Research Article

Lipid Regulation and Cardiovascular Biomarkers of Native Chickens Fed a Combination of Maggot, *Indigofera* and Turmeric

Sri Purwanti[1*], Wempie Pakiding[1], Marhamah Nadir[1], Nurhayu[2], Kusumandari Indah Prahesti[1], Sitti Nurani Sirajuddin[1], Jasmal Ahmari Syamsu[1,4], Andi Mushawwir[3]

[1]Faculty of Animal Science Universitas Hasanuddin, South Sulawesi, Indonesia; [2]Institute for Agricultural Technology (BPTP) of South Sulawesi, Indonesia; [3]Department of Animal Nutrition and Feed Technology, Animal Science Faculty, University of Padjadjaran, Indonesia; [4]Research and Development Center for Livestock Resources and Tropical Animal, Universitas Hasanuddin, Makassar.

Abstract | Feed plays an important role in the development of local chicken in Indonesia. Feed quality (feed additive and feed supplement) is directly related to an animal's ability to maintain tissue temperature and metabolism, both of which affect the cardiovascular function and energy metabolism in chickens. This research aims to determine the value of lipid regulation and cardiovascular biomarkers of native chickens given a combination of maggot, *indigofera* and turmeric. A total of 120, 4-week-old male indigenous native chickens with an average initial weight of 42-45 g were divided into 3 treatment groups and fed diets, namely P0 (basal feed), P1 (basal feed + maggot mash 25%+ *indigofera* mash 5%+ turmeric mash 2.5%), and P2 (basal feed + maggot mash 20% + *indigofera* mash 10% + turmeric mash 2.5%). The result of this experiment showed that the cardiovascular biomarkers including the CRP (C-reactive protein) high sensitivity, H-FABP (heart-type fatty acid-binding protein), homocysteine, and Gamma-glutamyl transpeptidase found higher (p<0.05) in R0 than R1 and R2. Moreover, the level of adiponectin, apolipoproteins, HDL (high-density lipoprotein) cholesterol, LDL (low density lipoprotein) cholesterol, triglycerides and NEFA (non-esterified fatty acid) was found significantlyr (p<0.05) better in R0 compared to the R1 and R2 groups. These results suggested that inclusion of maggot, *indigofera* and turmeric in the diet of local chickens may affect the lipid regulation and cardiovascular biomarkers.

Keywords | Cardiovascular, Lipid, Native chicken, Larvae, Herbs

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***Correspondence** | Sri Purwanti, Faculty of Animal Science Hasanuddin University, South Sulawesi, Indonesia; **Email:** sripurwanti@unhas.ac.id
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INTRODUCTION

South Sulawesi is a centre for the development and production of local chicken in Indonesia. The topography of Sulawesi varies from the lowlands to the highlands contributing to the biodiversity of plants and animals. Nevertheless, the rearing of local chicken is widespread in all these topographies. Previous research shows that local chicken productivity is strongly influenced by topography or altitude, including other factors such as feed and genetics. Feed plays an important role in animal metabolism, especially in local chickens, whether they are given protein source feed or feed additives in the form of phytobiotics. A range of studies have demonstrated the potential metabolic changes in livestock fed protein and phytobiotic supplements. Drannikov et al. (2020) highlights the benefits of a protein feed supplement derived from red clover plants, which can improve feed intake and normalize the acid-base environment. Gheisar and Kim (2018) and Purwanti et al. (2014) both emphasize the positive effects of phytobiotics on animal performance, including improved

protein digestibility, energy utilization, and growth promotion. Bagno and Kim (2018) further underscores the potential of phytobiotics in livestock feeding, particularly in improving feed digestibility, antimicrobial efficacy, and growth stimulation. These findings collectively suggest that protein and phytobiotic supplements can have a significant impact on the metabolic processes of livestock, potentially leading to improved productivity and health. On the other hand, the results of research on the use of maggots and herbs have been reported by researchers, among others the combination of 25% maggot, 2.5% turmeric, and 5% *Indigofera zollingeriana* in the basal diet can be used as a feed additive as an anti-bacterial (Purwanti et al., 2023), increasing lymphocyte cells to produce antibodies in native chicken in the starter phase (Muhammad et al., 2023), and maggot and *Indigofera* as a substitute for fish meal in native chicken rations (Riswanda et al., 2023; Kasmira et al., 2023). Other research shows that supplementing quail with turmeric powder up to a dose of 54 mg/quail/day (containing 7.97 per cent curcumin) enhances the metabolism and distribution of lipids to different organs through enterohepatic recirculation, abdominal fat deposits, and ovarian follicles (Saraswati et al., 2013).

A combination of maggot, *indigofera*, and herbs in the diet of native chickens has the potential to regulate lipid metabolism. Studies have shown that garlic and ginger, when used in combination, can improve growth and reduce serum cholesterol and triacylglycerol in broiler chickens (Ademola et al., 2009). Similarly, garlic supplementation has been found to decrease total cholesterol, triglycerides, LDL (low density lipoprotein), and VLDL (very low density lipoprotein), while increasing HDL (high density lipoprotein) in chickens (Prasad et al., 2009). *Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil have been shown to lower plasma triglycerides, LDL, and cholesterol, while increasing HDL in laying ducks (Arini et al., 2017). Plant extracts, including those from broccoli, have been found to regulate lipid metabolism in chickens, improving antioxidant capacity and reducing lipid peroxidation (Ding et al., 2023). Lignocellulose in the diet can also influence lipid metabolism, reducing triglycerides and LDL (Boguslawska-Tryk et al., 2016). However, no previous study has reported cardiovascular biomarker-associated heart physiology in the native chicken. Therefore, this experiment aimed to evaluate cardiovascular biomarkers and lipid regulation in native chickens fed diets with different feed treatments.

MATERIAL AND METHODS

Animal sample and study site

In this study, 120 4-week-old male indigenous native chickens were used with an average initial weight ranging from 42 - 45 grams and using cage system. The size of the experimental unit cage for each treatment measuring 2 x 2 m. The birds were divided into 3 treatment groups of 40 heads each and fed diets, namely P0 (basal feed); P1 (basal feed + maggot mash 25%+ *Indigofera* mash 5%+ turmeric mash 2.5%); P2 (basal feed + maggot mash 20% + *Indigofera* mash 10% + turmeric mash 2.5%). The composition of raw materials and feed composition are listed in Table 1 and Table 2. Nutrient requirements for the ration are based on the needs of native chickens in the Indonesian National Standard for Native Chick SNI 7783.2:2013 (SNI, 2013). This study was conducted at the Poultry Production Laboratory, Faculty of Animal Science, Hasanuddin University, South Sulawesi, Indonesia according to international animal ethics rules.

Blood and sample analysis

Using an Ethylenediaminetetraacetic acid (EDTA) tube and a sterile syringe, blood samples (5 mL) were drawn at weeks 4, 8, 12, 16, 20, and 24. The animals' front tail vein was used to draw blood samples early in the morning, before feeding time. The drawn blood sample was placed right away in an ice gel-filled thermos. The specimens were brought to the lab. Within 30 minutes of blood sample collection, the samples were centrifuged at 3,000 g for 10 minutes. The resultant plasma was then promptly frozen at –20°C until analysis. Afterwards, biochemical kits (Randox Laboratories LTD, UK, and Biolabo Biochemistry, France) were used to analyze the cardiovascular biomarkers from blood plasma including sPLA2-IIA (group IIA Secretory Phospholipase A2), H-FABP (Heart-Type Fatty Acid-Binding Protein), homocysteine, Gamma-glutamyl transpeptidase, and CRP (C-Reactive Protein) high sensitivity. Commercial biochemical kits were also used to analyze the triglycerides, HDL (high-density lipoprotein) cholesterol, LDL (low density lipoprotein) cholesterol, and apolipoprotein for lipid regulation. All analytical procedures were performed as instructed in the user handbook included with the biochemical kits (Randox Laboratories LTD, UK, and Biolabo Biochemistry, France).

Statistical analysis

All data collected were presented as average (mean) ± standard deviation (SD). To examine differences in cardiovascular biomarkes and lipid regulation, the analysis of variance and then the differences between treatments were analyzed by the Least Significance Difference (LSD) test (Steel and Torrie, 1980). All statistical analysis procedures were performed with the SPSS statistical software for Windows (Version IBM 21; SPSS Inc., Chicago, IL), with the significance level set to $p < 0.05$ for all tests.

Table 1: Nutrient Composition of Feed Ingredient

Ingredients	ME (Kcal/kg)	CP (%)	EE (%)	CF(%)	Lysine (%)	Methionine (%)	P (%)	Ca (%)
Yellow corn	3291.27	9.88	1.79	5.70	0.06	0.18	0.60	0.02
Cassava Flour	3200.00	2.00	12.70	11.40	0.07	0.01	0.40	0.33
Rice Bran	1451.85	10.60	13.66	27.80	0.00	0.00	1.48	0.05
Pile	3500.00	1.88	15.62	0.25	0.00	0.00	0.05	0.31
Coconut Meal	1525.00	16.00	15.00	16.00	0.00	0.00	0.75	0.03
Maggot	3596.401	46.14	21.88	13.12	0.00	0.00	0.934	1.285
Indigofera	2617.41	36.18	4.74	11.11	2.05	0.67	0.58	0.13
Dicalcium phosphate	0.00	0.00	0.00	0.00	0.00	0.00	21.00	16
$CaCO_3$	0.00	0.00	0.00	0.00	0.00	0.00	0.04	39
L-Lysine	0.00	62.00	0.00	0.00	99.0	0.00	0.00	0.00
DL-Methionine	0.00	58.78	0.00	0.00	0.00	99.00	0.00	0.00

Note: 2.5% turmeric mash was added as a feed additive (Purwanti, 2014).
*The results of Laboratory of Livestock Biotechnology Integrated Laboratory Test, 2022.
** Purwanti, 2021.

Table 2: Composition of the dietary treatments

Ingredient	Feed Treatment		
	P0	P1	P2
Feed Composition			
Yellow Corn (%)	Commercial Feed as a control	10.20	22.50
Cassava Flour (%)		8.00	9.90
Rice Bran (%)		27.00	15.00
Pile (%)		18.00	17.00
Coconut Meal (%)		2.50	1.00
NaCl (%)		0.50	0.50
Premix (%)		1.50	1.50
Maggot (%)		25.00	20.00
Indigofera (%)		5.00	10.00
DCP (%)		1.00	1.00
$CaCO_3$(%)		1.00	1.00
L-Lysine (%)		0.30	0.30
DL-Methionine (%)		0.30	0.30
Total		100.00	100.00
Turmeric		2.50	2.50
Nutrient Composition			
ME (Kcal/Kg)	-	3006.89	2970.40
Crude Protein (%)	23.15	20.97	20,47
Extract Ether (%)	7.36	8.08	7.11
Crude Fibre (%)	5.37	8.90	8.79
Lysine (%)	-	0.48	0.59
Methionine (%)	-	0.45	0.49
P (%)	-	1.11	1.14
Ca (%)	-	0.93	0.87

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

RESULT AND DISCUSSION

Cardiovascular parameters

The effect of feed treatment on the concentration of cardiovascular biomarkers in native chicken blood plasma was shown in Table 3. It shows that all cardiovascular parameters, including CRP high sensitivity, H-FABP, homocysteine, and Gamma-glutamyl transpeptidase in local chicken expressed significantly different (p<0.05) at P0 group than in P1 and P2 groups. Table 3, also showed that the expression of cardiovascular biomarkers in local chicken at P2 was not significantly different (p>0.05), except CRP high sensitivity and homocysteine level which were higher (p<0.05) in the P2 than P1 group.

A high daily heart pace rate in local chickens has an impact on increasing cardiovascular cellular damage. Biomarkers of heart damage have been reported, among others with CRP (Zhu et al., 2017). CRP is a protein that is released by the liver and is produced in large amounts during infections and heat stress (Zhao et al., 2017). Conversely, in inflammation that occurs in the process of developing atherosclerosis, an increase in CRP concentration is much smaller (Wheeler et al., 2014; Xu et al., 2015). Nevertheless, the increase is quite significant when compared to normal conditions.

High-sensitivity C-reactive protein (hs-CRP) measures the low amount of CRP in the blood. This test used to determine the risk of heart problems, especially those that were combined with other risk factors such as cholesterol, age (Kayadoe et al., 2019), and blood pressure (Song et al., 2015). This test used in this investigation to find out the effect of an increased risk of sudden cardiac problems of the native local chicken, such as heat stress. However, the relationship between high CRP levels and heart disease risk is not well understood (Kou et al., 2016).

Homocysteine is a natural amino acid, which, when at high levels in the blood, can increase the risk of clogged arteries (arteriosclerosis). Based on the result of this investigation native local chicken with high homocysteine levels triggers arteriosclerosis in veins, such as deep vein thrombosis (Fournel et al., 2017) and pulmonary embolism or in the arteries (Ikewaki., 2014). It is known that a high amount of homocysteine can damage the lining of blood vessels. This damage can cause arteriosclerosis. The report of previous studies also showed a close relationship between heat stress and homocysteine levels. Vanzin et al. (2011) found that oxidative stress, which is often associated with heat stress, is increased in patients with high homocysteine levels. Ledda et al. (2020) further supported this by showing that exposure to toxic heavy metals, which can be a result of heat stress, can influence homocysteine metabolism. Obradovic et al. (2017) highlighted the role of homocysteine in cardiovascular diseases, which can be exacerbated by heat stress. These findings suggest that heat stress can lead to increased homocysteine levels, which in turn can contribute to the development of cardiovascular diseases.

Many previous studies have shown that biological markers of phospholipase A2 (PLA2) have been found to play an important role in the inflammatory pathway (Lee et al., 2016). PLA2 is classified in the acute phase protein group. PLA2 is reported to trigger the host's inflammatory response to infection (Pruzanski and Vadas, 1991). Touqui and Alaoui-El-Azher (2001) reported that PLA2-IIA, a type of PLA2, is associated with various inflammatory diseases. However, Boilard et al. (2010) presented a contrasting view, suggesting that a specific type of PLA2, group V sPLA2, may have an anti-inflammatory role in immune complex-mediated arthritis. These findings highlight the complex and multifaceted role of PLA2 in the inflammatory response to infection.

As an inflammatory mediator, the release of arachidonic acid from the phospholipid membrane of cells is catalyzed by intracellular PLA, thereby initiating prostaglandin and leukotriene synthesis (Johnson et al., 2015). As a result, this stimulated an increase in many physiological responses in animals, such as vasodilation in the heat-stressed broiler chicken, and inhibition of platelet aggregation.

The release of sPLA2-IIA in low altitude or high temperatures can be induced by several specific proteins among other inflammatory cytokines, such as the group of interleukins (interleukin IL-6, IL-1β, and also tumour necrosis factor/TNF-α). These proteins were key factors in the process of neutrophil adhesion and migration. While the exact role of SPLA2-IIA for livestock is still being discussed. Although, studies using small clinical trials have shown that sPLA2-IIA plasma levels show a positive correlation with stress (Mushawwir et al., 2021).

The H-FABP biomarker of the heart in this study was analyzed. Zhao et al. (2017) showed that heart-FABP is a low molecular weight (15 kDa) cytoplasmic protein found in high concentrations in cardiac muscle tissue. Heart-FABP is released from the liver during cell necrosis faster than other markers (Zhu et al., 2017; Hernawan et al., 2017). With its small size and its location in the cytoplasm, it can be expelled rapidly into the bloodstream after damage to the heart muscle. Plasma H-FABP levels does not only rise early but are normalized after 24 hours it is possible to detect recurrent myocardial infarction (Slimen et al., 2016). The advantage of H-FABP was dominant in the early stages of myocardial infarction. The initial combination of H-FABP after the onset of symptoms of discharge, rapid

Table 3: The effect of feed treatment on cardiovascular biomarkers concentrations in the blood plasma of native chicken.

Cardiovascular biomarkers	Feed treatment		
	P0	P1	P2
CRP high sensitivity (mg/L)	18.83±1.34[a]	10.73±1.03[b]	9.68±1.04[c]
H-FABP (ng/mL)	7.24±1.01[a]	5.62±0.62[b]	4.17±0.13[b]
Homocysteine (µmol/L)	16.52±3.06[a]	15.84±5.04[a]	9.23±0.16[b]
Gamma-glutamyl Transpeptidase (IU)	48.41±2.57[a]	26.46±3.07[b]	23.93±2.02[b]
sPLA2-IIA (ng/dL)	89.05±3.04[a]	46.81±2.07[b]	45.19±3.38[b]

CPR High Sensitivity = C-Reactive Protein; H-FABP= Heart-Type Fatty acid-binding Protein; sPLA2-IIA = Secretory Phospholipase-A2-IIA

[abc]Means in the same row with a different letter of superscripts are significantly different (p<0.05); Values are given in Mean±SD

Table 4: The effect of feed treatment on lipid regulation in the blood plasma of native chickens.

Lipids Regulation	Feed treatment		
	P0	P1	P2
Adiponectin ((µg/mL)	11.03±0.44[a]	9.25±0.25[b]	9.31±0.09[b]
Apolipoprotein A-I (g/L)	2.68±0.19[a]	2.01±0.14[a]	1.98±0.11[b]
Apolipoprotein A-II (g/L)	2.81±0.89[a]	2.13±0.78[a]	1.03±0.08[b]
Apolipoprotein B (g/L)	1.93±0,08[a]	1.16±0.07[a]	1.13±0.08[a]
Apolipoprotein C-II (g/L)	2.67±0.11[a]	2.52±0.09[b]	2.47±0.81[b]
Apolipoprotein C-III (g/L)	2.18±0.59[a]	1.74±0.60[a]	1.72±0.09[a]
Apolipoprotein E (g/L)	2.88±0.08[a]	2.46±0.07[b]	2.39±0.11[b]
Cholesterol HDL (mg/dL)	89.35±3.04[a]	76.84±3.05[b]	74.16±2.96[c]
Cholesterol LDL (mg/dL)	101.67±4.06[a]	98.73±3.95[b]	91.38±4.02[c]
Cholesterol total (mg/dL)	191.78±5.02[a]	187.36±7.14[a]	174.92±4.02[b]
Triglycerides (mg/dL)	268.61±5.07[a]	282.47±5.62[b]	283.15±5.25[b]
NEFA (mg/dL)	78.81±4.06[a]	68.25±3.26[b]	68.07±4.13[b]

HDL = High-Density Lipoprotein; LDL=Low Density Lipoprotein; NEFA = Non-Esterified Fatty Acid

[abc] Means in the same row with a different letter of superscripts are significantly different (p<0.05); Values are given in Mean±SD

screening of the kidneys from blood circulation, and high cardiac characteristics show strong power for diagnostic tools that are useful in the early detection of heart muscle damage.

Lipid Regulation

The response of native chicken on lipid regulation at different feeds is shown in Table 4. Lipid transport protein levels include types of apolipoproteins, HDL, and LDL in the control group (P0), which showed significantly higher (p< 0.05) compared to P1 and P2. Lipid degradation was higher in chicken given diet P0 (without additive). This phenomenon was indicated by a decrease in triglyceride levels and an increase in NEFA levels in their blood plasma. The results in Table 4, as a whole, also showed that the rate of lipid regulation was higher and significantly different (p<0.05) in local chicken at P0 than those at P1 and P2. The specific proteins which function as lipid transport were generally not different (p>0.05) in the chicken fed diets P1 and P2. Research has shown that the absence of additives in chicken diets can lead to higher lipid degradation. Sanz et al. (2000) observed lower fat deposition and higher beta-oxidation in chickens fed unsaturated fat diets. Kanatt et al. (2008) reported that antioxidants combined with low-dose gamma irradiation reduced lipid peroxidation in chicken meat. These studies collectively suggest that the absence of additives can lead to higher lipid degradation in chickens, potentially impacting their health and meat quality.

Apolipoprotein or apoprotein is known as a protein group in lipoprotein. The function of apolipoprotein is to transport fat into the blood (Sato et al., 2016; Adriani and Mushawwir., 2020) because fat is not soluble in water, then the way it is transported in water-based blood, this fat will be bound by a protein which then forms a complex called a lipoprotein that can mix with water. Sierra-Johnson et al. (2009) reported that apolipoprotein consists of apolipoproteins A-I, A-II, B, C-I, C-II, and E. Apolipoprotein B (apo B), showing protein structure for particles atherogenic, VLDL, IDL, LDL, small dense LDL (sdLDL). Whereas apo A-I is the main structural protein for HDL

and reflects the atheroprotective side of lipid metabolism (Tan et al., 2015; Kamil et al., 2020). Both of these apolipoproteins can also indicate cardiovascular risk more accurately than LDL-C and other lipids. The apo B/apo A-I ratio is strongly associated with the risk of myocardial infarction (MI) (Walldius and Jungner 2006, Walldius and Jungner 2004).

The level of apolipoprotein E (apoE) found increased in the current study in P0 group. This lipid transport was a protein constituent of plasma lipoprotein which has several functions including its role in cholesterol metabolism and as an important ligand in lipoprotein clearance. Apolipoprotein E was first identified as a constituent of VLDL that functions as triglyceride transport from the liver to peripheral tissue (Quispe et al., 2015).

The correlation of lipid regulation with heart physiology, reported by Zahner et al. (2004), who demonstrated an associated lipid regulation with biomarkers of heart failure. In cattle, CRP levels increased with increasing age, indicating that high levels of apoE occur before inflammation occurs (Jiang et al., 2019; Tanuwiria et al., 2020). Previous investigation also showed that the biological activity of apoE can be influenced by modifications to its structure and or quantity (Emoto et al., 2013). Structural changes can occur in the apoE polymorphism, which encodes apoE2, apoE3, and apoE4. Apolipoprotein E2 showed lower affinity to LDL receptors, resulting in apoE clearance which was slower and increases plasma apoE levels (Emoto et al., 2013; Sato et al., 2016). This situation was responded by regulating LDL receptors in the liver to reduce cholesterol levels. Apolipoprotein E4 was instead taken more efficiently, resulting in lower apoE levels and increasing cholesterol levels, and both are related to altitude (Qu and Ajuwon, 2018).

Therefore, variations in genetics that affect lipid metabolism change the risk of cardiovascular disease. Although, heat stress enhanced lipid metabolism effectively, the degradation of triglycerides (showed by the decrease in plasma triglycerides into NEFA), based on the results of this study, illustrated that lipid regulation was an efficient alternative to supply energy precursors.

Adiponectin was a good indicator for estimating the complications of metabolic syndrome. Many studies show the use of adiponectin in the body as a marker for metabolic syndrome (Catapano et al., 2016). Decreased plasma adiponectin (hypoadiponectinemia) is associated with an increase in Body Mass Index (increased incidence of obesity), decreased insulin sensitivity (increased incidence of diabetes), and unwanted fat profile.

CONCLUSION

The high sensitivity of CRP, H-FABP, adiponectin, apolipoproteins, HDL and LDL cholesterol, triglycerides, NEFA, homocysteine, and gamma-glutamyl transpeptidase showed higher levels in native local chickens fed a basal diet without maggots, *indigofera*, and turmeric than in native chickens fed maggots, *indigofera*, and turmeric. This suggests that these compounds may affect biomarker-for lipid regulation and cardiovascular function

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CONFLICT OF INTEREST

There are no conflict interest for the work reported in this article

NOVELTY STATEMENT

This article covers lipid regulation and cardiovascular biomarkers of native chickens fed a combination of maggot, indigofera and turmeric.

AUTHORS CONTRIBUTIONS

Sri Purwanti was involved in all phase of this research, including conceptualization, design research, implementation, data analysis, paper drafting. Wempie Pakiding, Marhamah Nadir, Nurhayu, Kusumandari Indah Prahesti and Sitti Nurani Sirajuddin handled the investigation, data analysis and review. Jasmal Ahmari Syamsu contributed to the data analysis and writing of the original draft, and Andi Mushawwir helped manuscript review and editing.

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