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Hematology Status of Nellore Crossbreed Through Feed Supplementation Containing Go Pro, Rumen-Protected Nutrients, and Fecal Microbiota Transplantation

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Abstract Optimal meat production can transpire from healthy livestock. This study optimized ruminant meat production by increasing rumen microbial proliferation, using rumen non-degradable nutrients, and maintaining microbial balance through the application of Go Pro (feed addictive product developed in our laboratory), rumen un-degradable nutrients (RUN), and fecal microbiota transplantation (FMT). The hematological profile of 15 Nellore crossbred cattle (8 months old; weighed 199.53 ± 22.82 kg) fed on Go Pro, RUN, and FMT was determined. The cattle were allotted into three feeding groups: T1 (40% forage + 60% Go Pro-supplemented concentrate); T2 (RUN supplementation); and T3 (T2 diet + 5 g FMT). Feed was given at the rate of 3% dry matter/body weight and access to drinking water *ad libitum* was provided. Hematology values are still within the normal range. The observed variables were hemoglobin values (11.78-12 gr/dL), hematocrit (32.7-34.52 %), leukocytes (9.75-14.25 10^3/µL), neutrophils (1.93-2.54 10^3/µL), eosinophils (0.32-0.48 10^3/µL), and lymphocytes (7.38-10.12 10^3/µL). The combination of Go Pro and RUN can reduce allergy-causing substances, and no side effects such as allergies and parasitic infections transpire from FMT. The combination of Go Pro, RUN and FMT can maintain cattle health.

Keywords: hematology, microbiota, ruminant, feed supplement, immunology

Abstrak Produksi daging yang optimal dapat terjadi pada ternak yang sehat. Pada ternak ruminansia, produksi daging dapat optimal dengan meningkatkan proliferasi mikroba rumen, menggunakan nutrisi yang tidak dapat terurai di rumen, dan menjaga keseimbangan mikroba melalui penerapan Go Pro (pakan aditif yang dikembangkan di laboratorium kami), *rumen un-degradable nutrients* (RUN), dan *fecal microbiota transplantation* (FMT). kami mengevaluasi profil hematologi sapi yang diberi Go Pro, RUN, dan FMT. Lima belas ekor sapi persilangan Nellore, berusia sekitar 8 bulan dengan berat badan rata-rata 199,53 ± 22,82 kg, dibagi menjadi tiga kelompok pakan: T1 menerima pakan yang terdiri dari 40% hijauan dan 60% konsentrat yang disuplementasi dengan Go Pro; T2 menerima pakan yang sama dengan suplementasi RUN; dan T3 menerima pakan T2 dengan 5 g FMT. Pakan diberikan dengan takaran 3% bahan kering/berat badan, dengan akses air minum *ad libitum*. Nilai hematologi masih berada dalam kisaran nilai hematologi normal. Variabel yang diamati meliputi: nilai hemoglobin 11,78-12 gr/dL; hematokrit 32,7-34,52 %; leukosit 9,75-14,25 10^3/µL; neutrofil 1,93-2,54 10^3/µL, eosinofil 0,32-0,48 10^3/µL, limfosit 7,38-10,12 10^3/µL. Kombinasi Go Pro dan RUN mampu mengurangi zat penyebab alergi. Penggunaan FMT tidak menimbulkan efek samping, seperti alergi dan infeksi parasit. Pemberian kombinasi Go Pro, RUN dan FMT mampu menjaga kesehatan.

Kata kunci: hematologi, mikrobiota, ruminansia, suplemen pakan, imunologi

INTRODUCTION

The increasing demand for meat to accommodate the growing human population must be balanced with a higher meat production. Meat production of ruminants can be increased through feed that can improve nitrogen utility by rumen microbes for the rumen microbial proliferation process. We developed Go Pro, a slow-released N-source feed additive. Enhanced rumen microbial proliferation will increase protein sources provide high biological value for ruminants to produce meat according to Prasetiyono (2021).

The production of meat in ruminants can also be enhanced through supplements known as Rumen Undegradable Nutrients (RUN) that exhibit resistance to ruminal degradation. The incorporation of RUN into feed enhances the absorption of protein in the post-rumen organs, providing carbon component for the synthesis of methionine and choline in the digestive tract following the rumen (Prasetiyono *et al*., 2020[a]; Prasetiyono *et al*., 2020[b]). The combinations of scientifically tested RUN that have evidently enhanced production levels include rumen-protected protein (RPP), rumen-protected methionine (RPM), and rumen-protected choline (RPC). Improved nutrient provision can enhance intermediary metabolic processes and the protein synthesis. The enhancement of domestic meat production can be achieved through effective management of gastrointestinal tracts to optimize nutrient absorption and support immune function. Kim *et al*., (2021) report that the utilization of fecal microbiota transplantation has decreased the presence of harmful bacteria, increase the population of beneficial bacteria, improve nutrient absorption, and eventually increase carcass percentage and final weight. The efficacy of fecal microbiota transplantation is contingent upon the richness and variety of microbes present in the fecal matter used for the procedure (Wilson *et al*., 2019). The digestive system condition can be determined by observing the absence of infection and inflammation, the equilibrium between harmful bacteria and beneficial bacteria, as well as the sufficiency of essential nutrients. Nutrient adequacy and livestock health can optimize livestock productivity as depicted in the blood hematology status of livestock. The purpose of this study was to determine the effect of using Go Pro, Rumen Undegradable Nutrient, and Fecal Microbiota Transplantation on livestock health as described by hematology status.

Materials And Methods

Ethical Approval

Protocols for managing experimental animals and feeding treatments were carried out in accordance with the proper handling procedures for experimental animals that can minimize animal stress. This protocol had been reviewed and approved by the animal welfare and utilization committee with protocol number 59-04/A-09/KEP-FPP.

Fecal Microbiota Transplantation Procedure

Donor cow feces came from selected cows that showed high levels of productivity in our previous studies. These cows were screened for complete fecal analysis using the microscopic method and subjected to metagenomic testing.

Table 1. Complete Fecal Analysis

Variable	Result
Macroscopic	
Consistency	Solid
Blood	Negative
Mucus	Negative
Color	brown
Microscopic	
Erythrocytes	Negative
Leukocytes	Negative
Entamoeba Histolytica	Negative
Ancylostoma Dueodenale	Negative
Trichuris	Negative
Ascaria	Negative
Bacteria	Positive
Fungi	Negative
Food Scraps	Positive
Lipid	Negative
Epithelium	Negative
Other	Negative

Fecal samples were collected at the interior of the feces. A total of 80 grams of feces, 80 grams of glycerol, and 20 grams of physiological NaCl were mixed to a paste-like consistency, then filtered, inserted into an empty size 0 capsule shell, and coated with a 00-sized capsule. The purpose of fecal microbiota transplantation using capsules was to facilitate FMT administration and reduce stress caused by FMT. Materials for fecal microbiota transplantation were stored at approximately -20°C in the freezer (Saha and Khanna, 2021).

Experimental Design

This research used 15 Nellore crossbred cows, approximately 8 months old, with an average body weight of 199.53 ± 22.82 kg. The cows were allotted randomly to five groups and offered with three treatments. The categorization was determined according to the initial body weight at the commencement of the research. We ran an initial feed consumption test and found that cows were only capable of consuming feed with a dry matter to body weight ratio of less than 3%. Therefore, the feed provided during the study was adjusted to 3% accordingly. Water was made available freely for consumption. The cattle were initially weighed at the commencement of the study and continued to be weighed on a monthly basis in order to adjust their feed according to body weight. The nutrient content of feed ingredients was analyzed using the proximate method at the Feed Nutrition Science Laboratory, Diponegoro University. The nutrient composition of the concentrate is presented in Table 2.

Table 2. Concentrate composition

Concentrate composition	T1	T2	T3
		(%)	
Material:			
Peanut Shell	30,0	30,0	30,0
White Polar	19,5	21,0	21,0
Copra Meal	17,0	15,0	15,0
Palm oil meal	28,0	23,0	23,0
Mineral Mix	1,00	1,00	1,00
Calcite	1,00	1,00	1,00
Salt	1,00	1,00	1,00
GoPro	2,50	1,00	1,00
Rumen Protected Protein (RPP)	0,00	7,00	7,00
Nutrient content in the concentrate			
Dry Matter (DM) (%)	90,0	89,0	89,0
Ash content (AC) (%)	11,6	11,9	11,9
Crude Protein (CP) (%)	15,9	16,0	16,0
Total Digestible Nutrient (TDN) (%)	62,4	62,0	62,0
lipid (L) (%)	6,43	6,54	6,54
Crude Fiber (CF) (%)	23,9	23,0	23,0
Ca (%)	0,78	0,80	0,80
P (%)	0,62	0,60	0,60
Feed Supplement			
Rumen Protected Choline (%)	0.00	0,43	0,43
Rumen Protected Methionine (%)	0.00	0,31	0,31
Fecal Microbiota Transplantation (g)	0.00	0.00	5.00

Note: The Rumen Protected Choline is produced by Shangdong Fy Feed Technology Co Ltd. The Rumen Protected Methionine is produced by the Novus. Mineral mix, GoPro, Rumen Protected Protein is produced by CV. Berkah Intan Sentosa. Concentrates for all treatments were arranged based on nutrient requirements in the National Research Council (NRC) Table with iso TDN and iso CP.

Table 3. Supporting Data

Variable	Treatment		
	T1	T2	T3
Average Daily Gain (kg/d)	0.468[c]	0.600[b]	0.732[a]
In Vivo Lipid Digestibility (IVO LD) (%)	39.98[a]	46.36[ab]	52.44[b]
Blood HDL Cholesterol (mg/dl)	0.288[a]	0.290[a]	0.192[b]

Note: Different superscripts in the same line show a significant difference (P<0.05). T1 = 40% forage + 60% Go Pro-fortified concentrate, T2 = 40% forage + 60% RUN-fortified concentrate, and T3 = 40% forage + 60% RUN & FMT-fortified concentrate.

The administered treatments consist of T1 (40% forage + 60% Go Pro-fortified concentrate), T2 (40% forage + 60% RUN-fortified concentrate), and T3 (40% forage + 60% RUN & FMT-fortified concentrate). T1 showed that microorganisms could utilize the slow-release nitrogen (Go Pro) for the microbial proliferation process, resulting in increased production of high biological value microbial protein. T2 showed that cows fed on nutrients with equal amount but different quality from that of T1, and supplemented with feed additives (specifically RPC and RPM) to increase their metabolic rate. T3, which was essentially T2 FMT supplementation, expected to enhance the supplementary effect by improving the digestive tract condition and intermediary metabolism and optimizing nutrient absorption.

Supporting Data

Supporting data are presented in Table 3.

Collection and Testing Sample

Blood samples were collected at the end of the study using a 20-cc syringe, then the blood was put into a 3 ml vacutainer EDTA tube. Blood hematologic status was analyzed using a veterinary 5-diff Hematology Analyzer (Weis and Wardrop, 2010).

Statistical Analysis

The data obtained were analyzed statistically using the analysis of variance method with the F test. Any significant effects at the 5% level would be subjected to Duncan test.

Results And Discussion

Hematology

Hematological profile data (Table 4) showed a normal status. Previous studies reported that normal hematologic status consists of 8.4-12.0 gr/dL hemoglobin & 21.0-48.0% hematocrit (Yang *et al*., 2022); 4.9-10 10^6/µL erythrocytes (Sofyan *et al*., 2020); 4.95-14.44 10^3/µL leukocytes & 8-487 10^3/µL platelets (Yang *et al*., 2022); 1.7-6 10^3/µL neutrophils & 0.1-1.2 10^3/µL eosinophil (Borges *et al*., 2014); 1.8-8.1 10^3/µL lymphocytes (Sofyan *et al*., 2020); 36.0-50.0 fL MCV, 16-20 RDW, & 4.6-9.7 fL MPV (Yang *et al*., 2022); 14.0-19.0 pg MCH (Sofyan *et al*., 2020); & 38,0-43,0% MCHC (Borges *et al*., 2014).

The normal hematological status values indicated that the treated cattle are in good health. Normal hematologic values suggest no disturbance in cattle health after treatment (Yang *et al*., 2022). Erythrocytes, hemoglobin and hematocrit level, which did not show significant differences across treatments illustrated that the use of the treatment feed did not disrupt the erythropoiesis process that occurred due to metabolic disorders and nutrient deficiencies (Gattermann *et al*., 2021).

Cattle fed on T3 ration containing FMT experienced an increase in leukocyte values (P<0.05) compared to cattle fed the T1 and T2 rations, indicating an immune response in cattle after FMT administration. The increase in leukocyte values was probably due to differences in lymphocyte values after FMT, which tend to be higher than other treatments. The elevation in lymphocytes is attributed to the increased fat digestibility and blood HDL cholesterol (Table 3). Increased feed fat digestibility and blood HDL cholesterol levels indicated a greater supply of unsaturated fatty acids in the body. Unsaturated fatty acids, such arachidonic fatty acids are the primary constituents of Eicosanoid compounds. Eicosanoids, such as prostaglandin E2 (PGE2) can inhibit the suppressor function of T-cells (Mougiakakos *et al*., 2010). The increased lymphocyte values represent a positive immune response and enhanced immunity and health of livestock, thereby optimizing livestock productivity. This is supported by the data of daily body weight gain in livestock given the T3 ration compared to T2 and T1. The elevated leukocyte value within normal limits illustrates that the livestock are still in good health (Yang *et al*., 2022). There was no difference in eosinophil values between cattle fed on T3 ration containing RUN + FMT and cattle fed on T2 ration containing only RUN. In other words, FMT does not negatively affect the health of cattle with fully-developed rumen. Also, feces used in making FMT might contain little or no pathogenic microorganisms. Eosinophil values that were not significantly different after FMT supplementation illustrated that FMT is safe for livestock and does not cause allergic reactions and parasitic infections (Klion *et al*., 2019; Nur *et al*., 2023). This also illustrates that the donor feces used in making FMT do not contain parasites that can interfere with the function of the digestive tract (Table 1).

Hematology values that remained within normal ranges illustrated that FMT did not incur negative effects on animal health. FMT can reduce or maintain the digestive tract, thereby enhancing nutrient absorption and reducing the entry harmful substances into body that could otherwise impact productivity and animal health (Kim *et al*., 2021). A healthy digestive tract and metabolic activity will keep hematologic levels within normal range.

Conclusion

The combination of Go Pro, RUN and FMT is feasible to maintain animal health and increase animal immunity. The combination of Go Pro and RUN can reduce substances that cause allergies, whereas FMT does not impose side effects, such as allergies and parasite infections.

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