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Abstract. This study aimed to determine the blood profile, pH value of the small intestine and nutrient digestibility of native chickens fed functional diets with different energy and protein levels. The study used 192 DOC of native chickens with rearing up to 14 weeks old. The study was designed in a factorial completely randomized design with two factors. The first factor was the energy level (E_1 = 2,800 kcal, E_2 = 3,000 kcal), and the second factor was the protein level (P_1 = 18%; P_2 = 19%; P_3 = 20%; P_4 = 21%), each treatment had 4 replications. The feed ingredients used as functional feed in the ingredients of the ration i.e. yellow corn, rice bran, fish meal, soybeans grain, moringa leaves flour, turmeric flour, and Topmix® with the feed composition according to the treatment applied. Blood sampling, measurement of pH duodenum segment of the small intestine, and measurement of nutrient digestibility were performed on chickens after 12 weeks of age. The variables observed were blood profile, small intestinal pH, and nutrient digestibility. Data were analyzed using analysis of variance according to the design applied. The results showed that the effect of interaction between energy and protein level had a non-significant effect ($P>0.05$) on blood profile (hemoglobin, leucocyte, erythrocytes, platelets), small intestine pH, and nutrient digestibility. Similarly, also found the influence of a single factor of each energy and protein level. However, the treatments influenced a significant effect ($P<0.05$) on hematocrit and blood bilirubin values, and a highly significant effect ($P<0.01$) on total blood cholesterol. The study concluded that energy levels and protein levels had influenced significantly on blood profile, but not in pH intestine and nutrient digestibility.

1. Introduction

The productivity of native chickens can be done by improving the quality and quantity of the feed. Good quality feed in poultry diets is that which contains nutrients based on the requirement of the life phase of its growth by taking into account the balance of protein and metabolic energy of feed. The balanced nutrient elements in the feed can provide optimal growth. In addition, the balance of nutrients that accelerate the rate of metabolism also by inclusion feed with contain phytobiotics. A functional feed is a type of feed formulation with certain phytobiotic content which aims to promote

growth and egg production in poultry. There is evidence that functional feed ingredients can improve the meat yield of broiler [1].

The process of nutrient absorption in digestive organs can affect the blood profile because the elements of nutrients that have been absorbed in the blood will be used by the body in the process of metabolism. Blood functions to carry oxygen and as a medium for transporting nutrients in the body. Blood consists of fluid in the form of plasma (55%) and solids (45%), the solid portion consists of erythrocytes, leukocytes, and platelets. Blood plasma contains protein, water, ions, gas, and the rest of metabolism. The water content in blood plasma is 91% and functions as thermoregulation in blood circulation [2]. The protein content in the diets can affect hemoglobin levels in the blood. Guyton and Hall [3] reported that protein components, especially amino acids, glycine, and Fe minerals are constituents of hemoglobin. Hemoglobin levels are influenced by oxygen and the number of erythrocytes in the blood. There is a tendency that if oxygen in the blood is low then the body is stimulated to increase the production of erythrocytes and hemoglobin [4].

In stress conditions, there is a decrease in the number of erythrocytes, hematocrit values, and hemoglobin levels while the number of leucocytes tends to increase [5]. These stressors can be influenced by feed and ambient temperature. High environmental temperatures can interfere with the process of homeostasis and metabolism, it can influence the normal function of chicken organs [6]. Stress conditions can cause interference with several physiological effects including blood profile and pH conditions in the small intestine so that it can affect the digestibility of nutrients in the digestive organs during metabolism.

Digestion is a proportional amount of nutrients absorbed in the digestive organs [7]. Measurement of digestive value depends on the activity that occurs in the digestive organs consisting of enzyme activity, absorption, and microflora activity [8]. The digestibility value of nutrients from the diets consumed is influenced by the crude fiber content of the ration and the percentage of protein of the feed as well as the amount of protein intake [9]. Besides that, digestibility is also influenced by the level of feeding, animal species, lignin content, nutrient deficiency, food processing, and disorders in the digestive organs [10]. This study aimed to determine the blood profile, nutrient digestibility, small intestine pH of native chickens fed different energy and protein levels in the diets.

2. Research methods

2.1. Experimental chicken

Experimental chickens used 192 DOC (day old chick) of native chicken (commercial ‘ayam kampung super’) from PT.Citra Lestari Farm, Surabaya, that rearing up to 14-week-old. The chickens were kept in a slat cage, the feeder and drinking water were placed inside the plot.

2.2. Experimental diets

The functional feed ingredients used in the diet formula consisted of yellow corn grain, rice bran, fish meal, soybeans, moringa leaves flour, turmeric flour, palm oil, and Topmix®. Proximate analysis of feed ingredients was analyzed using the AOAC method [11]. The composition of feed ingredients and diet formulations for each treatment are shown in table 1.

Table 1. Feed stuffs composition and nutrient contents of the treatments

Feed stuffs Composition	Treatments (EM_1 = 2,800 kcal)				Treatments (EM_2 = 3,000 kcal)			
	P_1	P_2	P_3	P_4	P_1	P_2	P_3	P_4
Yellow corn grain (%)	60	60	56.5	56	61	60.5	59	56.5
Rice bran (%)	13	11.5	12	11	8	8	7.5	7
Soybean grain (%)	10	10	12	10	14	11	11	14
Fish meal (%)	9	10.5	11.5	15	7	10.5	12.5	12.5
Moringa olievera Leaves flours (%)	5	5	5	5	5	5	5	5
Curcuma flours (%)	1	1	1	1	1	1	1	1
Coconut oil (%)	1	1	1	1	3	3	3	3
Top Mix (%)	1	1	1	1	1	1	1	1
Total	100	100	100	100	100	100	100	100
Nutrient Contents[1)]								
Energy Metabolism (kcal)	2815	2835	2830	2840	3006	3001	3006	3017
Protein (%)	18.25	19.00	20.15	21.33	18.19	19.07	20.10	21.02
Fat (%)	5.13	5.14	5.43	5.20	5.53	5.17	5.20	5.60
Fiber (%)	6.33	6.07	6.17	5.84	5.72	5.51	5.37	5.39
Ash (%)	5.24	5.29	5.58	5.92	4.45	4.87	5.12	5.17
Ca (%)	0.62	0.70	0.76	0.93	0.53	0.70	0.80	0.81
P (%)	0.66	0.68	0.71	0.78	0.55	0.64	0.69	0.68
Lysine (%)	0.75	0.81	0.86	1.00	0.65	0.80	0.88	0.89
Methionine (%)	0.32	0.34	0.35	0.40	0.28	0.33	0.36	0.36

[1]Calculated from proximate analysis of each feedstuff [12]. EM=metabolism energy

2.3. Experimental design and treatment

The study was designed using a completely randomized factorial design with 2 factors. The first factor was the metabolic energy level of the diets consisting of EM_1 (2,800 kcal) and EM_2 (3,000 kcal), the second factor was the protein level consisting of P_1= 18%; P_2= 19%; P_3= 20%; P_4= 21%, and each treatment received 4 replications. The treatment composition was as shown in table 1. Feed treatments were given to chickens aged 1–12 weeks old, and the samples for blood profile were taken at 12 weeks old, measurement of nutrient digestibility at 12–13 weeks old, and measurement of small intestinal pH at 13 weeks old.

2.4. Observed variables

The variables observed were blood profile, small intestine pH, and nutrient digestibility.

2.4.1. Blood profile. Blood samples were taken from each treatment group when chickens were 12 weeks old, with a total sample of 32 birds, and each treatment was taken 4 animals. Blood samples were taken from the axillary vein (wings) as much as 3 mL using a syringe tube which was then inserted into a vacutainer tube containing ethylene diamine tetra acid (EDTA) anticoagulation. Then it was taken to the Laboratory Health Central Sulawesi Province for analysis of blood profiles.

2.4.2. Small intestine pH. At 13 weeks old, 32 chicken samples fasted for 8 hours with water offered ad libitum. Bodyweight was recorded per chicken before slaughtered at jugular vein and carotid artery. After bleeding, scalding, plucking, and washing, the feet, head, and neck were removed. Then portions of the small intestine consisting of duodenum, jejunum, and ileum were collected. The contents of the small intestine were removed and collected in a measuring cup and 10 mL of distilled water as a solvent up to homogenized. The measurement of small intestine pH is done by dipping the cathode pH meter in the measuring cup and left 4–5 minutes and then reading the pH value.

2.4.3. Nutrient digestibility. Digestibility sample measurements used 32 chickens aged 12 weeks by taking 4 samples from each treatment. The experiment chickens were transferred to the individual

metabolism cages with the size of 35cm×35 cm×27.5 cm (length×width×height) for digestibility trial. The methods used were a total collection. The chickens were allowed 5 days adjustment period in the cage followed by 7 days of fecal sample collection. Samples were air-dried and cleaned to remove feather and other contaminants. Feces collected from each sample during 7 days were mix up to homogenized and taken to the laboratory for proximate analysis.

2.4.4. Data analysis. The data were analyzed with the analysis of variance (ANOVA) of the SPSS 16 application program according to the research design used on each variable obtained. Differences between treatment means were tested according to Duncan's multiple range test [13].

3. Results and discussion

3.1. Results

3.1.1. Blood profile. The effects of experimental diets on blood profile for each treatment were presented in table 2.

Table 2. Average blood profile for each treatment.

Blood Profile	Energy (kcal)	Protein (%)				P
		P_1	P_2	P_3	P_4	
Hemoglobin (g/00 mL)	EM_1	11.10[a]	8.70[b]	10.20[a]	10.55[a]	*
	EM_2	10.30	10.30	10.45	11.20	ns
Leucocyte ($10^3/mm^3$)	EM_1	130.06[a]	129.80[a]	113.90[b]	114.95[b]	*
	EM_2	124.10[a]	122.30[a]	107.40[b]	105.15[b]	*
Erythrocyte ($10^6/mm^{3)}$	EM_1	2,530.00[a]	1,940.00[b]	2,325.00[a]	2,330.00[a]	*
	EM_2	2,370.00	2,210.00	2,250.00	2,400.00	ns
Hematocrit (%)	EM_1	35.25[a]	26.45[b]	30.65[a]	31.05[a]	*
	EM_2	30.75[a]	30.55[a]	30.35[a]	32.60[a]	*
Platelet	EM_1	70,000.00[a]	70,500.00[a]	56,000.00[b]	53,500.00[b]	*
	EM_2	49,000.00[a]	43,000.00[b]	40,000.00[b]	36,500.00[b]	*
Total cholesterol	EM_1	121.00[a]	111.50[b]	125.50[a]	109.50[b]	**
	EM_2	124.50	128.00	124.00	119.50	ns
Total bilirubin	EM_1	0.26[a]	0.22[a]	0.42[b]	0.45[b]	*
	EM_2	0.43[a]	0.20[b]	0.58[a]	0.31[b]	*

[1)]Results of analysis at the Laboratory Health, Central Sulawesi Province
[*]= significantly (P<0.05); [**] = high significant (P<0.01); [ns] = non significant (P >0.05); [a,b] means with different superscripts in the same row are significantly different (P < 0.05) by Duncan's multiple range test; EM=metabolism energy

3.1.2. pH value of intestine. The general description of small intestine pH with the duodenum samples from each treatment was shown in table 3.

Table 3. Average pH in the small intestine of each treatment.

Energy (kcal)	Protein (%)				Mean	P
	P_1	P_2	P_3	P_4		
EM_1	6.25	5.96	6.11	6.07	6.10	**ns**
EM_2	6.11	6.24	6.09	6.07	6.13	**ns**
Mean	6.18	616	6.10	6.07		

[ns] non-significant difference (P>0.05); EM=metabolism energy

3.1.3. Nutrient digestibility. Measurement of nutrient digestibility is an attempt to determine the amount of feed that can be absorbed in the digestive organs. The results of the feed digestibility trials were shown in table 4.

Table 4. Average nutrient digestibility of each treatment.

Digestibility	Energy (kcal)	Protein (%)				P
		P_1	P_2	P_3	P_4	
Dry matter (%)	EM_1	83.61	80.33	79.98	80.08	ns
	EM_2	83.52	80.31	78.72	79.88	ns
Crude protein (%)	EM_1	73.34	74.44	73.54	74.20	ns
	EM_2	72.62	71.36	74.37	74.77	ns
Crude fat (%)	EM_1	93.64	88.95	88.21	91.39	ns
	EM_2	91.52	91.75	87.09	87.32	ns

ns =non significant (P>0.05), EM=metabolism energy

3.2. Discussion

3.2.1. Blood profile. The effect of interaction between energy and protein levels of blood profile was shown in table 2. There were no significant differences (P>0.05) on blood hemoglobin (Hb), leucocyte, erythrocyte, and platelets. However, the blood hematocrit and bilirubin were found a significant difference (P<0.05) and highly significant (P<0.01) on blood cholesterol. In the treatment of a single factor, the energy levels were shown no significant effect (P>0.05) on the Hb, erythrocytes, hematocrit, and cholesterol. However, found high significant effect (P<0.01) on leucocytes and platelet. The average level of leucocytes and platelets in the feed energy content of 2,800 kcal (EM_1) is 122,180/m^3 and 62.500, giving a significant difference (P<0.05) higher than the feed energy level of 3,000 kcal (EM_2), i.e 112,240/m^3 and 42,125. The leucocyte value in this study was higher than the normal range of 20,000–30,000/mm^3 [14]. Leucocytes are part of the body's defense system that can move after its formation, leucocytes enter the bloodstream and go to the parts of the body that need it. The high value of leucocytes in this study was probably caused by the chicken sample of the study under stress during blood collection. An increase in the number of leukocytes indicates that the body's high ability to respond to infections or foreign bodies. Soeharsono et al. [15] suggested that a high number of leukocytes indicates the body is able to fight infection.

The treatment of protein (single factor) showed no significant effect (P>0.05) on platelets. However, it had a significant effect (P<0.05) on Hb, hematocrit, cholesterol, and a highly significant effect (P>0.01) on leucocytes and erythrocytes. This study indicated that increased levels of protein in ration gives an indication of reducing levels of erythrocytes and leucocytes in super native chicken blood with a range of erythrocytes 1.94–2.75 (10^6/mm^3) and leukocytes 105.15–130.06 (10^3/mm^3) while the range normal according to Swenson and William [14], founded erythrocytes 2.50–3.20 (10^6/mm^3) and leukocytes 20,00–30,00 (10^3/mm^3). Ulupi and Ihwantoro [16] reported that erythrocyte profiles in chickens kept in open cages were 2.65±0.30 (native chickens) and 2.61±0.31 (commercial laying hens, respectively) while leukocyte levels were 22.24±6.11 (native chicken) and 27.34±4.88 (commercial laying hens). Factors that can affect the number of erythrocytes are the levels of nutrients in the feed [17]. In this study there was a trend of increasing levels of erythrocytes with increasing levels of protein but still within the normal range. The treatment that gave the highest levels of erythrocytes was 2.75 (10^6/mm^3) at a metabolic energy level of 2,800 kcal and protein of 21%. Hematocrit values are in the normal range of 26.45–35.25% while the normal range is 30–33% [14]. Martinez et al. [18] stated that laying pullets had blood cholesterol levels in the range of 102.85-113.59 mg/dL; hemoglobin (Hb) 102–107.50 g/L; hematocrit 0.33-0.36 µ/L. According to Akintomide

et al. [19], in finisher chicken blood phase PCV content (27.13–29.22%), RBC (2.24–2.56.106/mm^3), Hb (9.05–9.73 g/100 mL).

3.2.2. intestine pH. The effect of interaction between energy and protein levels on blood profile was presented in table 2. There were no significant differences (P>0.05) were found in the pH value of the intestine parameter. Similarly results also found in the treatment of energy and protein in a single factor. This study indicated that the pH of the small intestine is not affected by the energy levels and protein content of the feed. The pH value in each part was in the duodenum around 4.17–5.68; the jejunum section ranges from 5-6 and the ileum section ranges from 5.83–6. According to Gauthier [20], the normal digestive pH in each part of the small intestine is different, in the duodenum pH 5-6, jejunum 6.5–7, and ileum 7–7.5. Furthermore, Nanung et al. [21] reported that the pH of 43-day old broiler digestive organs, crop (4.94–5.28), proventriculus (2.84–3.79), jejunum (5.93–6.19), ileum (7.10–7.47), and caeca (96.79–6.83).

3.2.3. Nutrient digestibility. The effect of interaction between energy and protein levels on blood profile is shown in table 4. There were no significant differences (P>0.05) were found in dry matter, protein, and fat. Similarly results also found in the treatment of energy and protein in a single factor. These are probably influenced by the feed ingredients used in the ration formulation are the same. The average value of digestibility in super native chickens as a result of this study was: dry matter digestibility in the range of 78.72–83.61%; protein digestibility of 71.36–74.77%, and fat digestibility of 88.21–93.64%. According to Johnson et al. [22] that the average digestibility of broilers aged 21 days is dry matter digestibility of 62.00–63.6%; protein digestibility of 61.60–63.90%; fat digestibility of 85.80–90.10%. The occurrence of these differences may be caused by differences in animal treated and feed ingredients used. Another effect is also influenced by the level of feeding, animal species, lignin content, nutrient deficiency, feed processing, and disorders in the digestive tract organs [10]. Legawa et al. [23] reported that the digestibility of dry matter in male chickens was 86.29–91.12%. Whereas Kwari et al. [24] stated that the digestibility of feed in broiler chickens aged 5 weeks, i.e., dry matter digestibility of 87.34–89.56%, protein digestibility of 87.06–95.87%, fat digestibility of 83.65–92.95%, and crude fiber digestibility of 65.62–68.70%.

4. Conclusion and recommendation

This study concluded that the use of different energy and protein levels in the diets affected the blood profile (hematocrit, blood bilirubin, and total blood cholesterol). However, it did not affect the intestinal pH and digestibility of feed (dry matter, protein, and fat). The treatment that gave the best results from this study was the feed formula EM_1P_1 (EM content of 2,800 kcal and 18% protein). Recommendations from the results of this study are that in the growth rate of super native chickens, it is recommended to use a feed formula with an EM content of 2,800 kcal, with a protein content of 18%.

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