)	3.31	3.23	0.03	0.14
TDN intake (kg/d)	5.44	5.18	0.06	0.002

CP = crude protein, EE = energy intake, CF = crude fiber, NFE = nitrogen free extract, TDN = total digestible nutrient

Table 5. Blood metabolite profiles of Peranakan Ongole cattle fed with finishing ration containing molasses or vinasse-molasses

Parameter	Molasses	Vinasse-molasses	SEM	P-value
Glucose (mg/dL)	82.2	81.4	0.74	0.65
Cholesterol (mg/dL)	46.4	50.3	5.24	0.73
Triglyceride (mg/dL)	27.8	36.6	4.54	0.36
Blood urea nitrogen (mg/dl)	17.4	21.4	0.98	0.03

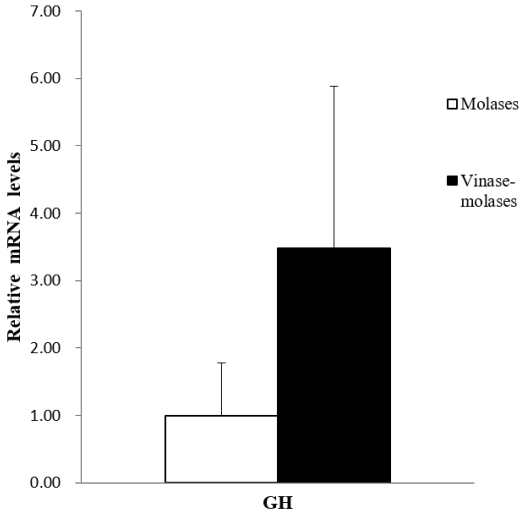

Figure 1. mRna levels of *GH1* in blood of Peranakan Ongole cattle fed finishing diet containing molasses (n=5) or vinasse-molases (n=5)

Vinasse-molasses contains high sugar and starch, which may improve the palatability. Moreover, sugar and starch are rapidly digested in the rumen resulting an increase of ruminal volatile fatty acid concentration, especially propionate [13]. Later, propionate is absorbed into the bloodstream and used as precursor for hepatic gluconeogenesis for producing glucose and energy. Thus, animal may have filled their energy requirement resulting in lower total dry matter intake. Moharrery *et al*. [14] reported a negative slope in increasing of daily starch intake on ruminal starch digestibility. Furthermore, vinasse-molasses is more palatable than molasses for cattle due to most of protein is combined with amide compounds [3]. In accordance with our result, Maneerat *et al*. [7] reported that utilization of vinasse on bagasse-vinasse mixture reduce DMI of steers in compare to rice straw-molasses diet.

Our study demonstrated that replacement of molasses as much as 15% with vinasse-molasses resulted in no significant differences on percentage of weight gain, though it was 19.8% higher. Thus, vinasse-molasses diet may provide adequate energy intake which effectively support growth performance of cattle probably by improving the feed digestibility. Vinasse-molases in the diets indirectly impact the rumen microbial population by providing minerals and microbial nitrogen that enhanced digestibility [3]. In accordance with our result, Maneerat *et al*. [7] stated that utilization of vinasse on bagasse-vinasse mixture diet had no significant effect in final body weight in compare to rice straw-molasses diet. Thus, vinasse-molasses can replace molasses in the finishing diet of beef cattle.

Blood metabolites reflects the energy status of cattle. On this study, blood glucose, triglyceride, and cholesterol did not differ between molasses and vinasse-molasses groups. The level of blood glucose was not different between treatments, indicating the body mechanism to regulate the blood glucose level within the normal range. In addition, it may indicate the similar gluconeogenesis rate when cattle offered with molasses or vinasse-molasses since starch fermentation will

produce more propionic acid which is a precursor for glucose synthesis [15]. Similar with our study, supplementation of vinasse-molasses until 12% in the ration did not change blood plasma glucose concentration of Mahabadi kids [8].

Our results showed no different in blood triglyceride and cholesterol levels indicating vinasse-molasses may fulfill the energy requirement of cattle. In low glucose status, the fat stored as energy deposit in the tissue were mobilized to the liver where it is either oxidized or re-esterified as triglyceride. Also, hepatocytes secrete triglyceride and cholesterol in very low-density lipoprotein which are circulated into intermediate-density lipoprotein by hydrolysis of triglyceride. Triglycerides are further used to fulfill energy requirement in other tissues. In accordance with our result, feeding molasses distillers soluble did not affect concentration levels of blood cholesterol and triglycerides in lambs [16]. Our study suggests that 15% Vinasse-molasses may maintain the energy status of cattle showed in no different in blood glucose, triglyceride, and cholesterol levels compare to molasses.

Results of our study showed that Vinasse-molasses increase the blood urea nitrogen levels. In ruminants, feed nitrogenous compounds were digested into ammonia in rumen, then rumen microbes synthesize bacterial proteins from the ammonia to stabilize rumen ammonia levels [17]. Next, digestible protein (nitrogen) is absorbed either in rumen-reticulum or small intestine. Puppel and Kuczyńska [18] reported that several factors including protein intake and rumen degradability, dietary amino acids composition, carbohydrate amount and rumen degradability may affect urea nitrogen level. The blood urea nitrogen is produced from amino acids and protein catabolism, which also negatively correlated to protein utilization. In accordance with our result, the higher blood urea nitrogen may indicate low protein utilization in the body. It is supported with no difference in mRNA levels of *Growth hormone I* and weight gain percentage in this study. In addition, hepatic urea nitrogen may be recycled via saliva secretion into the gastrointestinal tract [19]. Eismann and Tedeschi [20] reported that higher values of urea nitrogen in blood is correlated with the low intakes of dietary nitrogen. In this study the vinasse-molases utilization in concentrate diet resulted in lower CP intake (nitrogen intake) resulting in higher blood urea nitrogen. The recycled urea nitrogen provides nitrogen to support rumen microbial growth and preserves nitrogen [21]. Thus, low CP intake and the adequate protein pool in the body resulting higher blood urea nitrogen in vinasse-molasses diet. Taken together, the 15% vinasse-molasses utilization in the diet may fulfill the energy requirement of cattle, however it has not been able to provide a significant increase in the growth rate of PO cattle.

Energy homeostasis is controlled by a complex regulatory system in somatotrophic axis that may affect feed intake and body weight. Growth hormone is one of the hormones contributes in coordinating nutrient metabolism. Growth hormone is a peptide hormone synthesized by somatotrophin cells in pituitary gland that stimulates growth, cell reproduction and regeneration in human and animals [22]. The growth hormone (*GH1*) is considered as one of most important molecules that coordinates many physiological processes including bone and skeletal muscle growth in beef cattle [4].

In this study, no different in *GH1* mRNA levels was followed by no difference in weight gain percentage and blood plasma glucose concentration between treatments. Previously reported, circulating glucose concentration correlated with the blood GH levels in cows [23]. Growth hormone increases protein synthesis, thereby increasing nitrogen retention [24]. The pituitary gland senses the metabolic changes in the body and adjusting the GH releases. In this study, the replacement of 15% molasses with vinasse-molasses resulting lower CP intake resulting in no difference in blood glucose, while the blood urea nitrogen elevated. In line with our study, protein intake reduces causes depletion of insulin-like growth factor, insulin, and Ca, whereas the GH concentrations were not change in young goats [25]. A study in lambs exposed only severe dietary energy and protein restriction may causes significant

increase in GH transcription [26]. Therefore, 15% vinasse-molases in concentrate diet may support energy adequacy, while did not change mRNA levels of *GH1*.

In addition, the mRNA levels of *GH1* was analyzed only at the end of finishing period may also contribute to no great influence on the *GH1* mRNA levels. Miceikiené *et al*. [27] reported that Growth hormone had great influence on liveweight gain indicators only during first half of fattening period. In conlussion, replacing molasses with vinasse-molasses in the finishing ration may not have any negative effect on *GH1* mRNA levels of Peranakan Ongole cattle at the end of finishing period. Further study was needed to determine effect of vinasse-molases on *GH1* mRNA expression in finishing period.

CONCLUSION

Replacing Molasses with Vinasse-molasses on finishing diet reduced dry matter intake of Peranakan Ongole cattle. Its increased blood urea nitrogen, but did not change the *GH1* mRNA expression resulting no difference on weight gain percentage of Peranakan Ongole cattle. Vinasse-molasses as an alternative energy source ingredient may replace molasses in finishing cattle diet.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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