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Effect of dietary black soldier fly larvae *(Hermetia illucens)* and bioconversion product of cocoa pod husk on performance and hematological profile of sheep

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Abstract. Bioconversion of cocoa pod husk (CPH) is a biological processing technique using black soldier fly (BSF) larvae to improve the quality of the CPH while being able to produce added value in the form of BSF larvae as a protein source of animal feed. The aim of this study was to evaluate *in vivo* complete feeds containing BSF larvae and bioconversion product of CPH on performance and hematological profile of sheep. This study used a randomized complete block design (RCBD) with 5 treatments and 4 replications, so that total number of sheep used were 20 heads, conducted for 12 weeks period. The treatments were: R1 (complete feed, control without BSF and bioconverted CPH), R2 (5% BSF), R3 (20% bioconverted CPH), R4 (2.5% BSF + 10% bioconverted CPH), and R5 (5% BSF + 20% bioconverted CPH). The BSF and bioconverted CPH were used to replace soybean meal and coffee husk, respectively. Data were analyzed by analysis of variance and continued with a post-hoc Duncan's test. Results revealed that replacement of soybean meal with BSF larvae and replacement of coffee husk with bioconverted CPH in the complete feed did not significantly affect performance of sheep, i.e., live weight gain, feed consumption and feed conversion ratio). Furthermore, different dietary treatments showed similar response on hematological profile of sheep. It can be concluded that BSF larvae and bioconverted CPH may be used to replace soybean meal and coffee husk, respectively, without any adverse effects on performance and hematological profile of sheep.

1. Introduction

Indonesia is the third largest cocoa producer after Ghana and Ivory Coast. The total cocoa production in Indonesia reached 639,138 tons [1]. The composition of cocoa pod husk (CPH) is around 75% of the total weight of cocoa [2]. Based on this composition, CPH production is around 1,917,414 tons. An abundant of CPH production has the potential to be used as animal feed. Utilization of CPH as animal feed is generally not optimal [3]. The main problem of using CPH as animal feed is the high crude fiber of 12% -19% dry matter and low protein [4]. The CPH processing has not yet provided optimal value added.

Bioconversion of organic matter can convert separate or mixed waste into biomass rich in protein and fat by utilizing insect [5]. The use of insects as agents in the bioconversion process of CPH has not been evaluated to date. On the other hand, insect products as protein sources have been widely discussed by researchers in the world. Protein sources from insects are more economical and environmentally friendly [6].

Black soldier fly (BSF) is a type of insect that can be used in CPH bioconversion. These flies originated in America and subsequently spread to subtropical and tropical regions [7]. BSF larvae can convert large amounts of organic matter (up to 15 kg) per day [8]. Wardhana reported that BSF larvae are high-protein feed sources (40-50%), meanwhile, prepupae of BSF contain of 36–48% protein and 31–33% fat % [9-11]. The BSF larvae is very potential to be used as a protein supplement for ruminants. BSF Larvae ether extract (EE) so that it can supply essential fatty acids that livestock need. Although BSF larvae have low digestibility (dry matter digestibility 54-56% and organic matter digestibility 52-54%), BSF larvae have low methane emissions [12]. The bioconversion method using BSF can be applied to CPH. BSF larvae products can be a source of protein in ration formulations replacing fish meal or soybean meal [9]. Nutrient content is equivalent to fish meal and soybean meal. The use of BSF larvae flour has been applied as chicken feed [13] and fish feed [6].

So far, there is no research data on the use of BSF in bioconversion of CPH. Furthermore, to date, there is lack of experimental data that perform in vivo evaluation of combination between BSF larvae as a source of protein and CPH bioconversion product as a source of fiber in sheep's complete feed formulation. Therefore the aim of this study was to evaluate in vivo complete feeds containing BSF larvae and bioconversion product of CPH on performance and hematological profile of sheep.

2. Methods

2.1. Treatment

Twenty male sheep (6-8 months, BW=20.42 ± 3.57 kg were used in the present experiment. The experiment was used a randomized complete block design (RCBD) with 5 treatments and 4 replications, conducted for 12 weeks' period. The treatments were: R1 (complete feed, control without BSF and bioconverted CPH), R2 (5% BSF), R3 (20% bioconverted CPH), R4 (2.5% BSF + 10% bioconverted CPH), and R5 (5% BSF + 20% bioconverted CPH). The BSF and bioconverted CPH were used to replace soybean meal and coffee husk, respectively. Formulation of the experimental rations is presented in Table 1.

Sheep were kept in individual pens 1.5 × 1.0 m with free access to fresh water and consumption of ration was measured daily. The study consisted of 8 days of adaptation to the diets and 90 days of data collection. Animals were fed two equal portions at 08:00and 13:00 h daily. The sheep were measured their weight at the beginning (initial body weight, IBW), middle, and the end (final body weight, FBW) of the experiment, i.e., every month during the 3 months period. The total weight gain (TWG) was determined by the difference between IBW and FBW. Daily weight gain was determined by dividing the TGW by the duration of the treatment (90 days).

2.2. Blood collection

In the final day of the experiment, each sheep was restrained for blood collection. A pair of 5 ml blood samples were withdrawn into test tubes via jugular veinpuncture; one for haematological analysis and the other for leukocyte differentiation evaluation. The sterile test-tubes containing EDTA as anticoagulant were labelled in accordance to the corresponding animal number and then placed in a cooler bag containing ice cubes before being moved to the laboratory within an hour of collection for the determination of haematological parameters. Blood tubes without anticoagulant, were al-lowed to coagulate in kaylite holders at 37 °C for serum separation, centrifuged at 3500 rpm at 21 °C for 10 min and was stored at−20 °C. The hematological estimates including red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), and haematocrit (PCV). Data obtained were analyzed by analysis of variance and continued with a post-hoc Duncan's test.

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Table 1. Ingredients and composition (%) of the treatments diets.

Item	CP	R1		R2		R3		R4		R5	
	(%)	%	CP	%	CP	%	CP	%	CP	%	CP
Palm kernel meal	16	22.5	3.6	22.5	3.6	22.5	3.6	22.5	3.6	22.5	3.6
Coconut meal	26	5	1.3	5	1.3	5	1.3	5	1.3	5	1.3
Rice bran	9	10	0.9	10	0.9	10	0.9	10	0.9	10	0.9
Onggok	3	15	0.45	15	0.45	15	0.45	15	0.45	15	0.45
Molasses	3	5	0.15	5	0.15	5	0.15	5	0.15	5	0.15
Urea	280	0.5	1.4	0.5	1.4	0.5	1.4	0.5	1.4	0.5	1.4
Gaplek meal	3	15	0.45	15	0.45	15	0.45	15	0.45	15	0.45
Soybean meal	40	5	2	0	0	5	2	0	0	2.5	1
BSF larvae	39	0	0	5	1.95	0	0	5	1.95	2.5	0.98
Coffee husk	10	20	2	20	2	0	0	0	0	10	1
Bioconverted CPH	10	0	0	0	0	20	2	20	2	10	1
Calcium	0	1.5	0	1.5	0	1.5	0	1.5	0	1.5	0
Salt	0	0.5	0	0.5	0	0.5	0	0.5	0	0.5	0
Total		100	12.3	100	12,20	100	12,25	100	12,20	100	12.3

3. Results and discussion

3.1. Performance of sheep

After the ninety-day trial, changes in body weight, consumption and feed conversion ratio (FCR) were evaluated and the results were shown in Table 2. Diet treatments were not significant affecting on all parameters of performance (feed intake, final body weight, total gain, average daily increase, and (FCR).

Table 2. Effects of diet on sheep performance, weight gain, final body weight, feed consumption and Feed Conversion Ratio (FCR).

Variables	Ration Treatment (R)					SEM	P-Value
	R1	R2	R3	R4	R5		
Weight gain (kg/sheep) (month)							
1-2	3.75	4.15	2.97	3.73	3.27	0.26	0.265
2-3	4.22	3.60	3.15	5.50	4.37	0.36	0.082
1-3	7.97	6.40	6.12	9.23	7.65	0.52	0.109
Final body weight (kg/sheep)	29.40	31.45	25.27	31.60	27.75	1.00	0.087
Feed consumption (kg/sheep) (month)							
1-2							
2-3	28.05	27.16	27.90	31.83	27.72	1.08	0.271
1-3	35.52	31.10	29.62	36.00	33.07	1.14	0.137
	87.95	73.46	77.82	94.06	82.37	3.52	0.122
FCR (month)							
1-2	8.17	7.23	10.03	9.21	8.71	0.56	0.217
2-3	7.65	6.57	9.44	7.00	8.30	0.46	0.089
1-3	11.37	12.76	12.86	10.57	11.03	0.55	0.285

Substitution of 50% soybean meal by BSF larvae in the complete feed (R5) and substitution of 100% soybean meal by BSF larvae in the complete feed (R2) and (R4) were not significant effect on consumption, PBB and FCR. This shows that although the substitution of soybean meal by BSF larvae in ruminant diets generally results in a lower nutritional value *in vitro* [12] but it does not affect the *in vivo* test response to the parameters of consumption, PBB and FCR in sheep. Substitution of 50% coffee husk by cocoa bioconverted BSF larvae in the complete feed feed (R5) and substitution of 100% soybean meal by BSF larvae in the complete feed feed (R3) and (R4) do not have a significant effect on consumption, weight gain and FCR in sheep. These results indicate that both bioconverted CPH and

BSF larvae could partially replace coffee husk and soybean meal in the diet of sheep, respectively, without any negative effect on their performance.

3.2. Hematological parameter

The effects of diet on hematological of sheep was evaluated in this study and results are shown in Table 3 and Table 4. The RBC, Hb, and haematocrit showed similar responses among the dietary treatments and there were no significant differences among treatments (Table 3). The WBC were significantly difference among treatments ($P<0.05$). Component WBCs were also evaluated and results are shown in Table 4. All dietary treatments were not significantly affected leukocyte differentiation (lymphocytes, monocyte, neutrophils, eosinophils, and basophils). These also indicate that inclusion of bioconverted CPH and BSF larvae in sheep diets do not cause detrimental effects on their haematological profiles.

Table 3. Effect of diet treatment on red blood cells (RBC), white blood cells (WBC), hemaglobin and haematocrit (PVC).

Treatment	RBC	WBC	Hb	PCV
R1	15.62	13.07B	10.70	29.00
R2	15.10	13.15B	9.76	32.00
R3	14.56	15.43B	11.75	33.62
R4	15.97	8.36A	9.66	30.33
R5	15.69	8.71A	10.97	34.00
SEM	0.43	0.77	0.28	0.96
P-value	0.415	0.001	0.120	0.411

Table 4. Effect of diet treatment on sheep blood leukocyte differentiation.

Treatment	Lymphocytes	Monocyte	Neutrophils	Eosinophils	Basophils
R1	55.75	1.82	36.48	5.11	0.81
R2	48.23	1.84	42.71	6.52	0.69
R3	52.60	2.00	40.43	4.14	0.81
R4	67.00	2.75	25.89	3.53	0.02
R5	58.18	1.62	34.03	5.37	0.79
SEM	2.97	0.19	3.00	0.60	0.01
P-value	0.469	0.529	0.557	0.690	0.277

4. Conclusions

BSF larvae and bioconverted CPH may be used to replace soybean meal and coffee husk, respectively, without any adverse effects on performance and hematological profile of sheep.

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