Utilization of *Black Soldier Fly* larvae juice as a probiotic supplement for broiler chickens: effect on performance, immunity, haematology, and economic feasibility

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Abstract. Currently, various types of feed additives are being explored as alternatives to growth-promoting antibiotics, and larvae juice is emerging as a promising candidate. This study aimed to evaluate the potential of microorganisms contained in larvae juice as probiotics and determine the optimal dose for male broiler chickens. A total of 250 male Cobb broiler chickens were randomly divided into five treatments with five replications, each containing 10 chickens placed in separate cages. The treatments given included: positive control (PC), negative control (NC), larvae juice 1 mL/L (LJ1), larvae juice 3 mL/L (LJ3), larvae juice 5 mL/L (LJ5). Analysis revealed the predominance of gram-positive bacteria, such as *Bacillus* and *Actinomyces*, in the larvae juice probiotics. Giving probiotic larvae juice at a dose of 5 mL/L in drinking water significantly increases body weight and average daily gain, thereby providing a positive effect on saving feed costs.

In conclusion dosage 5 mL/L are considered safe for broilers and do not adversely affect their physiological vital organs, immune system, digestive organs, or blood profile.

1 Introduction

The efficiency of feed nutrient absorption can be optimized by adding feed additives to the ration for increase feed utilization. Feed additives that are often used are antibiotics growth promotor, on the other side, there are residues in livestock products that can harm consumers and cause antibiotic resistance [1]. A ban on its use is imposed based on Minister of Agriculture Regulation Number 14 of 2017 concerning Classification of Animal Medicines and the mandate of Law Number 18 of 2019 concerning Animal Husbandry and Animal Health [2].

Currently, many types of feed additives are used as an alternative to growth promotor antibiotics and one potential feed additive is probiotics. The efficiency of using maggots or *Hermetia illucens* as an additional ingredient in poultry feed is still being developed. Maggots contains polypeptides which can inhibit gram-negative bacteria and yeast [3]. Cecropin and defensin are two types of active peptides from maggots which have the highest concentration

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and activity. Cecropin inhibits the growth of gram-negative bacteria and defensins play a role in the humoral immune response [4,5,6].

Studies on the use of *Hermetia illucens* as a probiotic show the potential of lactic acid bacteria from maggots which play a role in suppressing the growth of pathogenic bacteria such as *Escherichia coli* and are indicated to have the ability to stick and colonize surfaces animal intestines as one of the requirements for probiotics [7]. Based on several studies [3,4,5,6,7], the diversification of maggot products, one of which is larvae juice, has the potential to be used as a feed additive containing positive bacteria that can help the digestion of broiler chickens. Therefore, this study aimed to evaluate the potential of microorganisms contained in larvae juice as probiotics and determine the optimal dose for male broiler chickens.

2 Materials and methods

This study continued the in vitro step experimental by Fitri et al. [8], where larvae juice was evaluated with in vivo experimental. This research was carried out from February to March 2023 at the IPB-Pokphand close-house research. Probiotic larvae juice was produced by PT Bio Cycle Indo, which is made from the extraction of fresh maggot using a pressing method to collect the larvae juice for the filtration process. Filtrate larvae juice added effective microorganisms and anaerobic fermentation was carried out for 40 days. The juice from fermentation was filtered and packed. Metagenomic molecular analysis was done to identify positive microorganisms in larvae juice at Indonesia Genetica Science Laboratory.

In vivo probiotic treatments added to drinking water, there are 5 treatments PC (positive control = without probiotic); NC (negative control = probiotic commercial 1 g/L); LJ1 (probiotic BSF larvae juice 1 mL/L); LJ3 (probiotic BSF larvae juice 3 mL/L); LJ5 (probiotic BSF larvae juice 5 mL/L). This study used 250 male broiler chicken strain Cobb with code CP 707 grade A (was vaccinated with Newcastle Disease). Rearing was doing for 32 days (7 days adaptation 25 days treatment).

During the experimental period all broiler fed with the same feed from PT Charoen Pokphand Indonesia with code (crumble BR11+) starter phase (1-21 days) and finisher phase (pellet BR12) (22-32 days). Drink water was given twice per day at 06.00 am and 04.00 pm. This study experimental procedure approved according guidelines for the care and using of animals as established by animal welfare and experimentation ethic committee IPB University which number 015/KEH/SKE/II/2023.

Measurement feed and water consumption of broilers were taken daily by weighing the provided and remaining. Broiler performance was assessed by measuring body weight gain final body weight, mortality and calculating the feed conversion ratio. Blood, vital organs (hearth, kidney, and liver), and immunity organs (bursa of fabricius, spleen, and lymph) samples were taken when the broiler aged 32 days in the morning. Haematology profile analysis using the Sastradipradja and Hartini method [9]. Cost analysis includes feed cost (starter and finisher), probiotic cost, gross income, income over feed cost, and index performance.

Experimental design used in this study is Completely Randomized Design (CRD), the differences among treatment analysis by Duncan's. The data processing was performed using IBM SPSS Statistics version 26, the significant differences were found between treatments, a further test, specifically Duncan’s post-hoc test, was utilized [10].

3 Results

3.1 Identification microorganisms of larvae juice

Identification of positive microorganism in larva juice by metagenomic by sequencing DNA based on 16S gene region reached a species and strain-level identification, which were later identified as *Bacilli* (Figure 1) and *Actinomycetia* (Figure 2) present in the results (Table 1).

Table 1. Identification positive microorganisms of larvae juice

Name	Tax rank	Tax ID	Max	Read clasifications kraken clade reads	Max 1	Read clasifications kraken taxon reads
Bacilli	Class	91061	19743	19743	4	4
Actinomycetia	Class	1760	1200	1200	76	76

Figure 1. Species of *Bacilli* in larvae juice

The *Bacilli* identified in larvae juice consists of several types of species that's *Bacillales* (*Paenibacillus* 23%, *Pullulanibacillus* 8%, *Caldibacillus* 6%, *Weizmannia* 4%, *Heyndrickxia* 3%, *Tuberibacillus* 1%, *Cohnella* 0.9%, *Scopulibacillus* 0.8%, *Bacillus* 0.8%, *Margalitia* 0.8%) and *Lactobacillales* (*Ligilactobacillus* 19%, *Lactiplantibacillus* 16%, *Lactecaseibacillus* 5%, *Lactobacillus* 3%, *Pediococcus* 2%, *Weissella* 2%). Another positive microorganism is *Actynomycetia* (*Catenulisporates* 0.5%, *Jiangellaceae* 0.3%, *Micromonosporaceae* 0.3%, *Bifidobacteriaceae* 0.1%).

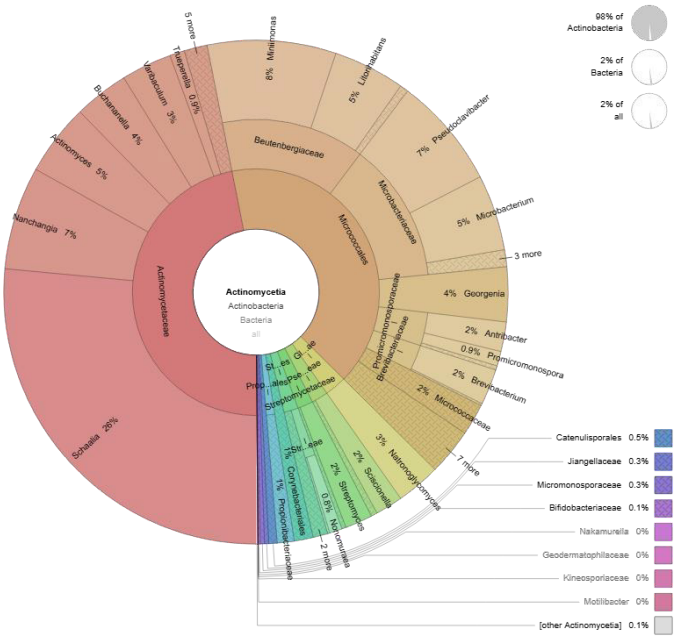

Figure 2. Species of *Actinomycetia* in larvae juice

3.2 Broilers performance at 32 days

Analysis of the variety in broiler performance showed that treatment did not significantly ($p>0.05$) affect water consumption, feed consumption, and FCR, but significantly affect body weight gain and final weight ($p<0.05$, Table 2).

Table 2. Broilers performance at 32 days

Parameters	PC	NC	LJ1	LJ3	LJ5
Water consumption (g/chick)	4609.49 ± 304.72	4291.00 ± 157.33	4602.57 ± 198.38	4309.98 ± 432.48	4608.43 ± 165.15
Feed consumption (g/chick)	3028.93 ± 137.63	2895.09 ± 149.10	2928.88 ± 151.29	2960.00 ± 172.90	3024.47 ± 85.62
Body weight gain (g/chick)	1783.48 ± 75.93^{b}	1706.95 ± 115.20^{b}	1711.34 ± 164.34^{b}	1712.28 ± 65.03^{b}	1952.57± 125.19^{a}
Final body weight gain (g/chick)	1942 ± 79.42^{b}	1863 ± 111.50^{b}	1894 ± 116.10^{b}	1874 ± 61.18^{b}	2112 ± 125.45^{a}
FCR	1.70 ± 0.08	1.70 ± 0.10	1.73 ± 0.21	1.73 ± 0.16	1.55 ± 0.12
Mortality (%)	0	0	0.4	0	0.4

PC = drinking water without probiotic; NC = drinking water with commercial probiotic 1 g/L; LJ1 = drinking water with probiotic larvae juice 1 mL/L; LJ3 = drinking water with probiotic larvae juice 3 mL/L; LJ5 = drinking water with probiotic larvae juice 5 mL/L. FCR = feed conversion ratio.

3.3 Vital organs

The percentage of internal organs observed is the heart, liver and kidneys (Table 3), which are vital organs that are sensitive to toxic and anti-nutritional substances. Measurement of

heart, liver and kidney organs in broiler chickens based on the results of analysis did not show a difference ($p>0.05$).

Table 3. Percentage organ weight of broilers at 32 days

Parameters (%)	PC	NC	LJ1	LJ3	LJ5
Hearth	0.52 ± 0.04	0.53 ± 0.05	0.54 ± 0.08	0.47 ± 0.06	0.53 ± 0.10
Liver	2.41 ± 0.20	2.21 ± 0.06	2.39 ± 0.27	2.52 ± 0.30	2.16 ± 0.23
Kidney	0.49 ± 0.15	0.57 ± 0.08	0.53 ± 0.09	0.60 ± 0.10	0.55 ± 0.10

PC = drinking water without probiotic; NC = drinking water with commercial probiotic 1 g/L; LJ1 = drinking water with probiotic larvae juice 1 mL/L; LJ3 = drinking water with probiotic larvae juice 3 mL/L; LJ5 = drinking water with probiotic larvae juice 5 mL/L.

3.4 Immune organs

Immune organs as indicators of pathogens or foreign substances that attack the immune system in the body of livestock, the organs of immunity measured are bursa of fabricius, spleen and lymph (Table 4). The statistical analysis of immune organs showed a significant effect ($p<0.05$) on the parameters of fabricius and lymph from each treatment given.

Table 4. Percentage immune organs of broilers at 32 days

Parameters (%)	PC	NC	LJ1	LJ3	LJ5
Bursa of fabricius	0.08 ± 0.03^{a}	0.07 ± 0.02^{ab}	0.04 ± 0.01^{b}	0.06 ± 0.02^{ab}	0.08 ± 0.04^{a}
Spleen	0.30 ± 0.10	0.28 ± 0.05	0.22 ± 0.08	0.25 ± 0.09	0.25 ± 0.12
Lymph	0.16 ± 0.03^{a}	0.12 ± 0.04^{ab}	0.11 ± 0.02^{b}	0.13 ± 0.02^{ab}	0.15 ± 0.03^{ab}

PC = drinking water without probiotic; NC = drinking water with commercial probiotic 1 g/L; LJ1 = drinking water with probiotic larvae juice 1 mL/L; LJ3 = drinking water with probiotic larvae juice 3 mL/L; LJ5 = drinking water with probiotic larvae juice 5 mL/L.

3.5 Haematology

Haematology of broiler blood can be observed in (Table 5) through red blood cell (RBC), haemoglobin (Hb). packed cell volume (PCV), and white blood cell (WBC). Haematology results showed that the addition of larvae juice to drinking water had a significant effect ($p<0.05$) on lymphocytes, monocytes and neutrophils (Table 6).

Table 5. Blood profile of broilers

Parameters	PC	NC	LJ1	LJ3	LJ5
Red blood cell 10^6 (cell/mm^3)	2.37 ± 0.34	2.87 ± 0.30	2.35 ± 0.30	2.62 ± 0.51	2.50 ± 0.27
Haemoglobin (%G)	8.88 ± 0.76	8.68 ± 0.74	8.84 ± 0.68	8.12 ± 1.19	9.32 ± 0.84
Packed cell volume (%)	25.00 ± 2.55	25.60 ± 2.07	24.20 ± 2.28	25.60 ± 0.89	26.00 ± 0.71
White blood cell 10^3 (cell/mm^3)	21.58 ± 4.54	20.06 ± 10.23	19.64 ± 3.23	23.22 ± 11.09	23.32 ± 8.63

PC = drinking water without probiotic; NC = drinking water with commercial probiotic 1 g/L; LJ1 = drinking water with probiotic larvae juice 1 mL/L; LJ3 = drinking water with probiotic larvae juice 3 mL/L; LJ5 = drinking water with probiotic larvae juice 5 mL/L.

Table 6. Differentiation leucocyte of broilers

Parameters	PC	NC	LJ1	LJ3	LJ5
Lymphocytes (%)	52.88 ± 1.82^{b}	56.86 ± 3.54^{a}	51.90 ± 1.00^{b}	52.94 ± 3.09^{b}	52.99 ± 2.16^{b}
Monocytes (%)	4.87 ± 0.84^{a}	3.86 ± 1.18^{ab}	3.16 ± 0.73^{b}	3.49 ± 1.62^{ab}	3.32 ± 1.19^{ab}
Neutrophils (%)	30.41 ± 2.17^{cd}	28.79 ± 2.28^{d}	36.56 ± 0.59^{a}	33.34 ± 2.36^{b}	31.89 ± 1.54^{bc}
Eosinophils (%)	10.08 ± 2.02	8.58 ± 3.06	7.15 ± 1.09	8.73 ± 1.81	10.03 ± 3.33
Basophils (%)	1.75 ± 0.62	1.90 ± 0.64	1.23 ± 0.49	1.48 ± 0.49	1.76 ± 0.66

PC = drinking water without probiotic; NC = drinking water with commercial probiotic 1 g/L; LJ1 = drinking water with probiotic larvae juice 1 mL/L; LJ3 = drinking water with probiotic larvae juice 3 mL/L; LJ5 = drinking water with probiotic larvae juice 5 mL/L.

3.6 Economic feasibility

Economic analysis can be shown from income value by calculating differences in chicken's price sale and rearing cost. The economic feasibility of broilers at 32 days is shown in (Table 7).

Table 7. Economic analysis of rearing broilers at 32 days

Parameters	PC	NC	LJ1	LJ3	LJ5
Total feed cost (IDR/chick)	24,586.22	23,499.79	23,775.39	24,027.43	24,549.44
Total probiotic cost (IDR/chick)	0	548.6	575.3	1,616.2	2,880.3
Gross Income (IDR kg/chick)	38,840	37,260	37,880	37,480	42,240
IOFCC (IDR/birds)	6,603.78	5,561.61	5,879.29	4,186.33	7,160.29
IP	357.09	341.14	342.93	337.95	422.80

PC = drinking water without probiotic; NC = drinking water with commercial probiotic 1 g/L; LJ1 = drinking water with probiotic larvae juice 1 mL/L; LJ3 = drinking water with probiotic larvae juice 3 mL/L; LJ5 = drinking water with probiotic larvae juice 5 mL/L. DOC = day old chicken; IOFCC = income over feed, probiotic, and chick cost; IP = index performance.

4 Discussion

Result of microorganism identification of larvae juice consist positive microorganism are *Bacilli* dan *Actinomycetia*. The positive microorganisms in probiotics aim to help enhance livestock performance. Probiotic balance the digestive system, and prevent several digestive diseases [11]. *Bacillus sp*. commonly utilized in probiotik commercial product, because of they thrive, adaptive, and anti-toxic [12]. *Bacillus sp*. can produce spore which help them adaptively and mobile in digestive tract that low pH and high temperature [13]. Probiotic microorganisms help suppress the negative bacteria of the secondary compounds produced. The condition of epithelial cells in the intestines that have fewer pathogens or negative

bacteria will be healthier in an optimal environment so that nutrient absorption activities will be better [14].

The treatment of probiotics from BSF maggots in drinking water had a positive impact on final weight and weight gain during maintenance. The average of broiler drinking water consumption ranges from 4291-4609 mL/chick and feed consumption 2895-3028 g/chick during maintenance within same range according to Hasan et al. [15]. The addition of probiotics doesn't affect the aroma and taste of drinking water also at the level of consumption [16]. The feed given has no difference both in nutritional content, shape, colour, and amount it doesn't affect the level of consumption.

The body weight gain (BWG) and final body weight (FBW) values are influenced by feed consumption factors, it is also suspected that added probiotics at level a 5 mL/L can create a more balanced intestinal environment to digest and absorb nutrients compared to other treatments, it's affects increase in BWG and FBW. Probiotic microorganisms help suppress negative bacteria from the secondary compounds produced. The condition of epithelial cells in the intestine that have fewer pathogens or negative bacteria will be healthier in an optimal environment, and it's better to absorb the nutrients [17].

The average percentage obtained in this study of vital organs as heart weight ranges from 0.47%-0.54%, liver 2.16%-2.52%, and kidney organ was at 0.49%-0.60% of body weight broilers, the size of the vital organs infected with toxic or anti-nutrient compounds will be enlargement and increase the percentage. All percentage of vital organs within at same range from several study [18,19,21]. The result of immune organ in this study has the lowest percentage of bursa of fabricius weight (LJ1), this allows the treatment to get a response from the broiler antibody system to the presence of foreign substances or pathogens so that the active formation of antibodies, lymphoid follicles that often produce B lymphocyte cell antibodies will deplete and shrink thereby reducing the weight of the bursa of fabricius [20, 21].

The number of lymphocytes in treatment (NC) is higher than in another, the function of lymphocytes is vital to respond to antigens by forming antibodies in the system and as a marker of physiological stress in broiler chickens [22,23]. The highest percentage of monocytes was in the control group, the average for all treatments ranged from 3.16-4.87%. Monocytes are mostly stored in the spleen (splenocytes) as reported by Yu et al. [24], presented in Table 4 the percentage of spleen weight having a higher number of (PC). Monocyte function to innate immune system of defence pathogen and response the inflammation. The percentage of neutrophils in (LJ1) treatments is higher than in other treatments, it is regarded as defence the innate immune system homeostasis and disease [25]. According to Table 7, adding 5 mL/L of probiotics larvae juice can save IDR 556.50/chick income compared to without probiotics. IOFCC is affected by feed consumption value, feed price, DOC price, final weight and selling price.

Conclusion

Larvae juice has the potential as a probiotic containing gram-positive bacteria such as *Bacillus* and *Actinomyces*. Giving probiotic larvae juice at a dose of 5 mL/L in drinking water significantly increases the final body weight of broiler chickens and the average body weight gain, thereby positively saving feed costs and also not adversely their haematology, vital organs and immunity.

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