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EFFECT OF COMBINATION OF *Indigofera zollingeriana,* BLACK SOLDIER FLY LARVAE, AND TURMERIC ON PERFORMANCE AND HISTOMORPHOLOGICAL CHARACTERISTICS OF NATIVE CHICKEN AT STARTER PHASE

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RESEARCH ARTICLE

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ABSTRACT: Sources of high protein feed ingredients can come from plants and animals (insects), namely *Indigofera zollingeriana syn.* and black soldier fly larvae. The addition of natural feed additives to feed can be obtained from turmeric phytobiotics which have many biological activities, such as anticancer, anti-inflammatory, antimicrobial and antioxidant. This study aimed to determine the effect of the combination of *Indigofera zollingeriana syn.*, black soldier fly (BSF) larvae and turmeric on the performance and histomorphology of the bursa Fabricius in the native chicken starter phase. The research design was completely randomized with 3 treatments and 5 replications with 6 chickens per unit. The combination treatments were P0 (Commercial feed as control); P1 (5% *Indigofera* flour + 25% BSF larvae flour + 2.5% turmeric flour) and P2 (10% *Indigofera* flour + 20% BSF larvae flour + 2.5% turmeric flour). Parameters measured in this study were performance (body weight gain, feed consumption, FCR) and bursa of fabricius histomorphology in native chickens. The results of the analysis of variance showed that the combination of *Indigofera zollingeriana syn.*, BSF larvae and turmeric had a significant effect on the performance of native chickens but could not match the performance of P0 (control feed). While the histomorphology of bursa Fabricius showed that the combination of *Indigofera zolliengeriana* flour up to 10% and 25% black soldier fly larvae flour in the feed identified the medulla width, cortex thickness and follicle width can increase lymphocyte cells to produce antibodies for native chickens at starter phase.

Keywords: Feed additives, *Indigofera zoillingeriana*, Insect, Native chicken, Larvae

INTRODUCTION

In native farming, to achieve fast growth and high productivity, it is necessary to feed that contains the needed nutrients and alternative feedstuffs (Ogbuewu et al., 2017; Truong et al., 2019). According to Varianti et al. (2017) that the quality of poultry feed is mainly estimated from the protein content (the higher and complete the amino acid content).

The protein content of the feed is related to growth rate because protein is used to form new tissue, maintain existing tissue and replace damaged tissue (Post and Hocquette, 2017). The protein content of feed has an important role in increasing protein consumption in chickens, according to Saputro et al., (2016) explaining if low protein consumption causes antibodies to be formed slightly so that the physiological function of chickens is disturbed, they are susceptible to diseases that affect the development of the bursa of Fabricius organs in chickens.

One source of herbal feed ingredients derived from plants is *Indigofera zollingeriana syn.* which is rich in nutrients; especially crude protein of 27.9% and 27.68% (Abdullah, 2010). Muhammad et al. (2021) stated that the substitution of soybean meal with *Indigofera zollingeriana syn.* up to a level of 15% (Soybean meal substitution PK = 4.23%) + 2.5 turmeric was able to increase the commercial cut of carcass and the percentage of the weight of the digestive tract of native chicken in the grower phase.

Another source of alternative feed ingredients derived from insect species is black soldier fly (BSF) larvae (*Hermetia illucens*) with a protein content of 40 - 50% (Lu et al., 2022). BSF larvae have a high lauric acid content and can function as a natural antimicrobial agent (Kim and Rhee, 2016). BSF larvae extract or maggot has inhibitory activity against the Gram-negative bacteria group (Sundari et al., 2022). Antimicrobial activity is very influential on the health and development of digestive tract organs and lymphoid organs (Derthi, 2012). Increasing protein consumption in native chickens can be done by using a natural feed additive made from herbs, namely curcumin. Curcumin is an active ingredient that is derived from turmeric phytobiotics, which have a wide range of biological benefits, including antioxidant, anti-inflammatory, anti-cancer, and anti-inflammatory properties (Araújo and Leon, 2001). Histopathological examinations of lymphoid organs after injection of Salmonella pullorum and *E. coli* showed that the use of turmeric at a level of 2.5% was able to maintain performance, internal organs and lymphoid organs in good condition (Purwanti et al, 2019).

This study aimed to determine the effect of the combination of *Indigofera zollingeriana syn*, black soldier fly (BSF) larvae and turmeric on the performance (feed consumption, weight gain and feed conversion ratio) and histomorphology of the bursa Fabricius in the native chicken starter phase.

Citation: Muhammad LN, Purwanti S, Pakiding W, Marhamah, Nurhayu, Prahesti KI, Sirajuddin SN and Mushawwir A (2023). Effect of combination of *Indigofera zollingeriana,* black soldier fly larvae, and turmeric on performance and histomorphological characterizes of native chicken at starter phase. *Online J. Anim. Feed Res*., 13(4): 279-285. DOI: https://dx.doi.org/10.51227/ojafr.2023.42

MATERIALS AND METHODS

From June to August 2022, at the Poultry Production Laboratory, Faculty of Animal Science, Hasanuddin University, South Sulawesi, Indonesia, 90 native chickens with the same body weight were randomly divided into 15 units for three treatments and five replications. First of all, the maintenance of native chickens in this study was through a brooding period of 100 aged chicks 1 day for 7 days. Domestic chicken rearing from day 8 to day 28 is the starter phase and day 29 to day 56 is the grower phase. The treatments groups consisted of P0 (Commercial feed as control); P1 (5% *Indigofera* flour + 25% BSF larvae flour + 2.5% turmeric flour) and P2 (10% *Indigofera* flour + 20% BSF larvae flour + 2.5% turmeric flour). The research began with the preparation of *Indigofera zollingeriana* shoot flour, the manufacture of turmeric flour and the manufacture of BSF (*Hermetia illucens*) flour. During maintenance, feed and drinking water were provided *ad libitum* based on treatment. The composition of the feed nutrients used during this study is in Table 1.

The parameters measured in this study were the performance and histomorphology of the bursa of the Fabricius organ of native chicken. Native chicken performance consists of feed consumption obtained from weighing the remaining feed given minus the remaining feed, weight gain can be obtained from the difference in body weight of chickens and feed conversion is obtained from the amount of feed consumed at a certain time divided by weight gain. The bursa of the Fabricius organ was obtained by slaughtering and cutting the samples in the starter phase (4 weeks) in each treatment. Slaughtering of chickens is done at the base of the neck by severing the respiratory tract (trachea), the feeding tube (esophagus) and the two neck veins (blood vessels on the right and left of the neck) with a single incision without lifting the knife. The slaughter process is done from the front of the neck and does not break the neck bone. According to Masyitha and Budiman (2017) the histological description of the bursa Fabricius includes the cortex, medulla, number, and diameter of follicles as well as the epithelial zone.

Table 1 – Nutrient composition

Ingredients	ME (Kkal/Kg)	CP (%)	EE (%)	CF%)	Lysine (%)	Methionine (%)	P (%)	Ca (%)
Yellow Corn	3291.27	9.88	1.79	5.70	0.06	0.18	0.60	0.02
Rice Bran	2730.00	13.40	5.10	11.50	0.42	0.30	2.50	0.20
Coconut meal	1525.00	16.00	15.00	16.00	0	0	0.75	0.30
BSF larvae	3596.40	46.14	21.88	13.12	0	0.00	0.93	1.28
Indigofera zollingeriana	2617.41	36.18	4.74	11.11	2.05	0.67	0.58	0.13
Cassava Flour	3200.00	2.00	12.70	11.40	0.07	0.01	0.40	0.33
Rice polish	1451.85	10.60	13.66	27.80	0	0	1.48	0.05
Heap	3500.00	1.88	15.62	0.25	0	0	0.05	0.31
DCP	0	0	0	0	0	0	21.00	16.00
$CaCO_3$	0	0	0	0	0	0	0.04	39.00
Lysine	0	0	0	0	99	0	0	0
Methionine	0	0	0	0	0	99	0	0

Turmeric flour as much as 2.50% as a feed additive (Purwanti et al., 2014). The Result Laboratorium of Livestock Biotechnology Integrated Laboratory Test, 2022.

Table 2 - Feed ingredients composition and nutrients value in the starter phase of the feed

Ingredients	P0	P1	P2
Yellow corn	Commercial Feed as feed control	47	46
Rice Bran		12.4	14.9
Coconut meal		6	4.5
NaCl		0.5	0.5
Premix		1.5	1.5
BSF larvae		25	20
Indigofera		5	10
DCP		1	1
CaCO3*		1	1
L-Lysin		0.3	0.3
DL-Metionin		0.3	0.3
Nutrient Composition			
ME (Kkal/Kg)	-	3006.89	2970.4
Crude Protein (%)	20	20.97	20.47
Crude Fat (%)	5	8.08	7.11
Crude Fiber (%)	5	8.9	8.79
Lysin (%)	1.2	0.48	0.59
Metionin (%)	0.45	0.45	0.49
P (%)	0.5	1.11	1.14
Ca (%)	0.6	0.93	0.87

Calculated based on the results of data analysis and calculation Table 1. P0 (Commercial feed as control); P1 (5% *Indigofera* flour + 25% BSF larvae flour + 2.5% turmeric flour) and P2 (10% *Indigofera* flour + 20% BSF larvae flour + 2.5% turmeric flour).

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Ethical regulation

Maintenance management in this study refers to the Australian Animal Welfare Standards and Guidelines for Poultry and slaughtering native chickens in this study refers to the Indonesian National Standard (INS) number 99002 of 2016 concerning halal slaughtering of poultry.

Statistical analysis

The research design was carried out using a Completely Randomized Design (CRD) with a significance level of 5%. If the data is significantly different at the P<0.05 on the measured parameters, then the differences between treatments were analyzed by an orthogonal contrast test.

RESULTS AND DISCUSSION

The results of the study on the combination of *Indigofera zollingeriana syn,* BSF larvae and turmeric on the performance of native chickens can be seen in table 2. The data in table 2 shows a significant effect (P<0.05), then proceed with further contrast tests which can be presented in table 3.

Table 3– Performance of native chicken fed a combination of *Indigofera zollingeriana*, larvae BSF and turmeric in the ration

Parameter \ Treatment	P0	P1	P2
Weight gain (g/bird/day)	9.47 ± 0.20	4.27 ± 0.29	3.46 ± 0.37
Feed consumption (g/bird/day)	18.32 ± 0.36	13.11 ± 0.56	11.41 ± 1.19
FCR	1.94 ± 0.05	3.08 ± 0.10	3.30 ± 0.04

P0 (Commercial feed as control); P1 (5% *Indigofera* flour + 25% BSF larvae flour + 2.5% turmeric flour) and P2 (10% *Indigofera* flour + 20% BSF larvae flour + 2.5% turmeric flour).

Table 4– Performance of native chicken (orthogonal contrast test)

Parameter		Significant
Weight gain	P0 Vs P1 and P2	114.289*
	P1 Vs P2	18.698*
Feed consumption	P0 Vs P1 and P2	118.227*
	P1 Vs P2	11.485*
FCR	P0 Vs P1 and P2	1186.18*
	P1 Vs P2	0.001^{ns}

*significant. ^{ns} non significant

Feed consumption

Based on Table 1 on feed consumption during the study were obtained from the average consumption of native chickens during the rearing phase. Feed consumption in the starter phase of native chicken in treatment P0 which was 18.32 grams/bird/day was higher than that of treatment P1 and P2. Namely 13.11 and 11.41 grams/bird/day. Further tests showed feed consumption in the starter phase, namely P0 Vs P1 and P2 showed a significant effect and P1 Vs P2 also showed a significant effect. This was because the P1 and P2 feed contained *Indigofera zollingeriana syn.* and BSF mangosteen which had high crude fiber content compared to the P0 treatment (control feed). The higher the crude fiber content, the faster the digesta rate, the shorter the digestion process in the digestive tract so that a lot of nutrients are lost to faces or excreta. This is in line with the opinion of Supriyanto et al. (2021) who reported that high crude fibre feed prevents chicks from utilizing nutritional feed properly and undigested crude fibre will carry nutrients out with faeces or excreta. Feeds that contain high crude fibre cannot be digested completely and will cause a full fast cache which results in limited feed consumption. The high crude fibre content in the ration will make the chickens feel full quickly because the crude fibre is bulky and will expand when exposed to water Supriadi et al. (2021). BSF flour contains high fibre so it can cause clumping of feed. Therefore, birds become full quickly and reduce the level of feed consumption (Rahayu et al. 2021).

Weight Gain

The increase in body weight gain is influenced by the type of feed given to the chickens. Table 2 shows that the body weight gain of treatment P0 (9.47 grams/bird/day) was much higher than that of treatment P1 (4.27 grams/head/day) and P2 (3.46 grams/head/day). This was due to the low level of feed consumption of P1 and P2 obtained during the maintenance of Native chickens in the starter phase. This is in line with the opinion of Nugraha et al. (2017) reported that body weight gain is closely related to feeding, in terms of quantity related to feeding consumption if feed consumption is disturbed it will interfere with growth.

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Further tests showed body weight gain in the starter phase, namely P0 Vs P1 and P2 showed a significant effect (P<0.05) and P1 Vs P2 also showed a significant effect (P<0.05). This is because the balance of energy metabolism and protein in the P0, P1 and P2 feed treatments is not much different, ranging from 143.39% - 145.10%. This is the opinion of Allama et al. (2012) who reported that the balance of food substances, especially protein and energy, is very important because it significantly affects the speed of body weight gain. The balance of energy and protein is intended to meet the minimum protein requirement because a lack of energy will convert protein into energy.

Chickens that received feeds with high energy and protein became efficient in changing feed to increase body weight gain, while chickens that received feeds with lower energy and protein were less efficient in using the feed to increase body weight gain (Eriko and Nur, 2015) According to Rahayu et al. (2021), protein plays an important role in chicken growth, because it contains essential and non-essential amino acids.

Feed conversion ratio

The relationship between feed consumption and body weight gain is determined by feed conversion. Low feed conversion values indicate better feed use efficiency. This means that the chickens are more efficient in consuming feed for meat production (Allama et al., 2022). Feed Conversion Ratio (FCR) in the starter phase showed that the mean of treatment P1 (3.08) and P2 (3.30) was higher than treatment P0 (1.94). Further tests showed that the FCR in the starter phase, namely P0 Vs P1 and P2, showed a significant effect (P<0.05) while P1 Vs P2 also showed a significantly different effect (P>0.05). The results of this study are different from Wahid et al. (2021) study which concluded that the substitution of fish meal with BSF maggot flour in native chickens was 25% FCR obtained by 4.62% while Supriadi et al. (2021) study reported that the use of 10% *Indigofera zollingeriana syn*. flour in chicken feed the village FCR obtained is 2.65. The difference in FCR in these three studies was due to the difference in feed consumption and body weight gain of each study, this was influenced by the different breeds of Native chickens used. This is in line with the opinion of Herlina et al. (2015) who said that the things that affect the consumption of rations are the breed of chickens, environmental temperature, production stage and energy in the ration. Feed conversion is closely related to feeding consumption and body weight gain.

Histomorphology of bursa Fabricius chicken native starter phase

The bursa of Fabricius is the main lymphoid organ in avian species. The bursa of Fabricius in birds has an important role as a central lymphoid organ for B lymphocyte differentiation. In addition, the bursa has B lymphocytes that have immune competence capable of producing local antibodies (Wu et al., 2013). The results of the histological study of the bursa of Fabricius of native chicken in the starter phase in each treatment can be seen in Figures 1, 2 and 3.

The bursa of Fabricius has follicles consisting of a medulla and cortex, according to Madej et al. (2013) saying that the development of the bursa of Fabricius occurs through the formation and colonization of the medulla during the embryonic stage, while the cortex develops after hatching. The figure below shows that the medulla in the P0 treatment was wider than in the P1 treatment but the medulla in the P2 treatment was wider than in the P0 treatment. This shows that there are more lymphocyte cells in P2 treatment compared to other treatments, this is in accordance with the opinion of Ebru et al. (2015) who reportede that the medulla only contains lymphocyte cells, these lymphocyte cells function to produce antibodies in chickens. The area of the medulla in the P2 treatment was influenced by the turmeric content in the ration, in the opinion of Nasrullah et al. (2020) saying that giving turmeric to chickens was thought to affect the increase in the number of lymphocytes. Curcumin has the ability to activate T lymphocyte cells and B lymphocyte cells which are part of lymphocytes.

The histological cortex of the bursa of Fabricius of native chicken in the starter phase showed that the P1 and P2 treatments were thicker than the control diet group. The width of the histological bursa follicles of native chickens in the starter phase showed that P1 and P2 were wider than those of the control diet. Ismiraj (2020) reported that most cell division occurs in the cortex, which the cortex consists of lymphocytes, plasma cells, and macrophages. In addition, Selim et al. (2021) reported that the width of the follicle which is higher than the bursa indicates an increase in the number of B lymphocytes and the formation of B lymphocytes for antibodies. The number of B lymphocytes in the treatment increased P1 and P2 due to the addition of immunomodulatory substances, namely *Indigofera zollingeriana syn*, black soldier fly larvae and turmeric. According to Sulistiyanto et al. (2019), an imunomodulator is a chemical substance, drug, or action of an immune system that can increase the body's immune system.

The results of the study on the number and diameter of the bursa of Fabricius follicles in the starter phase of native chickens can be seen in Table 4. The data in Table 4 shows a significant effect (P<0.05), then continued with the contrast test which can be seen in Table 5. The bursa of Fabricius has follicles consisting of a cortex and a medulla, each of which contains B lymphocytes. Aged B lymphocytes are released into the blood vessels to boost immunity. This release can be induced to produce optimum immunity. One way to generate immunity in chickens is to provide local/herbal feeds such as *Indigofera zollingeriana syn*, larvae BSF and turmeric. Table 3 shows the results of the calculation of the number of follicles in treatment P2 (41.87) which was higher than in treatment P0 (38.80) and P1 (31.40). The diameter of the follicles in the bursa can be seen in Table 3. It shows that the P2 treatment was higher than the P0 and P1 treatments. Further tests showed that the diameter of the follicles P0 Vs P1 and P2 showed a significant effect (P<0.05) and P1 Vs P2 also showed a significant effect (P<0.05). This is due to the content possessed by the combination of *Indigofera zollingeriana syn*, larvae BSF and turmeric which can help the growth of the bursa of Fabricius. *Indigofera zollingeriana*

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syn. contains anti-nutritional substances in the form of tannins which function as antibacterial (Azis et al., 2019). Larvae BSF has a high lauric acid content and can function as a natural antimicrobial agent (Kim and Rhee, 2016). Turmeric has many biological activities, such as anticancer, anti-inflammatory, antimicrobial and antioxidant (Araújo and Leon, 2001).

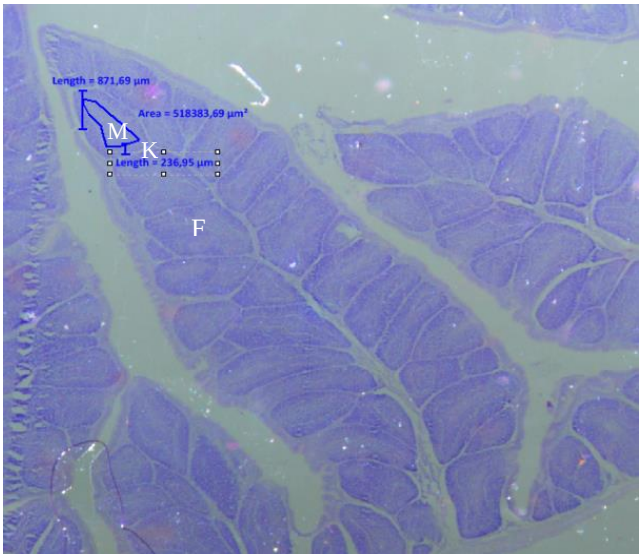

Figure 1 - Bursa Fabricius Feed Treatment P0. M = Medula; K = Corteks; F = Follicle

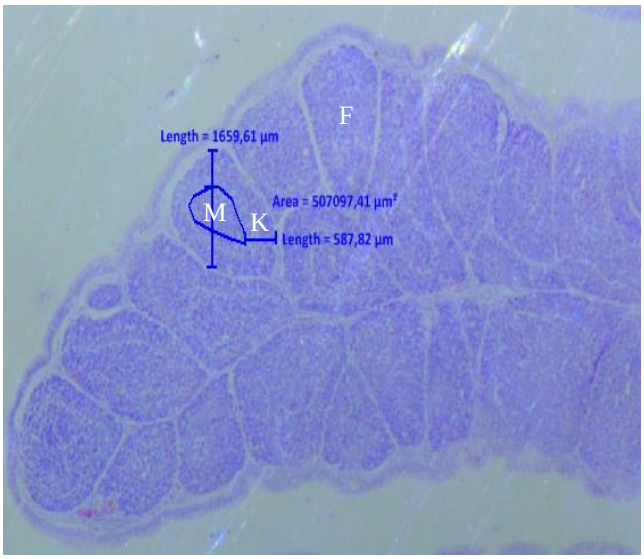

Figure 2 - Bursa Fabricius Feed Treatment P01. M = Medula; K = Corteks; F = Follicle

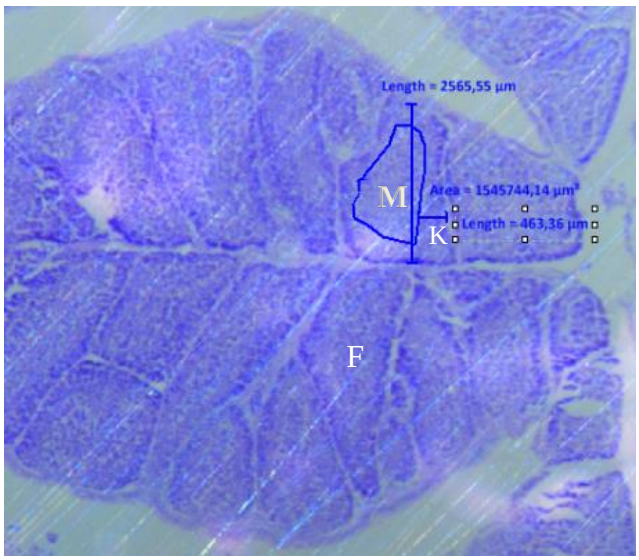

Figure 3 - Bursa Fabricius Feed Treatment P02. M = Medula; K = Corteks; F = Follicle

Table 5– The number and diameter of the bursa Fabricius follicle in the starter phase of native chicken

Parameter \ Treatment	P0	P1	P2
Number of Follicle	38.80 ±5.26	31.40 ± 12.25	41.87 ± 23.70
Follicle Diameter	1063.46 ± 219.63	1566.36 ± 221.83	1717.59 ± 489.97

P0 (Commercial feed as control); P1 (5% *Indigofera* flour + 25% BSF larvae flour + 2.5% turmeric flour) and P2 (10% *Indigofera* flour + 20% BSF larvae flour + 2.5% turmeric flour).

Table 6– Orthogonal contrast test of chicken native follicle diameter starter phase

Parameter	Folicle Diameter
P0 Vs P1 and P2	9.91*
P1 Vs P2	8.88*

*significant. ns non significant

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CONCLUSION

The combination of *Indigofera zollingeriana syn.* flour up to 10% and 25% black soldier fly larvae flour in feed did not produce native chicken performance (feed consumption, body weight gain and FCR) which was equivalent to commercial feed as a control. While the histomorphology of bursa Fabricius showed that the combination of *Indigofera zolliengeriana syn* flour up to 10% and 25% black soldier fly larvae flour in the feed identified the medulla width, cortex thickness and follicle width can increase lymphocyte cells to produce antibodies for Native chickens at starter phase.

It is necessary to be conducted trials on native chickens of other breeds to see the functionality of the prepared feed.

DECLARATIONS

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Authors' contribution

All authors contributed equally to this research work. All authors read and approved the final manuscript

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