



# The Effects of Dietary Inclusion of Miana Plant Flour (*Plectranthus scutellarioides* (L.) R. Br. on Serum Lipid Profile and Organ Weights of Broiler Chickens

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## ABSTRACT

Miana plant (*Plectranthus scutellarioides* (L.) R. Br. contains active compounds (such as steroids, flavonoids, saponins, and tannins) which can have several health benefits, including lowering cholesterol LDL and triglyceride as well as increasing feed consumption, body weight, and carcass weight of broilers. Therefore, the current experiment was conducted to evaluate the effect of Miana plant flour (*Plectranthus scutellarioides* (L.) R. Br. in the diet on blood serum lipid profiles and physiological organs of broilers. The experiment was performed on 100 day-old broiler chickens from strain Arbor Acres CP-707. The experiment was designed in a completely randomized design with five different levels of Miana plant flour (0%, 5%, 7.5%, 10%, and 12.5%) in broiler's diets as treatment, and each treatment was repeated four times. The diet was arranged iso-protein (21%) and iso-energy (2900 kcal/kg). The serum lipid profile measurement included the analysis of total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Physiological organ analysis entailed the percentage of liver weight, pancreas weight, gizzard weight, small intestine weight, and length of parts of the small intestine (duodenum, jejunum, and ileum) of broilers. The results showed that the inclusion of Miana plant flour in the broiler's diet could significantly affect total cholesterol, triglycerides, HDL, and LDL in the serum of broiler chickens, and it affected duodenum length significantly. Furthermore, the inclusion of Miana plant flour in the broiler's diet had an insignificant effect on the percentage of liver weight, pancreas weight, gizzard weight, small intestine weight, and length of each part of the small intestine (jejunum and ileum) on broilers. In conclusion, the inclusion of Miana plant flour as much as 12.5% in broiler's diets reduced total cholesterol, triglycerides, and LDL, and increased the HDL and duodenum length without adverse effects on the other physiological organs of broiler chickens.

**Keywords:** Broiler, Lipid profile, Lipoproteins, Miana plant, Physiological organs

## INTRODUCTION

*Plectranthus scutellarioides* (L.) R. Br. plant is widely grown in Asian countries. This plant is known by local names in Indonesia, such as Miana, Jawer Kotok, and Iler. According to the Decree of the Indonesian Minister of Agriculture, it is mainly used for medicinal properties and included in 66 biopharmaceutical plant commodities (Salim and Munadi, 2017). Previous researchers reported that Miana plants have pharmacological activities, such as antimicrobial, anthelmintic, antifungal, antibacterial, anti-inflammatory, antioxidant, antidiabetic, and antihistamine activities (Muljono et al., 2016; Novanti and Susilawati, 2017; Wakhidah and Silalahi, 2018).

Miana plants contain active compounds, such as tannins, saponins, anthocyanins, flavonoids, essential oils that can reduce plasma cholesterol and triglyceride levels (Al-Temimi and Choudhary, 2013; Warditiani et al., 2015; Kusuma et al., 2016). Tannin was reported to inhibit cholesterol biosynthesis, reduce cholesterol absorption in the intestine, and so cholesterol can be excreted out of the body (Al-Temimi and Choudhary, 2013). Furthermore, saponins compound was reported to bind cholesterol in the intestinal lumen, prevent cholesterol reabsorption, and bind bile acids to reduce the enterohepatic circulation of bile acids leading to increased cholesterol excretion (Alkanji et al., 2009; Khyade and Vaikos, 2009).

The compounds in the Miana plant are expected to make the digestive tract of broilers healthier because they can kill harmful worms and microbes so that digestive organs, such as the small intestine can develop properly. However, some of the active compounds found in Miana plants, such as tannins, alkaloids, and saponins, have been reported to be anti-nutritive at certain levels in poultry diets, and affect their physiological organs. Wicaksono et al. (2015) reported that damage found in the widening of the central vein of mice was thought to be caused by alkaloid compounds. The administration of 10% *Calliandra calothyrsus* leaf flour containing tannins reduced ration consumption, body weight

ORIGINAL ARTICLE  
 pii: S232245682200006-12  
 Received: 10 January 2022  
 Accepted: 26 February 2022

gain, protein consumption, and increased ration conversion of broilers (Wati et al., 2018). Furthermore, Mahata et al. (2021) reported Miana plant flour could be used up to 12.5% without adverse effects on broilers' performance.

Blood is a transporter of food substances and other substances obtained from absorption in the digestive tract to be distributed throughout the body. The lipid content in the blood serum can determine the lipid disposition in the cells of the body organs, including physiological organs. If the lipid content in the serum is too high, it may harm the physiological organs and interfere with livestock health. The accumulation of fat causes an increase in triglycerides that enter the spleen, thereby increasing the size and activity of the spleen (Windoro et al., 2020)

Based on the description of the active compounds in the Miana plant and the beneficial role of active compounds in animal health, the present research was carried out to evaluate the effect of Miana plant flour (*Plectranthus scutellarioides* (L.) R. Br. in the diet on blood serum lipid profiles and physiological organs of broiler chickens.

## MATERIALS AND METHODS

### Ethical approval

The broiler chickens in the current research were treated following the guidelines passed by the institutional ethics committee for the care of animals and were approved by the Animal Ethics Committee of the Universitas Andalas, Padang, Indonesia, with a code of 439/UN.16.2/KEP-FK/2021.

### Experimental birds

A total of 100 male day-old broiler chickens (DOC) of strain Arbor Acres CP-707 was purchased at a poultry shop in West Sumatra Province, Indonesia, for the current study.

### Experimental design

This experiment was conducted in a completely randomized design with different levels of Miana plant flour as treatments (0%, 5%, 7.5%, 10%, and 12.5%) in the broiler's diet, and each treatment was repeated four times. Miana plant flour was mixed with other feed ingredients according to the predetermined treatment level until homogeneity was reached and became the treatment feed in the current study.

### Experimental diet

The treatment diets were self-prepared, including soybean meal, meat flour, yellow corn, coconut oil, Bravo CP 511 (commercial diet), top mix, Miana plant flour (*Plectranthus scutellarioides* (L.) R. Br. (Table 1). All ingredients were mixed homogeneously before feeding the broiler. Broiler chickens were reared for an adaptation period from the second day up to day seven of DOC by giving a commercial diet (Bravo Cp 511) and it was then continued with a treatment diet containing Miana plant flour from 8-35 days.

**Table 1.** Composition of experimental diets used for different groups of broiler chickens

Feedstuffs	Composition of experimental diets				
	A	B	C	D	E
Yellow corn	53.50	48.75	46.75	44.75	42.75
Soybean meal	10.00	9.50	9.00	8.50	8.00
Coconut oil	0.00	0.75	1.25	1.75	2.25
Meat flour	14.00	14.00	14.00	14.00	14.00
Top mix	2.50	2.00	1.50	1.00	0.50
Miana plant flour	0.00	5.00	7.50	10.00	12.50
Bravo Cp 511	20.00	20.00	20.00	20.00	20.00
Total	100.00	100.00	100.00	100.00	100.00
<b>Diet nutrients content (%) and metabolizable energy (kcal/kg)</b>					
Crude protein	21.30	21.40	21.36	21.32	21.28
Crude fiber	3.19	4.12	4.58	5.05	5.51
Crude fat	4.05	5.13	5.80	6.47	7.15
Calcium	0.73	0.75	0.76	0.76	0.77
Available phosphorus	0.36	0.37	0.38	0.38	0.38
Metabolizable energy	2992.75	2948.94	2944.58	2940.21	2935.85
Crude protein	21.30	21.40	21.36	21.32	21.28
Lysin	0.22	0.20	0.17	0.14	0.12

A: 0% Miana plant flour, B: 5% Miana plant flour, C: 7.5% Miana plant flour, D: 10% Miana plant flour, E: 12.5% Miana plant flour

## Preparation of Miana plant flour

Miana plant was collected from several areas in West Sumatra Province, Indonesia. Miana plant was cut by pruning 25 cm height from the soil surface, cleaned, and dried in an oven at 60°C until the water reached 14%, then mashed. Miana plant flour furthermore was ready to use for poultry feed Modified method of (Bradley, 2010).

## The measured parameters

### Total cholesterol

The blood samples were taken from one broiler chicken of each replicate for the evaluation of serum parameters. Broiler blood samples were taken from each chicken in each treatment at the end of the study. The broiler chickens were slaughtered through the esophagus, trachea, and blood vessels carotid artery and jugular vein. Blood from each broiler was collected using 20 vacutainers with a volume of 10 ml for each treatment. Furthermore, the vacutainer was put into a cooler box and then taken to the laboratory. Blood from each vacutainer was centrifuged with a cold centrifuge at 4°C at a speed of 3500 rpm for 10 minutes. Then, the yellowish blood serum was separated at the top of the vortex tube from the red blood platelets at the bottom of the vortex tube. Furthermore, the blood serum was placed in an Eppendorf tube to analyze the content of total cholesterol, LDL, triglycerides, and HDL.

Total cholesterol in the blood serum of broiler chickens was measured by enzymatic colorimetric method/Cholesterol Oxidase-Peroxidase Aminoantipyrin/CHOD-PAP Method (Laboratory Stanbio, 2011). Blood serum was pipetted as much as 10 µl, and then 1000 µl DiaSys KIT LO reagent 60128 for cholesterol analysis was added. Serum and reagent were shaken to mix thoroughly and incubated for 10 minutes at 37°C, then read with a UV-1800 spectrophotometer (Shimadzu USA MFG Inc serial number A116349) at a wavelength of 546 nm. Cholesterol standards were prepared by mixing 10 µl of LOT 26521 cholesterol standard solution with DiaSys KIT LOT reagent 60128448 as much as 1000 µl. In the next step, it was homogenized and incubated at 37°C for 10 minutes, then read with a UV-1800 spectrophotometer (Shimadzu USA MFG Inc serial number A116349) at a wavelength of 546 nm. The total cholesterol was calculated using formula 1.

$$\text{Total cholesterol (mg/dl)} = \frac{\Delta a \text{ sample}}{\Delta a \text{ standard}} \times \text{standard concentration (mg/dL)} \quad (\text{Formula 1})$$

Where,  $\Delta a$  sample signifies sample absorbance,  $\Delta a$  standard accounts for standard absorbance, and Standard concentration is 200 mg/dL (Laboratory Stanbio, 2011).

### Triglycerides

Triglycerides in the blood serum of broiler were measured by the Glycerol Phosphate Oxidase (GPO-PAP, Fossati and Prencipe, 1982). This method is based on the enzymatic determination of glycerol using the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase. In this regard, 10 µl of blood serum was pipetted, and 1000 µl of DiaSys KIT LOT 60128416 reagent was added. Serum and reagents were shaken to mix well, then incubated for 10 minutes at 37°C. Furthermore, it was read using a UV-1800 spectrophotometer (Shimadzu USA MFG Inc serial number A116349) at a wavelength of 546 nm. The triglyceride standard was made by mixing 10 µl of standard solution for triglycerides LOT 24313, followed by adding 1000 µl of DiaSys KIT LOT 60128416 reagent, homogenizing and incubating for 10 minutes. In the next step, it was read by a UV-1800 spectrophotometer (Shimadzu USA MFG Inc serial number A116349) at a wavelength of 546 nm. The triglycerides were calculated by formula 2 as below:

$$\text{Triglycerides total (mg/dL)} = \frac{\Delta a \text{ sample}}{\Delta a \text{ standard}} \times \text{standard concentration (mg/dL)} \quad (\text{Formula 2})$$

Where,  $\Delta a$  sample signifies sample absorbance,  $\Delta a$  standard accounts for standard absorbance, and Standard concentration is 200 mg/dL (Laboratory Stanbio, 2011).

### High-density lipoprotein

High-density lipoprotein (HDL) in the blood serum of broiler was measured by the enzymatic colorimetric method/Cholesterol Oxidase-Peroxidase Aminoantipyrin/CHOD-PAP Method (Laboratory Stanbio, 2011). The volume of 250 µl blood serum was pipetted, then DiaSys KIT LOT 60128539 reagent was added for HDL analysis of 500 µl, then centrifuged for 10 minutes 2500 rpm, then centrifuged for 10 minutes at 2500 rpm. The supernatant formed after centrifugation was pipetted as much as 100 µl, and added with 1000 µl cholesterol reagent DiaSys KIT LOT 60128448. Furthermore, the solution mixture was incubated at 37°C for 10 minutes and read by a UV-1800 spectrophotometer (Shimadzu USA MFG Inc serial number A116349) at a wavelength of 546 nm.

The HDL standard was made by mixing 100 µl cholesterol standard (LOT 26521) with 1000 µl of DiaSys KIT LOT reagent 60128448. Furthermore, it was homogenized and incubated for 10 minutes at 37°C, then read with a UV-1800 spectrophotometer (Shimadzu USA MFG Inc. serial number A116349) at a wavelength of 546 nm. The HDL was calculated by formula 3 as below:

$$\text{HDL (mg/dL)} = \frac{\Delta a \text{ sample}}{\Delta a \text{ standard}} \times \text{standard concentration (mg/dL)} \quad (\text{Formula 3})$$

Where,  $\Delta a$  sample signifies sample absorbance,  $\Delta a$  standard accounts for standard absorbance, and Standard concentration is 200 mg/dL (Laboratory Stanbio, 2011).

### ***Low-density lipoprotein***

The LDL in the blood serum of broiler was measured by using the formula 4 of Friedewald et al. (1972) as below:

$$\text{LDL} = \text{Total cholesterol} - \text{HDL} - 1/5 \text{ Triglycerides} \quad (\text{Formula 4})$$

### ***Physiological organs***

Sampling and measurement of physiological organs of broilers were carried out at the age of 35 days.

Sample collection of physiological organs of broiler was taken from one bird for each treatment. This experiment consisted of 5 treatments and each treatment was replicated four times so that the total sample for physiological organs analysis was 20 samples. It was randomly selected from 100 chickens that were weighed and slaughtered following animal welfare laws. Before slaughter, the chickens fasted for 8 hours. After slaughter, physiological organs, such as the liver, pancreas, gizzard, small intestine (duodenum, jejunum, and ileum) were separated and weighed on a 0.001 g digital scale. Percentage of the weight of each physiological organ, calculated by the formula of Relative weight = (weight of organs/live weight) × 100% (Nastain et al., 2021). Furthermore, the lengths of the duodenum, jejunum, and ileum (cm) were measured.

### **Statistical analysis**

All data obtained in this experiment were statistically processed by analysis of variance (ANOVA). Differences among treatments were followed by analysis with Duncan's Multiple Range Test (Steel and Torrie, 1991). P value less than 0.05 was considered statistically significant.

## **RESULTS AND DISCUSSION**

Statistical results of total cholesterol, triglycerides, HDL, and LDL are shown in Table 2. The liver, pancreas, gizzard, and small intestine weight in percentage are depicted in Table 3. Furthermore, the analysis of the length of the parts of the small intestine (duodenum, jejunum, and ileum) is presented in Table 4. The inclusion of Miana plant flour in broilers' diets had a significant effect on total cholesterol, triglycerides, HDL, LDL in blood serum, and duodenum length of broilers ( $p < 0.05$ ). In contrast, it had no significant effect on the liver weight, pancreas weight, gizzard weight, small intestine weight, jejunum length, and ileum length ( $p > 0.05$ ).

The control diet (0% Miana) and inclusion of Miana (*Plectranthus scutellarioides* (L.) R. Br. plant flour as much as 5% had no significant effect on reducing total cholesterol, however, when Miana increased to 7.5%, 10%, and 12.5%, the total cholesterol in blood serum decreased. The decrease of total cholesterol in the current experiment was due to some compounds, such as tannins, saponins, anthocyanins, flavonoids, and essential oils, in the Miana plant that can reduce plasma cholesterol and triglyceride levels (Al-Temimi and Choudhary, 2013; Warditiani et al., 2015; Kusuma et al., 2016). Miana plant contains 18.15% tannin, which could inhibit cholesterol biosynthesis and reduce cholesterol absorption in the intestine leading to cholesterol excretion (Al-Temimi and Choudhary, 2013). As Zaubaidah et al. (2014) stated, tannin inhibits HMG-CoA reductase activity in cholesterol synthesis in cells so that HMG-CoA can not convert to mevalonate compound and cholesterol synthesis is inhibited. Saponins bind cholesterol in the intestinal lumen, prevent cholesterol reabsorption, bind bile acids to reduce the enterohepatic circulation of bile acids, and increase cholesterol excretion (Alkanji et al., 2009; Khyade and Vaikos, 2009). Furthermore, Miana plant (*Plectranthus scutellarioides* (L.) R. Br. contains anthocyanins as much as 0,435 mg/g (Ayu et al., 2018), while Jatmiko (2015) reported the anthocyanin content of Miana leaves as  $441.97 \pm 34.22$  mg/100 g. Kusuma et al. (2016) reported the inclusion of Dayak onion extract containing anthocyanins could reduce cholesterol and triglyceride blood in male rats. In addition, flavonoids were reported lowering cholesterol by reducing HMG-CoA reductase activity, acyl-CoA cholesterol acyltransferase (ACAT) activity, and reducing cholesterol absorption in the digestive tract (Rumanti, 2011; Sumardika and Jawi, 2012). It was predicted that the activity of some compounds found in the Miana plant reduced total cholesterol in the blood serum of broilers in the current experiment.

The active compounds in Miana plants, such as tannins and flavonoids, are reported to reduce triglycerides in the blood serum of broiler chickens. Miana plants contain active compounds, such as tannins, saponins, anthocyanins, flavonoids, essential oils that can reduce plasma cholesterol and triglyceride levels (Al-Temimi and Choudhary, 2013; Warditiani et al., 2015; Kusuma et al., 2016). The levels of Miana plant flour at 0% and 5% did not reduce triglycerides in the blood serum of broilers in this experiment. When levels were increased to 7.5%, 10%, and 12.5% in diets, triglycerides in the blood serum of broiler reduced, however, levels of 10% and 12.5% did not show another decline. According to Meirindasari et al. (2013), tannin compounds reduce triglycerides by decreasing the absorption of cholesterol and triglycerides in the small intestine and increasing the excretion of bile acids. The other mechanism of tannin reduces triglyceride by inhibiting adipogenesis and absorption in the intestine (Rosyadi, 2014). Flavonoid compounds were also reported to inhibit the fatty acid synthase enzyme activity, which is very important in fat

metabolism (Tian et al., 2011). Inhibition of fatty acid synthase can directly reduce the formation of fatty acids, thereby reducing the appearance of triglycerides.

Including Miana plant flour in the broilers' diet at a level of 12.5% was the best level to increase HDL in the blood serum of broiler chickens, but the lower levels of Miana flour decreased HDL in the blood serum of broilers. Flavonoid compound increased HDL of blood serum by influencing the synthesis of apolipoproteins (apolipoprotein A-1 and apolipoprotein A-2) as components of HDL. According to Zychlinski and Kleffmann (2014), apolipoprotein A-1 and apolipoprotein A-2 are the main structural protein components of high-density lipoprotein (HDL). Flavonoids also reduce plasma cholesterol and the formation of very-low-density lipoprotein (VLDL) synthesized by the liver, and consequently, increase HDL cholesterol levels (Narita, 2015).

The inclusion of Miana plant flour at the range of 7.5-12.5% significantly reduced LDL in the blood serum of the broilers in the current study. According to Babu and Liu (2008), flavonoid compounds could inhibit the synthesis of apolipoprotein B, one of the LDL constituent compounds; the higher the level of flavonoid content in the diet, the less the formation of apolipoprotein B, thereby suppressing the formation of LDL. According to Wang et al. (2006), flavonoids cause bile acids to be bound in the intestine so that the blood cannot reabsorb them, then the liver produces more bile to replace the lost bile using LDL cholesterol in the blood, thereby reducing the amount of LDL cholesterol in the blood. As Yunarto and Aini (2015) mentioned, flavonoid compounds reduce LDL by increasing bile acid excretion.

The inclusion of Miana plant flour in broiler diets did not affect the liver weight percentage. The content of essential oils found in Miana plants can function as antioxidants that protect liver cells from free radicals. Lee et al. (2005) and Hussain et al. (2008) found that the essential oil in basil leaves is anti-oxidant that can protect body cells from free radicals, including liver cells (hepatoprotective). Besides, the flavonoid, orientin, eugenol, and vicenin in basil leaves have hepatoprotective properties because they are also anti-oxidants that can protect liver cells from free radicals. Thus, the content of essential oils, flavonoids, and eugenol in the Miana plant in this study is thought to protect broilers' livers from the influence of other active substances, such as alkaloids that can increase the liver size. In the current study, it was revealed that the substances found in Miana plant flour did not harm the broiler's liver.

The flavonoids, tannins, and saponins found in Miana plant flour did not interfere with the pancreas weight percentage. Rohmah et al. (2016) stated that giving soursop leaf (*Annona muricata* L.) containing flavonoid, tamarins, tannins, and saponins did not affect the weight of the pancreas, and this was possible because these compounds were not directly related to the performance of the pancreas, whose function was to produce enzymes. The low tannin content in soursop leaf flour does not interfere with enzymes produced by the pancreas gland. The high tannin content in the diet can cause enlargement of the pancreas (Darmawan, 2008). In the current study, it was predicted that the concentration of saponins and tannins contained in Miana plant flour was still low, so broilers could tolerate it and no enlargement of the pancreas was observed.

The inclusion of Miana plant flour in the diet did not interfere with the gizzards and small intestines weight percentage of broilers. Active compounds in Miana plant flour, such as tannins, saponins, alkaloids, steroids, essential oils, and eugenol, act as anthelmintic, antimicrobial, and antibacterial compounds (Ridwan et al., 2006; Sangi et al., 2008; Muljono et al., 2016). Among the active compounds in Miana plant flour, such as tannins, saponins, alkaloids, are known to have anti-nutritional properties, and it is feared that they will affect the weight of the gizzard. Pangesti et al. (2016) reported that the inclusion of 5% jackfruit seed flour in feed containing saponin and tannin compounds had a very significant effect on gizzard weight. However, saponins and tannins concentration in Miana plant flour in the current study did not show any significant effect on gizzard weight. It was suspected that the dose of saponins and tannins in Miana plant flour given up to 12.5% in broiler's diet was still low, so that gizzard weight was not affected. The compounds in the Miana plant are expected to make the digestive tract of broilers healthier because they can kill harmful worms and microbes. Chaudhary et al. (2018) state that saponin compounds can increase the immunity, gut health, production, and meat quality, of poultry. Thus, the digestive organs, such as the small intestine, can develop properly, and their weight becomes more significant. Aji et al. (2017) stated that the small intestine weight of broilers fed noni fruit extract containing flavonoid compounds increased significantly; this was due to the ability of flavonoid compounds to improve microflora found in the broiler digestive tract. In the current study, the increase in the level of Miana plant flour in broiler diets did not show a difference in the intestine weight percentage. It is suspected that the dose of the active compound contained on Miana plant flour inclusion in broiler diets is still low, so it does not show a difference in the percentage of small intestine weight. The presence of microflora in the small intestine will affect the health and development of the small intestine and increase nutrient absorption.

The inclusion of Miana plant flour in the broilers' diet affected the duodenum length but did not affect the jejunum and ileum length of the broilers. This shows that the inclusion of Miana plant flour with different levels in the diets affects the length of the broiler duodenum. It is suspected that there is an influence of active substances, such as alkaloids, flavonoids, and tannins, in the Miana plant. Lenhardt and Mozes (2003) reported that the duodenum length is

closely related to the length of the villi and the relative weight of the duodenum, where the more extended the intestinal villi, the wider the surface for nutrient absorption and optimal absorption so that the duodenum is also heavier and longer. Other studies about the inclusion of guava leaves containing alkaloids, flavonoids, and tannins in broilers have been reported to lengthen the duodenal villi (Martinez et al., 2012; Fratiwi, 2015). The jejunum and ileum length of broilers fed Miana plant flour in the current study were not disturbed by the active substances found in the Miana plant.

**Table 2.** The serum lipid profile parameters of broiler chickens fed with different levels of Miana plant flour

Treatments (Miana plant flour%)	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
A (0)	170.17 <sup>a</sup>	151.24 <sup>a</sup>	46.64 <sup>c</sup>	93.28 <sup>a</sup>
B (5)	168.25 <sup>a</sup>	153.52 <sup>a</sup>	41.56 <sup>d</sup>	95.98 <sup>a</sup>
C (7.5)	154.30 <sup>b</sup>	135.95 <sup>b</sup>	52.02 <sup>b</sup>	70.35 <sup>b</sup>
D (10)	148.85 <sup>c</sup>	131.71 <sup>c</sup>	48.87 <sup>bc</sup>	73.64 <sup>b</sup>
E (12.5)	139.96 <sup>d</sup>	131.48 <sup>c</sup>	57.41 <sup>a</sup>	56.26 <sup>c</sup>
SE	1.33	1.03	1.06	1.55
P value	0.01	0.01	0.01	0.01

SE: Standard Error, HDL: High-density lipoprotein, LDL: Low-density lipoprotein. Different lowercase superscripts in the same column show a significant effect ( $p < 0.01$ ).

**Table 3.** The percent body weight of internal organs in broiler chickens fed with different levels of miana plant flour

Treatments (Miana plant flour%)	Liver weight percentage (%)	Pancreas weight percentage (%)	Gizzard weight percentage (%)	Small intestine weight percentage (%)
A (0)	2.00	0.23	2.19	3.54
B (5)	2.15	0.25	2.29	3.15
C (7.5)	2.07	0.22	2.17	3.42
D (10)	1.83	0.21	2.14	3.41
E (12.5)	1.60	0.19	1.93	3.32
SE	0.19	0.02	0.19	0.26
P value	0.05	0.05	0.05	0.05

SE: Standard Error

**Table 4.** Intestinal morphometric parameters of broiler chickens fed with different levels of miana plant flour

Treatments (Miana plant flour%)	Duodenal length (cm)	Jejunum length (cm)	Ileum length (cm)
A (0)	25.04 <sup>b</sup>	76.03	84.28
B (5)	27.60 <sup>ab</sup>	63.85	84.00
C (7.5)	28.38 <sup>a</sup>	73.13	70.80
D (10)	28.48 <sup>a</sup>	70.13	74.93
E (12.5)	28.61 <sup>a</sup>	70.38	74.88
SE	0.72	3.02	4.50
P value	0.05	0.05	0.05

SE: Standard Error. Different lowercase superscripts in the same column (duodenal length) show a significant difference ( $p < 0.05$ ).

## CONCLUSION

The inclusion of Miana plant flour as much as 12.5% in broilers' diets, reduced total cholesterol, triglycerides, and LDL, increased the HDL and duodenum length without adverse effects on the other physiological organs consisting of liver weight, pancreas weight, gizzard weight, small intestine weight, length of jejunum, and ileum in broiler chickens.

### Competing interests

All authors declare that they have no competing interest concerning the work presented in this manuscript.

### Authors' contributions

Maria Endo Mahata participated in all stages of the research, namely the research design, the conduct of the experiment, sample analysis, data analysis, writing, and editing of articles. Misra Weni participated in conducting the investigation, Yola Gusnanda was responsible for data analysis. Takayuki Ohnuma and Yose Rizal participated in the research and editing of the article. All authors participated in writing the article and checking the statistical analysis and finally approved the last version of the article for publishing.

## Acknowledgments

This research was funded by BASIC RESEARCH SKIMMED (SKIM PENELITIAN DASAR) in the 2021 budget. Contract number project from Ministry of Education, Culture, Research, and Technology: 104/E4.1/AK.04.PT/2021 and Contract number project from Research Institution and community service of Universitas Andalas: T/33/UN.16.17/PT.01.03/PD-Pangan/2021. We appreciated the Indonesian Education, Culture, Research, and Technology who provided us the opportunity and financial support to perform this research. The authors also thank the Research Institution and community service of Universitas Andalas, who have facilitated this research.

## Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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