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Effect of fermented purple sweet potato flour on physiological conditions and intestinal conditions of broiler chickens

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Abstract. The study investigated the effect of fermented purple sweet potato flour (PSPF) on intestinal and physiological health of broilers. A 189-day-old broiler chicks were divided into T0 (diet based on corn and soybean meal), T1 (diet containing 15% unfermented PSPF), and T2 (diet containing 15% fermented PSPF). Samples collection and measurement were conducted at day 35. The T2 chicks had greater ($p < 0.05$) weight gain than T1, but did not differ from T0. Feed conversion ratio (FCR) was better ($p < 0.05$) in T2 than in T1. The mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were higher ($P < 0.05$) in T2 than in T0 and T1 groups. Haemoglobin tended ($p = 0.08$) to be lower in in T2 than in T0 and T1 groups. Heterophils were higher ($p = 0.05$) in T2 than in T0 and T1 groups. Total cholesterol and high-density lipoprotein (HDL) were higher ($p < 0.05$) in T0 than in T1. Low-density lipoprotein (LDL) tended ($p = 0.06$) to be lower in T1 than that in T0. Total protein and globulin were higher ($p < 0.05$) in T0 than that in T1 and T2. Lactic acid bacteria (LAB) to coliform ratio in the ileum was higher ($p < 0.05$) in T2 than in T0. LAB counts tended ($p = 0.08$) to be greater in T2 than in other chickens. T1 tended ($p = 0.09$) to have a smaller number of lactose negative *Enterobacteriaceae* (LNE) in caecum as compared to that of T0 chicks. T2 tended ($p = 0.09$) to have a lower crypt depth than T0. In conclusion, feeding fermented purple sweet potato flour contributes for the better growth, feed conversion, immune defence, bacterial population and morphology of the small intestine.

Key words: bacteria, broilers, energy source, fermentation, immune response, intestine.

INTRODUCTION

Corn is the main energy source for poultry and contributes more than 70% of the energy needs of poultry (Sultana et al., 2016). The demand for corn increases every year along with the increase in the poultry population. To reduce the use of corn in feed formulations, several attempts have been made by broiler producers, one of which is by using alternative energy source feed ingredients. Despite their widespread availability, using alternative feed ingredients is often challenging due to their low nutritional quality and the presence of anti-nutritional compounds in the ingredients (Helda et al., 2021). Owing to these circumstances, specific processing techniques are required to raise the

nutritional value of these substitute feed ingredients. Fermentation is a commonly employed technique to enhance nutritional value and reduce the amount of anti-nutritional substances present in feed ingredients. Its application is relatively simple and cheap (Sugiharto & Ranjitkar, 2019). Apart from having an impact on improving nutrition, fermentation is also reported to increase the bioactive components in feed ingredients (Sugiharto & Ranjitkar, 2019). Furthermore, according to Sugiharto et al. (2018a), fermentation can raise functional value, which benefits broiler chickens' physiological state and general health.

One alternative feed stuff that can be used as an energy source for broiler chickens and also as a functional feed ingredient is purple sweet potato. Purple sweet potatoes have a relatively high carbohydrate content of 75–90%, which suggests that they could be utilized as an alternative feed ingredient to provide broilers with energy sources (Dapawisi et al., 2022). According to Kusuma et al. (2016), purple sweet potatoes are rich in vitamins, β-carotene and anthocyanins. Indeed, purple sweet potato also shows high antioxidant activity. The antioxidant properties in purple sweet potatoes is beneficial for health because it can ward off free radicals, oxidation in the body and clumping of blood cells. Natural antioxidants in purple sweet potatoes have the advantage of low molecular weight which is effective in suppressing reactive oxygen species (ROS) and preventing oxidative damage to biomolecules. With regard particularly to anthocyanins, because anthocyanins possess antibacterial properties, they can inhibit the growth of harmful bacteria, which in turn encourages the production of mucus by intestinal microbes and improves the digestibility of nutrients (Edi et al., 2018). The high level of carbohydrates in purple sweet potato is inversely proportional to the level of crude protein. Crude protein in purple sweet potatoes is only around 3.2% (Hartadi et al., 2005). Purple sweet potatoes also have anti-nutritional components, one of which is trypsin inhibitor, which can inhibit the work of the trypsin enzyme thereby reducing the level of protein utilization by broiler chickens (Anbuselvi & Muthumani, 2014).

Among the fermentation starters that are often used for the fermentation process is *Saccharomyces cerevisiae* (Nurhayati et al., 2019). *S. cerevisiae* is a yeast that is able to utilize sugar for its growth. *S. cerevisiae* as a fermentation starter is reported to increase protein content and also reduce crude fibre content. In this case, *S. cerevisiae* produces enzymes that can degrade complex carbohydrates into simpler carbohydrates (Kusuma, 2016). Apart from its potential as a fermenter, *Saccharomyces* is utilized extensively as a probiotic, potentially improving the well-being and productivity of chickens (Sugiharto, 2016). Based on the capabilities of this yeast, it was expected that using *S. cerevisiae* as a fermenter for feed ingredients would improve the nutritional quality of the feed ingredients while also serving as a probiotic for poultry. In this study, *S. cerevisiae* was used to ferment purple sweet potato flour. Overall, the demand for corn as the primary energy source in broiler chicken feed continues to rise, and it is frequently unmet because corn production remains unstable throughout the year. Such conditions therefore necessitate efforts to find alternative energy sources other than corn without compromising broiler chicken productivity and health. The scientific purpose of the present study was to look into how fermented purple sweet potato flour affected the intestinal and physiological health of broiler chickens. From the practical broiler production point of view, the present study aimed to find alternative energy source feed ingredients, to reduce the proportion of corn in the feed, for broiler chickens.

MATERIALS AND METHODS

Production of the fermented sweet potatoes

Purple sweet potatoes were purchased from farmers around Semarang, Central Java, Indonesia. Making purple sweet potato flour begun with washing and separating the rotten parts of the sweet potato. Purple sweet potatoes were cut into small pieces, then dried in the sun until dry. The dried purple sweet potato is then ground using a disk mill, then sifted to get purple sweet potato flour. Making fermented purple sweet potato flour begins with sterilizing the purple sweet potato flour using an autoclave at 121 °C for 15 minutes. Next, the sterilized flour was added with yeast containing 6.6×10^8 cfu/g of *S. cerevisiae* (5.5 g of yeast added for every 1 kg of purple sweet potato flour). Then purple sweet potato flour was mixed with water in a ratio of 1:2. In this study, considering that purple sweet potato flour has a high water absorption capacity (ability to absorb and retain water), more water was needed so that the fermentation process could take place optimally through solid state fermentation. Fermentation was carried out in the bucket aerobically for 2 hours at room temperature (~25 °C). Fermentation was halted when alcohol-like smell started to emerge. Fermented purple sweet potato flour was then sun-dried (at the temperature of ~34 °C) until dry (about 2 hours). After drying, a proximate analysis was carried out to determine the nutritional content of the material.

Broiler experiment

The broiler experiment was conducted to met animal welfare principles as approved by Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (No. 59-07d/A-16/KEP-FPP). In total, 189-day-old broiler chicks from the Cobb strain were used in this investigation. Using rice husk as litter, they were raised in an open-sided (naturally ventilated) broiler house for the entire of the rearing process. To manually regulate the temperature and humidity inside the broiler house, plastic curtains, light bulbs, and fans were used. The temperature was kept at 28–30 °C, and the humidity at 80–85%. During the study, the chickens were given light 24 hours a day. The birds, weighing 45.85 ± 3.04 g at arrival, were fed commercial pre-starter feed for seven days. The birds, weighing 142.17 ± 0.34 g, were divided into experimental treatment groups at random starting on day 8. From day 8 to day 21, the birds were fed formulated starter feed (Table 1), and from day 22 to day 35, they were fed finisher feed (Table 2). For the duration of the rearing process, food and water were given freely using round bottom feeder and manual drinker. With three treatment groups and seven replicates (each containing nine chicks), the dietary treatments were set up in a completely randomized manner. From day 8 to 35, the following treatments were given to the chicks: T0 (a diet based on corn and soybean meal), T1 (a diet containing 15% unfermented purple sweet potato flour), and T2 (a diet containing 15% fermented purple sweet potato flour). The chicks were raised in compliance with the Cobb broiler strain rearing guidelines. Using eye drops (day 4) and drinking water (day 18), the chicks received the Newcastle disease vaccination. On day 12, a drinkable vaccine against infectious bursal disease was also administered.

Both the total amount of feed consumed and the body weight were noted. To determine the feed conversion ratio (FCR) value, the ratio of accumulative feed intake (g) to total body weight gain (g)was computed. At the end of experiment, one male chick (in order to prevent gender bias), representing the average body weight of each experimental unit, was chosen to have blood withdrawn from the brachial vein. To produce serum, 3 mL of blood were placed in a non-ethylenediaminetetraacetic acid (EDTA) tube and 1 mL of blood was placed in an EDTA tube for routine blood testing. The same chicks as blood sampled were slaughtered. As soon as the chicken was slaughtered, its intestines were removed. Segments of the duodenum, jejunum, and ileum (about 2 cm) were placed in 10% buffered formalin (Leica Biosystems Richmond, Inc., Richmond, USA) in order to measure the morphology of the small intestine (villus height and crypt depth). After being placed in each sterile sample pot, the ileum and cecum digesta were examined further in the lab to determine the population of particular bacteria present in the intestine.

Table 1. Feed ingredients and nutritional compositions of broilers (day 8–21)

Ingredients (%)	T0	T1	T2
Yellow corn	53.5	36.2	36.4
Palm oil	2.32	2.39	2.55
Soybean meal	40.13	42.36	42.0
DL-methionine	0.19	0.19	0.19
Bentonite	0.75	0.75	0.75
Limestone	1.00	1.00	1.00
Monocalcium phosphate	1.30	1.30	1.30
Premix[1]	0.34	0.34	0.34
Chlorine chloride	0.07	0.07	0.07
Salt	0.40	0.40	0.40
Purple sweet potato flour	-	15.0	-
Fermented purple sweet potato flour	-	-	15.0
Nutritional compositions:			
ME[2] (kcal kg^{-1})	2,900	2,900	2,900
Crude protein	22.0	22.0	22.0
Crude fibre	5.47	4.56	4.75
Ca	1.14	1.09	1.09
P (available)	0.57	0.50	0.50

[1]The following nutrients are provided per kilogram of feed: 1,100 mg Zn, 1,000 mg Mn, 75 mg Cu, 850 mg Fe, 4 mg Se, 19 mg I, 6 mg Co, 1,225 mg K, 1,225 mg Mg, 1,250,000 IU vitamin A, 250,000 IU vitamin D_3, 1,350 g pantothenic acid, 1,875 g vitamin E, 250 g vitamin K_3, 250 g vitamin B_1, 750 g vitamin B_2, 500 g vitamin B_6, 2,500 mg vitamin B_{12}, 5,000 g niacin, 125 g folic acid and 2,500 mg biotin.

[2]ME (metabolizable energy) was calculated according to formula: 40.81 {0.87 (crude protein + 2.25 crude fat + nitrogen-free extract) + 2.5}.

T0: chicks were provided with corn-soybean meal-based diet, T1: chicks were provided with diet containing 15% unfermented purple sweet potato flour, T2: chicks were provided with diet containing 15% fermented purple sweet potato flour.

Laboratory analyses

The routine blood profile tests of the chicks were determined automatically by means of a Hematology Analyzer (Prima Fully-auto Hematology Analyzer, PT. Prima Alkesindo Nusantara, Jakarta, Indonesia) based on the manufacturer's instructions (Sapsuha et al., 2022). Serum lipid profiles (total triglycerides, total cholesterol, low-density lipoprotein [LDL], and high-density lipoprotein [HDL]) and creatinine and uric acid levels were measured using enzyme-based colorimetric techniques. Serum total protein and albumin levels were determined using spectrophotometric and photometric methods. To calculate the globulin concentration, the serum albumin value was

subtracted from the total protein value. All biochemical analyses of serum samples were performed in accordance with the manufacturer's instructions (DiaSys Diagnostic System GmbH, Holzheim, Germany).

Small intestinal segments were histologically examined using 5 μm sections of the ileum, jejunum, or duodenum stained with haematoxylin and eosin. The villous height and crypt depth in each segment were measured with an optical microscope equipped with a digital camera (Leica Microsystems GmbH, Wetzlar, Germany). Five measurements were used to determine the mean values of villous height and crypt depth for each sample. The bacterial population in the ileal and caecal contents was determined according to the total plate count method. After a 24-hour aerobic incubation at 38 °C, coliforms and lactose-negative *Enterobacteriaceae* (LNE) were counted as red and colourless colonies on MacConkey agar (Merck KGaA, Darmstadt, Germany). Lactic acid bacteria (LAB) were counted on de Man, Rogosa, and Sharpe (MRS; Merck KGaA) agar after a 48-hour anaerobic incubation period at 38 °C.

Table 2. Feed ingredients and nutritional compositions of broilers (day 22–35)

Ingredients (%)	T0	T1	T2
Yellow corn	61.74	44.49	44.6
Palm oil	2.41	2.46	2.63
Soybean meal	31.8	34.0	33.72
DL-methionine	0.19	0.19	0.19
Bentonite	0.75	0.75	0.75
Limestone	1.00	1.00	1.00
Monocalcium phosphate	1.30	1.30	1.30
Premix[1]	0.34	0.34	0.34
Chlorine chloride	0.07	0.07	0.07
Salt	0.40	0.40	0.40
Purple sweet potato flour	-	15.0	-
Fermented purple sweet potato flour	-	-	15.0
Nutritional compositions:			
ME[2] (kcal kg^{-1})	3,000	3,000	3,000
Crude protein	19.0	19.0	19.0
Crude fibre	5.57	4.67	4.85
Ca	1.12	1.07	1.07
P (available)	0.59	0.51	0.51

[1]The following nutrients are provided per kilogram of feed: 1,100 mg Zn, 1,000 mg Mn, 75 mg Cu, 850 mg Fe, 4 mg Se, 19 mg I, 6 mg Co, 1,225 mg K, 1,225 mg Mg, 1,250,000 IU vitamin A, 250,000 IU vitamin D_3, 1,350 g pantothenic acid, 1,875 g vitamin E, 250 g vitamin K_3, 250 g vitamin B_1, 750 g vitamin B_2, 500 g vitamin B_6, 2,500 mg vitamin B_{12}, 5,000 g niacin, 125 g folic acid and 2,500 mg biotin.

[2]ME (metabolizable energy) was calculated according to formula: 40.81 {0.87 (crude protein + 2.25 crude fat + nitrogen-free extract) + 2.5}.

T0: chicks were provided with corn-soybean meal-based diet, T1: chicks were provided with diet containing 15% unfermented purple sweet potato flour, T2: chicks were provided with diet containing 15% fermented purple sweet potato flour.

Statistical analyses

To evaluate the data gathered for the study, analysis of variance (ANOVA, SPSS version 16.0) was employed. Duncan'smultiple analysis was conducted when a significant effect ($p < 0.05$) was observed from the treatments. The implementation of tendency occurred when $0.05 \leq p < 0.10$.

RESULTS AND DISCUSSION

Chemical composition and antioxidant activity of non- and fermented purple sweet potato flour are presented in Table 3. The concentrations of crude protein, crude fibre, crude fat and crude ash increased with fermentation using *S. cerevisiae*. There was a

decline in antioxidant activity following fermentation. Typically, fermentation is identical with increasing the nutritional value of feed ingredients (Sugiharto & Ranjitkar, 2019). In line with the latter study, the fermentation carried out increased the crude protein, crude fat and crude ash content in purple sweet potato flour in this study. With protein, crude fat and crude ash content in purple sweet potato flour in this study. With respect to the crude fibre, fermentation using *S. cerevisiae* increased the crude fibre content of the purple sweet potato flour. In contrast, Sugiharto & Ranjitkar (2019) have confirmed that fermentation reduces the amount of crude fibre in feed ingredients. However, our results were consistent with that of Sugiharto et al. (2018a), who found that the fermentation of herbal medicine waste with *Bacillus* bacteria increased its fibre content. They also proposed that the breakdown of complex carbohydrates (polysaccharides) into simpler fibre (oligosaccharides), which led to an increase in crude fibre content and a decrease in carbohydrate content, was the cause of the increased fibre content in the fermented ingredients. In term of antioxidant activity, our finding showed that *S. cerevisiae*-fermentation reduced antioxidant activity of sweet potato flour. In agreement, Sugiharto et al. (2018b) noted the reduced antioxidant activity of the herbal medicine waste with *Bacillus subtilis.* One possible explanation for this situation could be the breakdown of phenolic compounds that occurs during fermentation. In fact, during the fermentation process, phenolics are reactive compounds that can be destroyed by enzymatic and/or non-enzymatic reactions (Sugiharto et al., 2018b).

Table 3. Chemical compositions and antioxidant activity of purple sweet potato flour*

Chemical compositions	Purple sweet potato flour	Fermented purple sweet potato flour
Crude protein (%)	2.55	3.43
Crude fibre (%)	0.60	1.89
Crude fat (%)	1.20	1.39
Crude ash (%)	3.89	4.98
Antioxidant activity (% inhibition)	1.55	0.27

*Analyses were conducted in duplicate, and not statistically analysed.

Fig. 1 shows the weight gain, feed intake and FCR of broilers. It was apparent that T2 chicks had greater ($p < 0.05$) weight gain than the chicks in T1 groups, but did not differ from those in T0 group. FCR was better ($p < 0.05$) in T2 than in T1, but did not vary from that of T0 chicks. There was no substantial effect of dietary treatments on feed intake of broilers. There were no dead or sick chickens found during this present study. The use of purple sweet potato flour, whether fermented or not, was intended to reduce the proportion of corn as an energy source for broiler chickens. In this study, the use of either fermented or non-fermented purple sweet potato flour had no detrimental effects on the broiler chickens' feed intake, weight gain, and FCR during the rearing phase. An interesting finding was seen in this research, in which broiler chickens that were fed fermented purple sweet potato flour had better weight gain and FCR than those that were fed non-fermented purple sweet potato flour. Hence, the benefits of fermentation for maximizing the use of purple sweet potato flour by broiler chickens can be confirmed by these data. As mentioned above, fermentation can improve the nutritional content of purple sweet potato flour. In this respect, improving the nutrient content may increase nutrient availability and chicken growth. Apart from improving nutrition, fermentation of purple sweet potato flour using *S. cerevisiae* could be associated with the use of probiotics in broiler feeds, which has an impact on improving intestinal health and

chicken function. Indeed, *S. cerevisiae* is one of the probiotic microbes that is reported to improve the growth performance of broiler chickens (Sugiharto, 2016). In particular, when it came to feed intake, feeding birds purple sweet potato flour or fermented purple sweet potato flour tended to reduce their feed consumption, even though the difference was not statistically significant. Our results were consistent with those of Maphosa et al. (2003), who observed a drop in broiler feed consumption when fed sweet potato flour because the sweet potato was not well-palatable or well-accepted by the broiler chickens.

The values of MCH and MCHC were higher ($p < 0.05$) in T2 than in T0 and T1 groups, while there was a tendency ($p = 0.08$) that haemoglobin values were lower in in T2 than in T0 and T1 groups. Moreover, there was a strong tendency ($p = 0.05$) that heterophils were higher in T2 than in T0 and T1 groups. The data on complete blood counts are listed in Table 4. Data in the current study showed that the chicks that received fermented purple sweet potato flour had lower MCH and MCHC values than the control group and chicks that received non-fermented purple sweet potato flour. The haemoglobin value was also lower in the fermented purple sweet potato flour group than in the purple sweet potato flour and control groups, which is consistent with these two parameters. According to earlier studies conducted by Pratama et al. (2022), feeding broiler chickens fermented wheat bran reduced the amount of MCH and haemoglobin in the chickens. They suggested that the decline in the risk of infection in chickens fed fermented ingredients was closely related to the decline in haemoglobin and MCH levels.

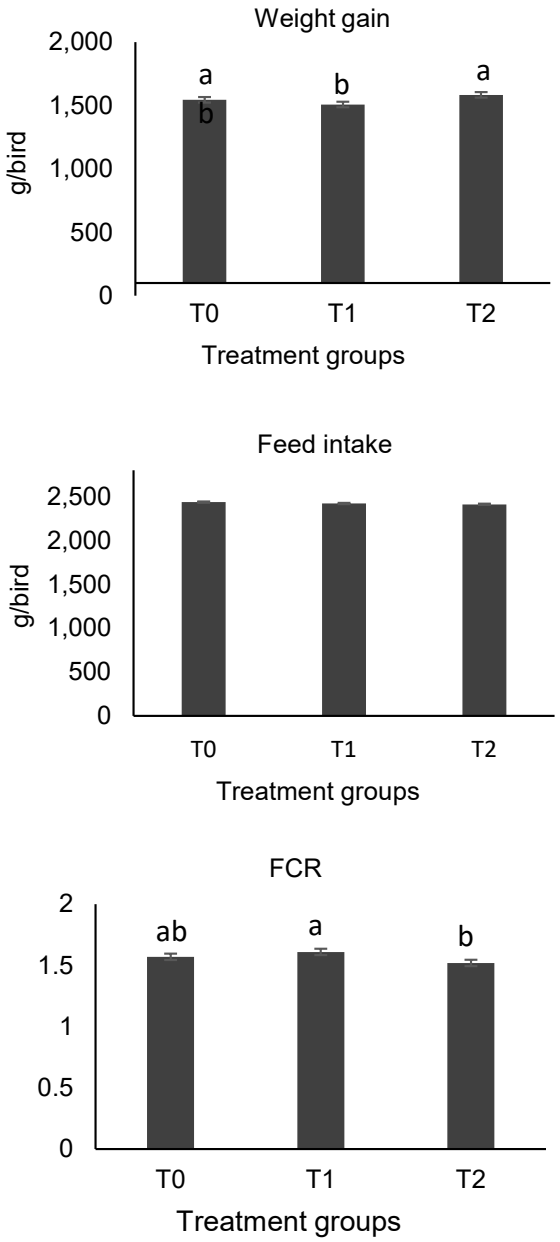

Figure 1. Weight gain, feed intake and FCR of broiler chickens.

[a,b]Means with divergent superscripts among the column differ significantly ($p < 0.05$). T0: chicks were provided with corn-soybean meal-based diet; T1: chicks were provided with diet containing 15% unfermented purple sweet potato flour; T2: chicks were provided with diet containing 15% fermented purple sweet potato flour; FCR: feed conversion ratio.

Haemoglobin typically functions as a transporter of oxygen used for energy metabolism. In many cases, infected chickens require more energy for recovery, so they have higher numbers of haemoglobin and MCH to transport the oxygen supplied to the cells. In regard to the infection, the heterophils value was significantly higher in the fermented purple sweet potato flour group compared to the control group. Hidayah et al. (2021) revealed that the high value of heterophils has an effect on bolstering chicken immunity, reducing the risk of infection in broiler chickens. The mechanism by which fermented feed can improve the immune system in chickens has been discussed in detail by Sugiharto & Ranjitkar (2019). They explained that fermented feed can improve the composition of bacteria in the intestine so that it has a positive impact on the immune response of broiler chickens.

Table 4. Complete blood counts of broiler chickens

Items	T0	T1	T2	SE	*p* value
Erythrocytes (10^{12}/L)	1.66	1.65	1.54	0.04	0.51
Haemoglobin (g dL^{1})	6.63	6.77	5.74	0.20	0.08
Haematocrits (%)	28.4	28.2	26.4	0.80	0.56
MCV (fl)	172	171	172	1.45	0.94
MCH (pg)	39.9[a]	40.8[a]	36.9[b]	0.58	0.01
MCHC (g/dL)	22.7[a]	23.5[a]	21.2[b]	0.34	0.01
RDW-SD (10^{-15} L)	48.9	47.5	34.8	3.22	0.14
RDW-CV (%)	9.81	9.63	13.6	1.75	0.60
Leukocytes (10^{9}/L)	57.9	62.7	63.7	2.02	0.48
Heterophils (10^{9}/L)	1.34	2.11	4.23	0.51	0.05
Lymphocytes (10^{9}/L)	56.6	60.6	59.5	1.73	0.64
Thrombocytes (10^{9}/L)	84.7	87.6	64.4	9.35	0.57

[a,b]Means with divergent superscripts within the similar row differ significantly ($p < 0.05$).
T0: chicks were provided with corn-soybean meal-based diet; T1: chicks were provided with diet containing 15% unfermented purple sweet potato flour; T2: chicks were provided with diet containing 15% fermented purple sweet potato flour; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-SD: red cell distribution width standard deviation; RDW-CV: red cell distribution width coefficient variation, SE: standard error of the means.

The data on serum biochemistry of broiler chickens are presented in Table 5.

Table 5. Serum biochemistry of broiler chickens

Items	T0	T1	T2	SE	*p* value
Total cholesterol (mg dL^{-1})	109[a]	78.7[b]	96.1[ab]	5.18	0.04
Total triglyceride (mg dL^{-1})	85.9	80.1	78.2	6.53	0.89
LDL (mg dL^{-1})	17.7	7.20	13.9	1.88	0.06
HDL (mg dL^{-1})	74.6[a]	55.4[b]	66.7[ab]	2.90	0.01
Total protein (g dL^{-1})	2.19[a]	1.10[b]	1.15[b]	0.15	< 0.01
Albumin (g dL^{-1})	0.46	0.49	0.51	0.08	0.96
Globulin (g dL^{-1})	1.74[a]	0.61[b]	0.64[b]	0.18	0.01
Uric acid (mg dL^{-1})	7.92	5.32	6.37	0.52	0.12
Creatinine (mg dL^{-1})	0.33	0.67	0.77	0.21	0.70

[a,b]Means with divergent superscripts within the similar row differ significantly ($p < 0.05$).
T0: chicks were provided with corn-soybean meal-based diet; T1: chicks were provided with diet containing 15% unfermented purple sweet potato flour; T2: chicks were provided with diet containing 15% fermented purple sweet potato flour; LDL: low-density lipoprotein; HDL: high-density lipoprotein, SE: standard error of the means.

The levels of total cholesterol and HDL were higher ($p < 0.05$) in T0 when compared to that in T1, but was not different from T2 chicks. The level of LDL tended ($p = 0.06$) to be lower in T1 than that in T0. Total protein and globulin were higher ($p < 0.05$) in T0 than that in T1 and T2 chicks. In this study, total cholesterol, LDL and HDL values were lower in the group of chickens receiving non-fermented purple sweet potato flour compared to chickens that received control feed. However, significant differences were not found when compared with chickens that received fermented purple sweet potato flour. Various factors influence the cholesterol profile in the serum of broiler chickens, one of which is the antioxidant content in the feed. Shen et al. (2019) confirmed that antioxidant components such as flavonoids and polyphenols can influence metabolism thereby reducing cholesterol content of broiler chickens. Given that purple sweet potato flour has an antioxidant capacity, using it in this study was probably going to enhance that capacity and had an effect on lowering HDL, LDL, and cholesterol in broiler chicken serum. In this study, the chickens fed fermented purple sweet potato flour did not experience significant reductions in cholesterol, LDL, or HDL. As previously mentioned, the antioxidant capacity of purple sweet potato flour is decreased during the fermentation process using *S. cerevisiae*. Because of this, there is little discernible effect of fermented purple sweet potato flour on the cholesterol profile.

The use of purple sweet potato flour or fermented purple sweet potato flour had an impact on reducing total protein and globulin in broiler chicken serum. In this study, serum albumin levels were not affected by the dietary intervention applied during the study. Considering that the globulin value is the difference between total protein and albumin, the decrease in total protein in broiler chicken serum was very likely related to the decrease in globulin in broiler chicken serum. Globulin is a precursor for immunoglobulin which is responsible for the immunity of broiler chickens. As previously mentioned, the low risk of infection in chickens was very likely the reason for the low need for globulin for the production of immunoglobulins. In line with this inference, Wu et al. (2018) reported that the use of oridonin improved *Salmonella*-induced immune responses and reduced immunoglobulin concentrations (IgA and IgG) in the jejunum of broiler chickens.

Table 6. Selected bacteria count in the ileum and caecum of broiler chickens

Items	T0	T1	T2	SE	*p* value
Ileum (log cfu/g)					
LAB	2.38	2.77	4.00	0.31	0.08
Coliform	1.58	1.29	1.53	0.26	0.90
LNE	1.00	1.00	1.32	0.11	0.38
LAB/coliform	1.84^{b}	2.25^{ab}	3.42^{a}	0.27	0.04
Caecum (log cfu/g)					
LAB	8.06	7.91	7.98	0.11	0.87
Coliform	5.11	4.39	4.52	0.14	0.10
LNE	2.80	1.33	2.03	0.28	0.09
LAB/coliform	1.59	1.83	1.82	0.06	0.26

^{a,b}Means with divergent superscripts within the similar row differ significantly ($p < 0.05$).
T0: chicks were provided with corn-soybean meal-based diet; T1: chicks were provided with diet containing 15% unfermented purple sweet potato flour; T2: chicks were provided with diet containing 15% fermented purple sweet potato flour; LAB: lactic acid bacteria; LNE: lactose negative *Enterobacteriaceae*; SE: standard error of the means.

The LAB to coliform ratio in the ileum was higher ($p < 0.05$) in T2 than in T0, but did not significantly different from that in T1 chicks (Table 6). The counts of LAB tended ($p = 0.08$) to be greater in T2 than in other treatment groups of chickens. In the

caecum, the T1 chicks tended (p = 0.09) to have a smaller number of LNE as comparedto that of T0 chicks. When compared with the control feed, the use of fermented purple sweet potato flour in the feed significantly increased the population of LAB and the LAB to coliform ratio in the ileum of broiler chickens. These results were as expected, as according to Sugiharto & Ranjitkar (2019), fermented feed with a high organic acid content can support the growth of LAB in the small intestine of broilers. A higher LAB to coliform ratio was observed in the group of chickens fed feed containing fermented purple sweet potato flour due to the high number of LAB in those birds and the comparatively constant number of coliforms in those birds across treatment groups. In this study, it was alsoseen that there was a lower population of LNE in the cecum of broiler chickens that received non-fermented purple sweet potato flour compared to controls. The reason for the lower counts of LNE in the caecum of chickens given purple sweet potato flour remains unclear. However, the content of anthocyanins (possessing antibacterial properties) (Edi et al., 2018) in purple sweet potato flour may inhibit the growth of LNE in the caecum of broiler chickens.

Table 7 shows the morphology of the small intestine of broiler chickens. The group of chicks in T2 tended (p = 0.09) to have a lower crypt depth as compared particularly to the T0 group. The villi height, crypt depth as well as the ratio of villi height to crypt depth did not differ in the duodenum and jejunum of broiler chickens. In this study, it was observed that the use of fermented purple sweet potato flour in feed was associated with the reduced crypt depth, especially in the ileum of broilers. Study shows that crypt depth increases are considered to be a result of damage to epithelial cells lining the villi or increased epithelial cell turnover requiring replacement cells from the crypt to prevent loss of absorptive surface area (Cloft et al., 2023). Based on these conditions, it can be inferred that the use of fermented purple sweet potato flour may result in reduced potential for damage to epithelial cells in the ileum. In this context, the use of fermented purple sweet potato flour could improve the ecological conditions in the intestine, and hence the potential for damage to epithelial cells in the intestine can be minimized (Sugiharto & Ranjitkar, 2019). The latter inference was actually supported by the fact in this study that feeding fermented purple sweet potato flour enhanced the LAB countsand LAB to coliform ratio in the ileum of broilers.

Table 7. Intestinal villous height and crypt depth of broiler chickens

Items	T0	T1	T2	SE	p value
Duodenum					
VH (µm)	1,202	1,304	1,390	61.6	0.48
CD (µm)	221	251	223	8.45	0.28
VH/CD ratio	5.47	5.29	6.39	0.31	0.30
Jejunum					
VH (µm)	1,235	1,307	1,194	80.9	0.85
CD (µm)	255	242	281	11.8	0.40
VH/CD ratio	4.74	5.55	4.25	0.30	0.20
Ileum					
VH (µm)	752	748	676	30.7	0.54
CD (µm)	235	197	178	10.9	0.09
VH/CD ratio	3.31	3.89	3.93	0.19	0.35

T0: chicks were provided with corn-soybean meal-based diet; T1: chicks were provided with diet containing 15% unfermented purple sweet potato flour; T2: chicks were provided with diet containing 15% fermented purple sweet potato flour; VH: villous height; CD: crypt depth; SE: standard error of the means.

Purple sweet potatoes are now becoming increasingly popular as a human food due to their high nutritional value and functional properties (Dereje et al., 2020). This increases the economic value of purple sweet potatoes. Based on these conditions, apart from the positive influence of the fermented purple sweet potato on broiler health and productivity, the use of purple sweet potato or fermented purple sweet potato as an energy source feed ingredient for broiler chickens was considered less efficient, particularly during seasons when corn supplies can meet broiler feed needs. However, the positive values of fermentedpurple sweet potato as a functional feed ingredient may be exploited to improve the physiological and health conditions of broilers, particularly after the ban on the use of antibiotic growth promoters in feed and efforts to reduce chemical-based feed additives for chickens.

CONCLUSIONS

Feeding fermented purple sweet potato flour contributes for the better growth, feed conversion, immune defence, bacterial population and morphology of the small intestine

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