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Research Article

Efficacy of concentrates containing tropical weed *Chromolaena odorata,* methionine hydroxy analog and palm oils in fattening male Bali cattle: A physiological study

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Abstract

Chromolaena odorata is a potential feed source but is low in methionine, which might affect the rumen function. Therefore providing efficient nutrient/s might improve rumen function. The study aimed to assess the efficacy of a concentrate containing Chromolaena odorata meal, hydroxy methionine analog (HMA), and palm oil on blood metabolites of fattened cattle. Sixteen Bali cattle (*Bos sondaicus*) with an average body weight of 130.5kg ± 32.5 were allotted into four dietary treatments (and four replicates) using a Completely Randomized Block Design with 4 cattle in each Group. The treatments were concentrates with no addition of palm oil and/or HMA as the control diet (Group I) , added with 3 g HMA (CMA) (Group II), added with 0.5% palm oil (Group III) and (CPO) added with a combination of 3 g HMA and 0.5% palm oil (CMO) Group IV). The concentrate was offered to the animals at a rate of 2% liveweight, whilst rice straws were provided ad libitum as the source of fiber. The concentrate contained 18% crude protein and 12 MJ ME/kg DM to target a live weight gain of at least 0.6 kg/day. Blood metabolites measured were total cholesterol levels, plasma glucose, plasma proteins, and plasma urea. Collected data were subjected to analysis of variance with the significance level set at an alpha value of ≤ 0.05. The results showed that providing concentrate-containing. *C. odorata* with or without HMA and/or palm oil (Group IV) only significantly affected blood plasma proteins, whereas other blood metabolites were unaffected. It might be concluded that there is no benefit of adding palm oil or methionine analog to chromolaena-based concentrate for fattened cattle since it will not affect the concentration of blood metabolites.

Keywords: Bali cattle, Blood urea, Palm oil, Plasma glucose, Plasma protein, Rice straw

INTRODUCTION

Chromolaena odorata is an invasive weed to native pastures in tropical areas of the world, yet it is a potential feed source due to its high biomass production that can reach 70 tons DM/ha/year (Mullik *et al.*, 2015), and high crude protein content which is in a range of 13-35% (Oematan *et al.*, 2016). The protein score is 88.2% (Apori *et al.*, 2000; Ngozi *et al.*, 2009), which is very high. On the contrary, this plant's organic biomass also possesses a variety of anti-nutritive compounds phenolics, alkaloids, and terpenoids (Singh, 2022). These secondary metabolites could be reduced by the bio-fermentation process (Ridla et al; 2016; Mulik *et al.,* 2016) before being used as a feed source for ruminants (Bira *et al.,* 2017). However, maximizing the rumen fermentation process is the next challenge to the use of bio-fermented *C. odorata* as ruminant animal feed. The rumen function of cattle given bio-fermented *C. odorata* might be less optimal since the concentration of methionine an essential amino acid, is very low. Ngozi *et al.* (2009) and Oematan *et al.* (2020) reported that methionine content in this plant only in a range of 0.22% to 0.0095% which potentially be the limiting factor in stimulating the growth of rumen microbes and hence the performance of cattle. Generally, the most deficient amino acids will disrupt the balance of amino acids in the ration. With a transamination system, an amino acid can be replaced by an analog such as an alpha hydroxy or alpha-keto acid. Methionine can be replaced with its analog in the form of a calcium salt known as dihydroxy methionine analog (Sutardi, 1980). Another potential limitation is high inefficiency in producing the rumen microbial crude protein, which is the ruminant animals' main protein source. The high protozoal population in the rumen is the main factor contributing to this inefficiency. Therefore, reducing the protozoal population by using palm oil might improve the protein production efficiency index.

Optimizing rumen function through the inclusion of hydroxy methionine analog (HMA) and palm oil in a concentrate given to fattened cattle aimed at the improvement of the performance of the animal. This improvement could be detected using blood metabolites such as blood glucose, blood plasma protein, and blood urea levels because they are related to nutritional status and cattle productivity. The supply of glucose and protein is closely related to various intakes and cattle's physiological state. When energy consumption is low, the glucose level in the blood is also low. In addition, urea levels in the blood can be used as an index of protein status and usage. This experiment was designed to evaluate the effects of providing concentrates containing HMA or palm oil or a mixture of the two substances on blood plasma metabolite profiles of fattened Bali cattle, *Bos sondaicus.*

MATERIALS AND METHODS

This study was carried out for 2.5 months and consisted of 2 weeks of adaptation and 2 months of data collection. Sixteen male Bali cattle (*Bos sondaicus*) with an initial body weight of 181±25.3 kg were assigned to four dietary treatments in four replicates using a completely randomized block design. The treatments were concentrates with no addition of palm oil and/or HMA as the control diet (Group I), or added with 3 g HMA (Group II), or added with 0.5% palm oil (Group III), or added with a combination of 3 g HMA and 0.5% palm oil (Group IV). The concentrate was offered to the animals at a rate of 2% liveweight, whilst rice straws were provided ad libitum as the source of fiber. The concentrate contains 18% crude protein and metabolizable energy of around 12 MJ/kg DM to target a liveweight gain of at least 0.6 kg/day (based on NRC, 2000). The concentrate consisted of 30% *C. odorata* silage flour, 10% yellow corn, 47% wheat bran, 0.5% coarse salt, and 0.5% vitamins-minerals premix. The quantity of the concentrate fed was 2% of the liveweight. Chopped rice straws and drinking water were available ad libitum. The nutrient composition of the concentrate in all treatments and the rice straw is presented in Table 1.

Blood metabolites measured were total cholesterol concentrations, plasma glucose, plasma proteins, hemoglobin, hematocrit, erythrocyte, and leucocyte. Blood samples were collected from each animal on the last day of the data collection period. A pair of 50 ml blood was drawn into 50 ml heparinized vacutainer tubes inserted into 20G2 vacutainer needle puncture into the jugular vein. One tube from each animal tube was centrifuged at 3000 rpm for 10 minutes to separate plasma from other blood materials. The plasma is then used for intended variables. Meanwhile, blood samples in the uncentrifuged tubes were used for blood cell quantification.

Blood samples analysis

The plasma concentrations of glucose, total cholesterol, blood urea nitrogen, and total protein were measured using the corresponding commercial kits in an automatic biochemical analyzer (Mindray BS-180, Biomedical Electronics Co. Ltd., Shenzhen, China). Hemoglobin in the blood was analyzed by Hemoglobin Colorimetric Detection Kit, Catalog number: EIAHGBC (Thermo Fisher Scientific; Life Technologies Corporation Carlsbad, CA 92008 USA). Blood cell counting was done using the TC20 automated cell counter (Bio-Rad) with the protocol described by Hsiung *et al.* (2013).

Data analysis

Collected data were subjected to analysis of variance using a univariate general learn model for a completely randomized block design. The treatment effects were detected at an alfa value of 0.05, whereas the Duncan Multiple range test was tested between treatment differences. The software used in the data analysis was SPSS v 25 (IBM, 2017).

RESULTS

Blood metabolites

Cholesterol is present in muscle and adipose tissue because it is an important component of cell membranes and can be stored as cholesterol esters in lipids. Differences in cholesterol content among species are generally caused by cholesterol absorption and biosynthesis variations, lipoprotein metabolism, diet, genetic variation, subcutaneous and intramuscular fat, and body weight (Dessi and Batetta, 2003). The highest data on blood cholesterol levels produced in this study was achieved by the supplementation treatment of a combination of hydroxy methionine analog and vegetable oil (Group IV), namely 216.78 mg/dl, followed by the treatment of hydroxy methionine analog supplementation (Group II), namely 215.40 mg/dl then by vegetable oil supplementation (Group III), which was 212.21 mg/dl and the lowest by treatment without supplementation (Group I), which was 200.16 mg/dL. The average blood cholesterol levels of male Bali cattle (Table 2) varied from 200.16 mg/dl to 216.78 mg/dl, averaging 211.14 mg/dl.

The results of statistical analysis showed that the ration without supplementation (Group I) and rations supplemented with hydroxy methionine analog (Group II), supplemented with vegetable oil (Group III) and supplemented with a combination of hydroxy methionine analog and vegetable oil (Group IV) in concentrate rations had the same effect (P>0.05) on blood cholesterol levels in Bali cattle research. Blood cholesterol concentrations that did not differ between treatments were due to the effect of the feed given having the same quality of nutrients (Table 1).

Supplementation of hydroxy methionine analog, vegetable oil, and their combination in concentrate rations has not been able to significantly reduce cholesterol levels, causing blood cholesterol in the body of Bali cattle to have the same effect between treatments. Other factors influencing blood cholesterol levels are the concentration of rumen fluid acetic acid and blood glucose resulting from the inter-treatment research rations have the same effect. Low blood glucose levels in the treatment without hydroxy methionine analog supplementation and vegetable oil (Group I) can spur an increase in blood cholesterol levels because, at low blood glucose levels, insulin secretion will be inhibited and cause blood cholesterol concentrations to increase. This is per the results of Syahrir (2010), which said that acetic acid is the main precursor of cholesterol biosynthesis. Low blood glucose levels can spur increased blood cholesterol levels.

According to Mitruka (1981), normal blood cholesterol in cows is in the range of 80 – 170 mg/dl. Thus, the blood cholesterol levels in the Bali cattle in this study were above the normal range, namely 200.16 mg/dL to 216.78 mg/dl. The average blood cholesterol levels produced in this study when compared with the results of the research by Syahrir *et al.* (2010) by giving concentrate made from mulberry leaves to Bali cattle, the results were higher, namely 211.14 mg/dl vs 168 mg/dl. Meanwhile, when compared to the results of a study by Maranatha *et al.* (2021) on Bali cattle, the results were higher, namely 211.14 mg/dL vs 56.65 mg/dl. However, when compared with the cholesterol contained in the serum of dairy cows, the results are lower, namely 211.14/dl vs 227.8 mg/100 ml, and in camels, the results are not much different, namely 211.14 mg/dl vs 106.4 mg/ 100 ml (Faye *et al.*, 2015). Differences in blood cholesterol levels from the results of this study and reports on the results of research conducted by researchers are due to the feed used, the physiological status of the research cattle, and other environmental factors.

Table 1. Nutrient content of the un-enriched chromolaena-based concentrate (CON), or enriched with 3 mg hydroxy metheonine analog (CMA), or 0.5% palm oil (CPO), or 3 g hydroxy methenine analog and 0.5% palm oil (CMO), and the rice straw used in the experiment.

Nutrient (%)	**Treatment**				**Rice Straw**
	CON (Group I)	**CMA (Group II)**	**CPO (Group III)**	**CMO (Group IV)**	
Dry Matter	95,21	95,93	95,64	95,36	94,65
Organic matter	85.73	86.26	85.95	83.38	81.91
Crude Protein	14,35	14,98	15,27	15,48	4,48
Crude lipid	6.39	6.61	7.09	7.41	1.16
Crude Fiber	17,16	16,67	15,41	15,26	33,89
Total carbohydrates	64.98	64.65	63.59	62.50	76.27
Metabolizable energy, MJ/kg DM	12,3	12,2	12,7	12,09	14,68

Table 2. Concentrations of blood metabolites of fattened cattle given an un-enriched chromolaena-based concentrate (CON) or enriched with 3 mg hydroxy metheonine analog (CMA), or 0.5% palm oil (CPO), or 3 g hydroxy methenine analog and 0.5% palm oil (CMO)

Variables	**Treatment diets**				**SEM**	***p*-value**
	CON (Group I)	**CMA (Group II)**	**CPO (Group III)**	**CMO (Group IV)**		
Total cholesterol; mg/dL	200.1	215.4	212.2	216.8	10.05	0.655
Plasm glucose; mg/dL	90.7	95.8	95.3	96.9	2.04	0.173
Plasma urea; mg/dL	44.9	46.4	47.0	47.7	1.11	0.391
Plasma proteins; g/dL	6.7a	7.3^{b}	7.5^{b}	8.5^{c}	0.16	0.003
Hemoglobin; g/dL	13.7	13.4	13.0	12.7	0.49	0.321
Hematocrit, %	41.2	40.3	39.1	37.9	1.47	0.443
Erythrocytes, 10^{6}/ml	10,92	10.8	10.2	10.0	0.66	0.742
Leucocytes, 10^{3}/ml	8.9	8.6	8.9	8.9	0.36	0.664

Levels of hemoglobin and blood cells

The concentration of blood hemoglobin (12.7 g/dL - 13.7 g/dL), hematocrit value (37.9% - 41.2%, erythrocytes value (10.0 x 106/µl - 10.9 x 106/µl), and leucocyte value (8.6 x 103/µl - 8.9 x 103/µl) on the blood of Bali cattle fed the tested diets. The inclusion of HMA and/or palm oil did affect ($p>0.05$) all these variables.

DISCUSSION

Treatment effects on blood cholesterol, glucose, urea, and proteins

The level of cholesterol in the blood relates to cholesterol absorption and biosynthesis, lipoprotein metabolism, diet, genetic variation, subcutaneous and intramuscular fat, and body weight (Dessi and Batetta, 2003). The normal blood cholesterol value for Bali cattle is 80 – 170 mg/dl (Mitruka, 1981). The current results are also higher than the findings of Syahrir *et al.* (2010), who reported a mean value of 168 mg/dL in Bali cattle given a mulberry leaves-based concentrate. Maranatha *et al.* (2021) also reported a far lower cholesterol level of 56.65 mg/dL in the blood of Bali cattle-fed grass-legume-food crop residue. Higher blood cholesterol levels in the present study could be affected by a high nutrient density, particularly metabolizable energy present study (12.09 – 12.7 MJ ME/kg DM). The nonsignificant difference in the blood cholesterol concentrations in the present study could be related to the similar quality of nutrients in the feed (Table 1). Inclusion of HMA and palm oil into the chromolaena-based concentrate either separately or in a combination of the two was not able to reduce cholesterol levels resulting in similar blood cholesterol among treatments.

Blood glucose indicates protein and energy adequacy in the animal's diet. For ruminant animals, glucose is needed in certain quantities for maintenance, tissue growth, and other production parameter. Glucose levels in the body are controlled through glycolysis, glycogenesis, and gluconeogenesis so that the concentration of glucose in the body is maintained relatively constant (Windi *et al.*, 2016). According to Mitruka (1981), the normal range of blood glucose levels in cattle varies between 43-100 mg/dL. The values found in the present study ranged from 90.70 mg/dL to 96.93 mg/dL (Table 2), which is categorized as normal. A study (Bira, 2016) on Bali cattle fed chrolomaena-based concentrate showed similar blood glucose levels (94.69 mg/dL - 82.47 mg/dL). Other studies on Bali Cattle (Tahuk *et al.*, 2017; Windi *et al.*, 2016) reported a range value of 58.3 mg/dL to 64.23 mg/dL). Higher blood glucose levels in this study compared with other studies indicate that the use of concentrate containing *C. odorata* and supplemented with HMA, palm oil, and their combinations provided sufficient protein and energy for the needs of Bali cattle. Whereas lack of response to HMA and palm oil addition into the chromolaena-based concentrate used in the current study suggests a lack of stimulation from the two substances on the production of glucose precursors for glucose extraction in the digestive tract and/or organ tissues metabolism.

In both the clinical setting and research on livestock production, monitoring protein status in ruminants by measuring blood urea nitrogen (UN) concentration has become a routine practice.

Roseler *et al.* (1993) and Giallongo *et al.* (2015) stated that blood urea concentration relates to three possible conditions in the rumen. Firstly, high protein breakdown in the rumen causes ammonia accumulation. Secondly, inadequate energy as the carbon source in ammonia assimilation and asynchrony of energy and protein resulted in low rumen in and utilizing the energy they consume. According to Byers and Moxon (1980) and Preston *et al.* (1978), the optimal plasma UN concentrations for growing feedlot steers are between 11 and 15 mg/dL, while the optimal concentrations for finishing cattle are between 7 and 8 mg/dL. The much higher blood urea nitrogen found in our study is expected since 2/3 of the feed offered was concentrate which contains high crude protein (16%) and dense energy (12 MJ ME/kg DM). Meanwhile, insignificant effects of HMA and palm oil inclusion into the concentrate (Table 2) due to similar protein and energy content of the tested diets (Table 1).

Plasma proteins consist of albumin, globulin, fibrinogen, glycoproteins, haptoglobulins, and lipoproteins that have various biological functions. Albumin plays a role in forming osmotic pressure in the plasma, which will prevent the loss of plasma from the capillaries, globulin plays a role in performing enzymatic functions in plasma and immunity. Fibrinogen plays a role in the process of blood clotting, glycoproteins play a role in the proliferation process in response to tissue damage, haptoglobulin plays a role in forming complex protein bonds to prevent iron loss and protect the kidneys from damage that the deposition of hemoglobin can cause while lipoproteins act as carriers of steroid hormones, vitamins, fat-soluble, glycerides, cholesterol, and their ester forms (Hariono, 1993). Feed proteins are sources of blood proteins. High or low concentration of total protein in the blood is highly dependent on the amino acids absorbed through the intestinal wallPartial plasma proteins in this study were not quantified and only measured as total plasma proteins. The normal plasma protein value for beef cattle is in the range of 5.7 – 8.1 g/dL (Radostits *et al.*, 2007). The total plasma proteins recorded in the present experiment (6.7 – 8.5 g/dL) are categorized as normal and suggest adequacy of protein supplied from diets (Table 1). Other studies on Bali cattle (Irfan *et al.,* 2014; Sandria *et al.,* 2019) reported similar values. Significantly higher ($p=0.003$) plasma proteins in cattle-fed enriched concentrates could be explained by more dense protein in these treatments (CMA, CPO, and CMO).

Effects of treatments on hemoglobin and blood cells

Hemoglobin (Hb) and erythrocytes are two plasma metabolites that go hand in hand because Hb plays a role in red blood cells as an oxygen binder for transportation to all tissues of the livestock body for metabolic purposes. Hb is a complex protein compound of iron that strongly bonds with oxygen and forms oxyhemoglobin (Kasthama and Marhaeniyanto, 2006). Therefore, one of the most important factors in the formation of hemoglobin is nutrition, especially amino acids (glycine and methionine), minerals (iron, copper), and certain groups of B vitamins, namely pyridoxine, folic acid, and cobalamin (Frandson, 1992). Interestingly, the current study is that HMA addition is expected to stimulate Hb and red blood cell formation due to the presence of methionine in the concentrate. However, the effect of HMA inclusion in the concentrate on the levels of Hb and erythrocytes was not significantly detected (P>0.05) in the analysis of variance. Lack of response in Hb and erythrocytes to HMA or palm oil could be related to two aspects. Firstly, the HMA might have been converted and utilized by rumen microbes during the fermentation process resulting in nonsignificant effects on animal tissue metabolism. Secondly, the adequate energy and amino acids supplied by the enriched concentrate stimulate gluconeogenesis by utilizing available amino acids and energy. As gluconeogenesis increases in fulfilling energy, Hb-forming amino acids (especially glycine and methionine) are preferred to enter the Krebs' cycle pathway for energy synthesis, which causes the rate of Hb formation to decrease (Atik *et al.*, 2020). As the rate of Hb synthesis declines, so as erythrocytes formation.

The level of Hb found in the present study (12.7 – 13.7 mg/dL; Table 2) is similar to the findings of Dewi *et al.* (2018), who recorded a range Hb value of 11 – 13.5 g/dL in Bali cattle fed varying degrees of protein and energy concentration. Septiarini *et al.* (2018) also reported a range of Hb levels of 9.9 – 15.8 g/dL in Bai cattle raised mainly on forage. For erythrocytes, Diparayoga *et al.* (2014) reported that the average erythrocytes in Bali cattle were 7.93 x 106/µl while Utama *et al.* (2001) found that the total erythrocytes of Bali cattle were 3.8 - 5.7 x106/µl. Lower erythrocytes 3.96 - 6.42 x106/µl were found in the transportation-stress of Bali cattle (Perayadhista *et al.*, 2022). The total erythrocytes in the present study (10.0 x 106 - 10.9 x 106/µl were much higher than the average value in the studies mentioned. The higher value in our study could be related to a better quality of feed used in present study.

Hematocrit is the number of erythrocytes in ratio to the total blood volume. Diparayoga *et al.* (2014) stated that the normal hematocrit percentage of Bali cattle was 32.87%, while Utama *et al.* (2001) stated that the hematocrit percentage in Bali cattle is 29-32.5%. The results obtained in the current study ranged from 37.9% - 41.2% (Table 2). The value of hematocrit in this study is included in the range of values reported by Perayadhista *et al.* (2022), which is 25.9 - 38.7% in Bali cattle. The average hematocrit percentage obtained in this study were higher than the normal range (29-32.5%) reported by Utama *et al.* (2001) because the diets used in the present study was a fattening diet that had very high nutrients to support a growth rate of >0.6 kg/day.

White blood cells (WBCs) or leukocytes play an important role in immune defense and include different subpopulations: neutrophils, eosinophils, and granulocytes, basophils, monocytes, and lymphocytes. Leukocytes are produced and mature in the bone marrow, and in the case of lymphocytes, in the lymph tissue. The number of leukocytes in the blood is only a fraction of the total population and is subject to wide fluctuations. The normal value of leucocytes for cattle is in the range of 4.9 x 103/µlto 12.0 x 103/µl (Wood and Quiroz-Rocha, 2010; Kraft and Dürr, 2005; George *et al.*, 2010). Meanwhile (Hartaningsih, 1983) proposed the normal range for Bali cattle is 2.3 x 103µL to 9.5 x 103/µl. The leucocyte level in the present study is categorized as normal since it ranged from 8.6 x 103/µl to 8.9 x 103/µl. Higher leukocyte levels were reported by Bunga *et al.* (2019) in Bali cattle feeding on a city waste dumping site in Kupang City, Indonesia. The leucocyte levels recorded in their experiment were 7.7x 10^3µL - 44.3 x 10^3/µl This reflects nutrient adequacy and the absence of health problems. In present study, insignificant treatment effect (P>0.05) suggested that the addition of HMA and palm oil improved the nutrient status of the cattle.

Conclusion

Adding 3 g HMA and 0.5% palm oil into a chromolaena-based concentrate feed to fattened Bali cattle will not be beneficial since most blood metabolites (cholesterol, glucose, urea, hemoglobin and blood cells) were not altered. The only blood variable affected was plasma protein level. Adding the two ingredients (HMA and palm oil) separately or combined can increase blood plasma protein levels.

Conflict of interest

The authors declare that they have no conflict of interest.

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