Research Article

Physiological Parameters, Intestinal Microbial Population and Internal Organ Weight of Broilers Supplemented with the Fungus Monascus Purpureus

Turrini Yudiarti*, Sugiharto Sugiharto, Endang Widiastuti, Hanny Indrat Wahyuni, Tri Agus Sartono, Maulana Hamonangan Nasution

Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia.

Abstract | Antibiotic growth promoters (AGPs) have been administrated to chicken feed for many years to prevent disease and increase growth rate. Since the use of antibiotics is now forbidden, replacements should be developed that can also be used in their place. One option is to use non-antibiotic feed additives generated from fungal ferments, which have been shown to improve gastrointestinal health and production. Fungal can be used as a feed additive, and their effectiveness has been demonstrated in boosting animal development and growth. Monascus purpureus is a fungus that has a potential and is commonly used in medicine. They produce many secondary metabolites that has been shown improve the nutritional quality, antibacterial properties and antioxidant activity. The obyective of this study was to see how adding the fermented product of the fungus Monascus purpureus as a feed additive affected to physiological parameters, intestine microbial population, and broiler internal organ weight. The method was one hundred and fifty un-sexed one-day-old chicks (DOC) were reared in an open cage. With three treatments, the study used a completely randomized design. The treatments consisted of a dose of the fungus Monascus purpureus as a feed additive (FA). These included T0 (basal diet/control); T1 (basal diet supplemented with 0.5% FA); and T2 (basal diet supplemented with 1% FA). Each treatment was repeated five times with a total of ten chiks each pen. Feed additive was provided to chicks beginning 8 days after rearing and was mixed in with the basal diet. Water was available at all times. The data were collected from chickens that were 42 days old. The results shown that the dose of feed additive up to 1.0% increase cholesterol value, total protein, MCHC, Coliform population in ileum and weight of heart also bursa of fabricius, whereas could reduce MCV, coliform population in caecum, and weight of pancreatic also caecum and no effect on broiler performance. The conclusion is supplementation of fermented product of the fungus Monascus purpureus as a feed additive that applied on boiler can improve the physiological parameters, intestinal microbial population and internal organ weight but no impact on performance.

Keywords | Fungal feed additive, Physiological parameter, Microbial population, Internal organ weight, Biochemical indices, Antibiotic growth promoters, Antioxidant, Broiler

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***Correspondence** | Turrini Yudiarti, Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia; **Email:** tyudirti@yahoo.co.id
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INTRODUCTION

Antibiotic growth promoters (AGPs) are a type of feed additive that is typically used to control disease and increase animal growth. For many years, that type of additive has been used in practically every part of the world. However, long-term use of such additives may have a negative impact on animal health. The consequences of

AGP include the development of host and gut microbiota resistance, as well as drug residues still present in meat (Hamid *et al*., 2019). Because of this, some countries including Indonesia (Yudiarti *et al*., 2019) have banned the use of AGP in broiler production (Silje *et al*., 2020). Concerns about this prompted then the study try to find a replacement that would allow broiler chickens to grow faster. Another option is to use different feed additives that can increase intestinal health and performance without causing harm to the animals and humans as consumers.

Probiotics are a type of feed ingredient that has long been recognized in the worldwide poultry feed industry (Silje *et al*., 2020). Probiotics is as 'cultures of microbial life that have a favorable effect on the host's gut microbial balance' (Khan and Naz, 2013). Furthermore, colonization of the intestine, competitive exclusion of other microbes, synthesis of specific metabolites, and immune system stimulation are all routes of action (Kabir, 2009). These probiotic feed additives will have an impact on the gut bacteria population, physiological processes, and internal organ growth.

Fungal probiotics are a type of probiotic that can be used as a feed additive, and their effectiveness has been demonstrated in boosting animal development and growth (Chuang *et al*., 2020). According to previous studies, the fungus *Chrysonillia crassa* stimulated the development of duodenal villi and reduced the counts of bacteria and fungi pathogens in the chicken's gastrointestinal system (Yudiarti *et al*., 2012). According to Chang *et al*. (2019), adding a Saccharomyces cerevisiae and Aspergillus oryzae fermentation product to broiler feed can effectively minimize the inflammatory response and reduce the amount of dangerous bacteria in the ileum. Furthermore, a co-fermented product containing Aspergillus oryzae and phytase increased villus height in the jejunum, while Teng *et al*. (2017) found that supplementing wheat bran with Saccharomyces cerevisiae increased villus height in the ileum.

Monascus purpureus is another fungus that has potential and is commonly used in medicine. It's been employed in the creation of 'angkak', which is a traditional remedy produced from fermented rice, for centuries. Isoflavones, edible pigments, fatty acids, dimerumic acid (antioxidant), enzymes, organic acids, monacolin K (lovastatin, anti-hypercholesterolemic agent), -aminobutyric acid (GABA, hypotensive agent), and vitamins are among the secondary metabolites that the fungus can produce, according to Kim and Ku (2018). Also, the red pigment has been shown to have antibacterial properties against the microorganisms Escherichia coli and Bacillus subtilis (Neera *et al*., 2017). Furthermore, it was shown that utilizing Monascus purpureus to ferment rice improved the nutritional quality as well as the antioxidant and antibacterial properties of the fermented product (Yudiarti *et al*., 2019). Overall, the aim of this study was to investigate how adding the fermented product of the fungus Monascus purpureus as a feed additive affected to physiological parameters, intestine microbial population, and broiler internal organ weight.

MATERIALS AND METHODS

Preparation of feed additive

Approximately 500 g of used rice purchased from the local market was soaked in water for an hour. Then it was drained and steamed for 60 minutes before being placed on a cooling tray. Monascus purpureus culture stock was regrown in PDA media on a plate and cultured for 7 days. Then 50 ml aquades were put into each fresh Monascus purpureus culture isolate. All spores on the surface medium were harvested with a spatula and placed in a sterile tube. After that, the steamed rice was inoculated with 50 ml of Monascus purpureus suspension (7.0 × 107 cfu/ml) and properly mixed. To incubate, the mixture was spread out on a tray and covered with aluminum foil. The incubation period was 7 days, followed by two days of sun-drying. The fermented product was crushed and sieved after drying (1-mm sieve) before use (Yudiarti *et al*., 2019).

In vivo experiment

A total of 150 un-sexed one-day-old chicks (DOC) were reared in an open cage and they are healthy. With three treatments, the study used a completely randomized design. The treatments consisted of a dose of the fungus Monascus purpureus as a feed additive (FA). These included T0 (basal diet/control); T1 (basal diet supplemented with 0.5% FA); and T2 (basal diet supplemented with 1% FA). Each treatment was repeated five times with a total of ten chiks each pen. Feed additive was provided to chicks beginning 8 days after rearing and was mixed in with the basal diet (Table 1). Water was available at all times. The vaccines were administered as follows: ND-AI via eye drops on the 4th day and ND-IB via drinking water on the 18th day. The data were collected from chickens that were 42 days old. Until the day 42, there were not any chicken that sick or dead.

The weight of internal organs, including heart, liver, proventriculus, gizzard, pancreas, spleen, thymus, bursa fabricius, duodenum, jejunum, ileum, and caecum, were measured. The microbial population was counted in the ileum and caecum digesta. Complete blood counts and serum biochemical analyses were used for hematological assessments. Blood was drawn from the veins of the bird's wings at days 42, and deposited in ethylenediaminetetraacetic acid-(EDTA) containing and anticoagulant-free vacutainers. The blood in the latter vacutainers was allowed to coagulate at room temperature before being centrifuged for 15 minutes at 448 g to produce

serum. The serum was kept frozen until the tests were completed. The diluted flash method was used to count the number of erythrocytes and leukocytes, and a Bürker chamber was employed to count the number of corpuscles. The automated erythrocyte sedimentation rate analyzer (Streck ESR-Auto Plus, Streck, Omaha, NE) was used to determine erythrocyte sedimentation. When producing blood smears, the differential leukocytes were determined using a light microscope with an immersion lens and the coverslip technique. Photometric test according to the biuret method utilizing the kit was used to determine total serum protein (total protein kit, DiaSys Diagnostic System GmbH, Holzheim, Germany). While a photometric test with bromocresol green (DiaSys Diagnostic System GmbH, Holzheim, Germany) was used to measure albumin levels.

The number of bacteria in the intestinal digesta was counted based on Engberg *et al.* (2004), with a few modifications. After a 24-hour aerobic incubation at 38°C, total bacteria were counted on a PDA. Coliform bacteria were calculated using MacConkey agar following a 24 hour aerobic incubation at 38°C as a red colony. After anaerobic incubation at 38°C for 48 hours, lactic acid bacteria (LAB) were enumerated on De Man, Rogosa, and Sharpe agar. The data were evaluated using analysis of variance, then Duncan's Multiple Range Test when the significant effect was found. The significance level was set at P<0.05.

Tabel 1: Composition of basal diet added with feed additive.

Items (%)	Starter			Finisher		
	T0	T1	T2	T0	T1	T2
Meat Bone Meal	4.70	4.70	4.70	2.35	2.35	2.35
Corn	54.8	50.8	47.0	58.5	54.6	50.7
Soybean Oil	1.55	1.25	0.8	3.25	2.90	2.50
Soybean meal	35.7	35.0	34.3	32.7	32.0	31.2
DL-methionine	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine	0.20	0.20	0.20	0.20	0.20	0.20
Limestone	0.50	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50
Premix	0.50	0.50	0.50	0.50	0.50	0.50
NaCl	0.25	0.25	0.25	0.25	0.25	0.25
Feed additive (FA/%)	0.00	0.50	1.00	0.00	0.50	1.00
Nutrient content						
Crude protein (%)	22.0	22.0	22.0	20.0	20.0	20.0
Crude fiber (%)	5.59	5.32	5.07	5.54	5.28	5.02
Metabolism energy (kkal/kg)	2901	2907	2905	3063	3066	3066

RESULTS AND DISCUSSION

Biochemical parameters

Statistical analysis revealed that supplying feed additive had an influence on broiler biochemical parameters (Table 2), and Duncan's test revealed that cholesterol level in T2 was significantly increased (P<0.05) compared to T0 but was not different to T1, and for total protein in T1 significantly increased (P<0.05) compared to T0 ,whereas was not different to T2.

Table 2: Biochemical Parameters of the chicken fed with feed additive.

Parameter	T0	T1	T2
Cholesterol	105±10.5^{b}	123±14.1^{ab}	139±20.5^{a}
HDL	81.4±8.79	104±23.9	74.6±49.3
LDL	11.0±9.08	19.4±12.8	24.1±19.1
Triglycerides	90.7±21.7	63.2±28.4	156±130
SGOT	259±24.8	292±54.7	381±21.8
SGPT	2.10±0.78	1.45±0.62	1.80±1.60
Abdomenal fat	29.6±8.08	24.6±11.7	26.0±12.3
Albumin	1.30±0.04	1.40±0.10	1.32±0.13
Total Protein	2.85±0.20^{b}	3.35±0.34^{a}	3.04±0.28^{ab}
Uric acid	5.19±1.53	6.37±3.32	3.72±1.76
Creatinin	0.03±0.01	0.02±0.02	0.03±0.02

Different superscripts within the same rows indicate significantly different (P<0.05),T0: chicks receiving basal diet without FA, T1 : chicks receiving basal diet contained 0.5% FA, T2 : chicks receiving basal diet contained 1.0% FA

Hematological parameters

Statistical analysis revealed that administering feed additive had an effect on broiler hematological parameters (Table 3), with Duncan's test revealing that hematological parameters were substantially different (P<0.05). For MCV in T0 significantly decreased (P<0.05) compare to T1 and T2. In contras for MCHC in T0 significantly increased (P<0.05) compare to T1 and T2.

Table 3: Hematological parameters of the chicken fed with feed additive.

Parameters	T0	T1	T2
Leucocyt (10^{2}/mm^{3})	73.7±7.64	76.3±19.5	65.6±5.94
Eritrocyt (10^{6}mm^{3})	2.88±0.23	3.14±0.58	3.06±0.22
Hemoglobin(g/dl)	9.40±0.84	10.8±1.92	10.7±0.84
Hematocrit(%)	38.5±3.41	38.9±7.27	37.9±2.56
Platelets	10.8±2.77	11.0±1.87	8.40±1.67
Lymfocyt (%)	71.0±8.21	73.0±18.0	62.9±5.92
Neutrophils (%)	2.70±1.04	3.30±1.82	2.70±0.57
MCV(fl)	135±3.39^{a}	125±2.91^{b}	124±2.12^{b}
MCH (pg)	32.4±1.79	34.4±1.79	34.9±1.48
MCHC (%)	25.4±0.90^{b}	27.7±1.06^{a}	28.2±0.73^{a}

^{a,b}Different superscripts within the same rows indicate significantly different (P<0.05). T0: chicks rceiving basal diet without FA, T1: chicks rceiving basal diet contained 0.5% FA, T2: chicks rceiving basal diet contained 1.0% FA.

Intestinal microbial population

Diet containing FA at 0.5% significantly increased (P<0.05) on coliform colonies in ileum in T1 compare to T0 and T2. But in caecum for T0 significantly decreased (P>0.05) compare to T1 and T2. However the dietary treatments had no effect (P>0.05) on the number of LAB colonies in ileum nor caecum (Table 4).

Table 4: The number of intestinal microbial population in ileum and caecum of the chicken fed with feed additive.

Item	T0	T1	T2
Ileum (log cfu/g)			
Coliform	6.70±11.0[b]	8.58±14.0[a]	8.40±13.8[b]
LAB	11.0±18.0	11.0±18.3	11.5±18.7
Caecum (log cfu/g)			
Coliform	9.06±14.8[a]	8.29±13.6[b]	7.82±12.8[b]
LAB	11.2±18.3	12.1±19.7	12.2±19.9

[a,b]Different superscripts within the same rows indicate significantly different (P<0.05). T0: chicks rceiving basal diet without FA, T1: chicks rceiving basal diet contained 0.5% FA, T2: chicks rceiving basal diet contained 1.0% FA, LAB: Lactic acid bacteria

Internal organs weight

Statistical analysis showed that there was an effect of giving feed additive to the average weight of internal organs of broiler (Table 5) and Duncan's test resulted the average weight of internal organs was significantly different (P<0.05). For heart was significantly incresed (P<0.05) in T2 compare to T0,but was no different to T1. For bursa fabricius in T1 was significantly incresed (P<0.05) compare to T0 and T2.

Table 5: Internal organs weight of chicken fed with feed additive.

Organ	T0	T1	T2
Heart	8.12±0.30[b]	8.72±0.69[ab]	10.1±2.00[a]
Liver	53.3±4.75	48.2±10.4	49.4±8.15
Proventriculus	9.73±1.02	8.52±1.15	8.13±1.36
Gizzard	31.0±2.16	32.9±4.46	30.8±4.40
Pancreas	5.43±2.16[a]	4.80±0.48[ab]	4.16±0.57[b]
Spleen	1.57±0.46	2.10±0.90	1.74±0.73
Thymus	2.12±0.97	2.64±0.91	2.94±1.29
Bursa fabricius	0.60±0.13[b]	1.18±0.28[a]	0.79±0.17[b]
Duodenum	10.0±1.41	8.59±1.82	9.61±2.13
Jejunum	24.8±4.75	19.5±3.97	21.1±1.96
Ileum	17.0±3.34	16.0±5.35	16.4±2.21
Caecum	11.4±3.40[a]	6.77±1.34[b]	8.01±1.83[b]

Different superscripts within the same rows indicate significantly different (P<0.05). T0: chicks rceiving basal diet without FA, T1: chicks rceiving basal diet contained 0.5% FA, T2: chicks rceiving basal diet contained 1.0% FA.

Feed additive which added at any level of treatments did not affect the biochemical parameters like HDL, LDL, triglycerides, SGOT, SGPT, abdomenal fat, albumin, uric acid and creatinin. Whereas the treatments level 1.0% gave a significantly different in cholesterol and total protein levels compare to the control. The addition FA in this study showed increased in cholesterol level as increased the dose of FA. In fact, cholesterol in the body actually is supported in two ways that are from synthesis in the body cell itself and also from feed. As stated by Ponte *et al.* (2004) that cholesterol is an important molecule that has a roles in membrane structure as well as being a precursor for the synthesis of molecules such as steroid hormones, vitamin D and bile acids. In addition, the cholesterol can be obtained directly from the diet (exogenous cholesterol) or it can be synthesized in cells (endogenous cholesterol). The findings showed that increased in treatment rates of FA increased also in cholesterol levels. FA contains of fungus Monascus purpureus, it means that increasing of treatment level also increased the number of fungus. According to Yudiarti *et al.* (2019) that this fungus has been used as a fermented starter of the used rice and it was proved that they could increase the lipid content on the product and the substance is derivated of cholesterol. If it looks for the level treatmen of FA is only 1.0 %, it might not to much effect in increasing cholestrol level. It might the main contribution is from the lipid content in FA as follows: T0 (4.03%), T1 (4.58%) and T2 (4.82%).

The findings showed that total protein in this current study also increased as increasing level of FA. As mention before that FA contains fungus Monascus purpureus and its potency that has been proved by Yudiarti *et al.* (2019) is increasing protein content of the product fermentation. This is also contributed by Tseng *et al.* (2000) that the fungus produces protease that is an enzyme which responsible for the formation and breakdown of proteins. Owing to that, the protease which is produced by the fungus makes improving the digestibility of nutrients and their absorption in the intestines of chicken especially in protein.

In this study, it was found that the administration of feed containing FA resulted in a decrease in the MCV value of broilers. The reason for the condition is not known, but it is most likely that the decrease in infection potential could be related to the decrease in MCV levels in FA-fed broilers. This opinion is supported by the study of Dar *et al.* (2014) where Eimeria tenella infection increased levels of MCV (macrocytic anemia) in broiler chickens. Our opinion is also supported by the fact that FA administration reduces the coliform gut population of broilers. The value of MCHC increased with the feeding diet containing FA to the chickens in this study. In accordance with our

findings, Sugiharto *et al.* (2020) recently reported that administration of fermented banana peels resulted in an increase in broiler MCHC levels. The last author suggested that fermented feed can increase hematopoietic activity in broiler chickens. Other possibilities may that the probiotic activity of the filamentous fungus Monascus purpureus used as fermentation starter effected on the protein digestibility and thus enhance the protein availability for haematopoietic activity. Note that protein is a crucial nutrient needed for red blood cells as well as haemoglobin synthesis (Zanetti *et al.*, 2013).

Feeding diet as containing FA to broilers was no affect on LAB populations, whereas decreased coliform in caecum. In agreement with this finding, Yudiarti *et al.* (2012) reported that the addition of 0.5 % dried culture of Chrysonillia crassa in feed could reduce the number of bacteria and fungi in caecum of the indigenous chicken. The decreased number of Coliform seemed due to the probiotic activity of the fungus Monascus purpureus. Indeed, Monascus purpureus exhibited antimicrobial activity in the intestine of poultry as reported previously by Yudiarti *et al.* (2019). In contrast, in ileum showed there is an increasing in Coliform bacteria. As stated above that feed additive in this study contains the fungus Monascus purpureus and the fungus has the ability of fermenting substrate (Yudiarti *et al.* 2019). Fermentation products for one microbe are different from other microbes. As mentioned by Lijuan *et al.* (2021) that different fermentation strains have different reactions with substrate and produce different fermentation products. Like *Bacillus subtilis* which has the ability to create an anaerobic environment in the intestinal tract, which was conducive to the growth and proliferation of lactic acid bacteria. Thus, there a possibility that the increasing the Coliform bacteria in ileum due to the fungus in this study produce substances that promote the growth of that bacteria.

Our present finding showed that chickens fed diet containing 1.0% FA had higher relative weight of heart (Table 5). The increased weight of the heart in the current study may due to the enlargement of the heart size, which is usually caused by the enlargement of cardio-muscular of chickens and those is triggered by the more activities of the chicken (Wiesje and Rajab., 2019). The reason for the increased cardio-muscular development was not exactly known, but possibly the increased activity of heart in circulating the blood throughout the body was associated with the increased cardio-muscular development. Note that feeding fermented feed as well as probiotic supplementation may be attributed to the increased nutrient digestibility and hence nutrient available for cellular metabolism (Sugiharto, 2017; Sugiharto and Ranjitkar, 2019). To reach the cells, the nutrients must be circulated, and in this case the heart plays a crucial role in the nutrient circulations (Wiesje and Rajab., 2019).

The finding showed that the effect of feed contained FA on the chicken decreased in pancreas. On the other hand these results differ from the findings of Olnood *et al.* (2015) that showing an increased relative weight of the pancreas with feeding probiotic Lactobacillus johnsonii to broilers at 21 days of age and Sugiharto *et al.* (2018) finding the dietary supplementation of the chicken feeds with 0.5% of multistrain probiotic preparation in combination with vitamins and minerals may improve the development and functionality of pancreas. Previous research from Su and Chang (2002) has also found the same result. They explained that pancreatic hypertrophy of animal including poultry are caused by the increasing secretion of pancreatic enzymes and those trigger by inhibitor trypsin (IT). Futher explain that IT is as an anti-nutritional compound which can inhibits proteolysis, reduces protein digestibility, increase the need for S amino acids, and makes enlarge of pancreas. Feed additive in this study contained the fungus Monascus purpureus and this fungus produces protease (Tseng *et al.*, 2000) that is the enzyme of pancreatic product. With the contribution of the fungus in secreting the pancreatic enzym, therefore can reduce the activity or work of the pancreas. The reducing of pancreas activity will reduce the weight of pancreas. This is indicated by the results that the weights of the pancreas T0, T1 and T2 are 5.43, 4.80, 4.16, respectively.

Bursa of Fabricius is a primary lymphoid organ in birds and plays a key role in the differentiation of B-lymphocytes (Bangyuan *et al.*, 2013; Xi *et al.*, 2017). Its development begins during incubation and reaches maximum size between 8 to 10 weeks of age, when the regression process starts, and is completed by 6 to 7 months of age. Several pathologic conditions (infectious diseases etc.) can directly impact bursa size, as well as mycotoxin contamination in feed (Cazaban *et al.*, 2015). The results showed that the bursa fabricius weight of the chicken fed FA increased compare to control. Different results are got by Bangyuan *et al.* (2013) that bursae fabricius weight of the chickens were treated with the feed that deficiency in Methionine at 35 days were significantly decreased. Methionine is involved in avian immune functions and dietary methionine can promote antibody production and cell-mediated immune responses in broilers. The same findings also found by Xi *et al.* (2017). They reported that the decreasing of the organ was caused when the chicken are fed a diet including corn mainly contaminated with aflatoxin. Aflatoxins are a group of cytotoxic and carcinogenic mycotoxins produced by Aspergillus flavus. As Cazaban *et al.* (2015) statement that toxin can cause adverse health effects including the development of organ immune. On the contrary in this study showed that the bursa fabricius weight of the chickens fed with FA increased. This might be supported

by Monascus purpureus and the fungus does not produces toxin but gives beneficial to the host by producing protease (Tseng *et al*., 2000). In addition the fungus also has proved that it can improve protein content especially methionine in fermentation product (Yudiarti *et al*., 2019).

In this study showed the weight of caeca of the chickens fed with FA decreased compare to control. Caecum is a part of the digestion tract which serves as a place microbial digestion with the function is to digest unabsorbed nutrients from the small intestine especially crude fiber (Varastegani and Dahlan, 2014). Increase in the weight of the cecum is caused increased activity of nutrient digestion which is not absorbed in the small intestine (Sharifi *et al*., 2012). As stated by Sharifi *et al*. (2012), Varastegani and Dahlan (2014) above that the increasing of caecum weight is caused by the increasing of the activity of nutrient digestion which unabsorbed from small intestine especially crude fiber. The feed additive in this study contains fungi that have the ability to degrade crude fiber (Yudiarti *et al*., 2019), so that with the activity of these fungi there is not much crude fiber left in the small intestine. Decreased crude fiber in the small intestine has an impact on the weight of the cecum.

These findings indicate that the administration of FA up to 1% has a positive effect on physiological processes but it has not yet effect on the performance of broilers. This may be the nutrient content in each treatment almost the same and these could be not enough to improve the performance of broiler, as seen in Table 1.

CONCLUSIONS AND RECOMMENDATIONS

The conclusion is supplementation of fermented product of the fungus *Monascus purpureus* as a feed additive on boiler can improve the physiological parameters, intestinal microbial population and internal organ weight but no on performance.

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Maulana Hamonangan Nasution is now deceased.

NOVELTY STATEMENT

The usefull of fungi to contribute in growing of chicken.

AUTHOR'S CONTRIBUTION

TRY designed, performed the work and wrote the manuscript, SS, EW, HIW, TAS and MHN performed the work and revised the manuscript and MHN performed the data analysis.

Conflict of interests

The authors have declared no conflict of interest.

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