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Research Article

Evaluation of Haematological Responses and Blood Biochemical Parameters of Heat-stressed Broilers with Dietary Supplementation of Javanese Ginger Powder (*Curcuma xanthorrhiza*) and Garlic Extract (*Allium sativum*)

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Abstract

Background and Objective: Temperature is one of the main factors affecting the performance of broilers. Feed efficiency and poor performance of broilers are a main problem for broiler producers in regions in which the temperature exceeds the thermoneutral zone or even the upper critical temperature. The current experiment was conducted to evaluate the potential of combining Javanese ginger powder (JGP) with garlic extract (GE) in improving haematological condition and blood biochemistry in heat-stressed broilers. **Materials and Methods:** One hundred and seventy-five, one-day-old unsex Cobb broiler chicks were used in the current study to evaluate the potential of combining Javanese ginger powder (JGP) with garlic extract (GE) in improving haematological condition and blood biochemistry in heat-stressed broiler. The dietary treatments consisted of Javanese ginger powder (JGP) and garlic extract (GE). The broiler samples were randomly allocated to 7 treatment groups: Control group = D0 (without JGP and GE), JGP of 10 g kg^{-1} basal diet (D1), JGP of 15 g kg^{-1} basal diet (D2), GE of 5 mL kg^{-1} basal diet (D3), GE of 10 mL kg^{-1} basal diet (D4), JGP of 10 g: GE of 5 mL (D5) and JGP of 10 g: GE of 10 mL (D6). Blood samples were collected and whole blood was used to analyse haematological parameters while the blood plasma was used to determine the concentration of biochemical parameters by an automatic biochemical analyser, using a commercial kit. **Results:** The current study showed decreased haematological condition and biochemical profile in heat-stressed broilers. Combining JGP and GE can improve the physiological condition (haematological and biochemical) in heat-stressed broilers. A combination of JGP with GE (D5 and D6) demonstrated supplemented levels that significantly enhanced ($p<0.05$) the haematological and biochemical profile in heat-stressed broilers. **Conclusion:** Based on the results of this study, combining JGP with GE resulted in supplemented levels that significantly enhanced the haematological and biochemical profile, therefore, we conclude that JGP and GE can be used to avoid heat-stress in broilers.

Key words: Natural extract, physiologic, broilers, heat stress

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The effect of temperature can be defined in terms of its impact on animal function. An animal typically spends most of its life in a range of temperatures that is optimal for physiological processes. Animals differ in their ability of tolerating changes in ambient temperature[1]. Stenothermic animals such as poultry are restricted to a narrow range of ambient temperatures.

Feed efficiency and poor performance of broilers are key problems for broiler producers in regions in which temperature exceeds the thermoneutral zone or even the upper critical temperature[2]. This issue is commonly experienced by broiler producers in tropical countries[3]. Heat stress results in significant economic problem due to declined growth, feed efficiency and immunity, while increasing the probability of mortality, especially of extensive broilers in which several weeks of production cost have been invested[2,4,5].

Natural substances have been reported in animal models for their effect on function, such as prevention of disease and, antioxidant effects[6,7]. Previous studies also have demonstrated that aromatic plants like garlic are effective alternatives to synthetic antibiotics in inducing layer performance[8-11] and, broiler[12-14]. Garlic in chicken meat has been shown to overcome the problem of lipid oxidation, reduce cholesterol levels and increase stability of lipids[15,16], thus demonstrating that garlic is effective for enhancing lipids.

Many reports have shown that garlic had a significant effect in various poultry species such as laying hens and, broilers. However, only limited published information is available regarding dietary Javanese ginger powder for poultry on the combination of both garlic and ginger to avoid heat stress in broilers.

The blood (extracellular fluid) functions to transport carbon dioxide, oxygen, nutrients and, metabolic waste and is the pathway of humoural transmission. Thus, the blood (haematological and biochemical parameters) should be considered the physiological state of an animal's body. Therefore, the current experiment sought evaluate the potential of combining Javanese ginger powder (JGP) with garlic extract (GE) in improving haematologic condition and blood biochemistry in heat-stressed broilers.

MATERIALS AND METHODS

The experiment was conducted from January 5, 2017 to March 28, 2017, at the Al Mustofa Broiler Farm, Sukabumi and Laboratory of Animal Physiology and Biochemistry, Department of Animal Nutrition and Feed Technology, Animal Science Faculty, Universitas Padjadjaran, Bandung, Indonesia.

Animal, experimental design and treatments: A total of one hundred and seventy-five, one-day-old unsex Cobb broiler chicks were used in the current study. The broiler samples were obtained from a local commercial breeding farm company, with an average body weight of 43.17±1.13 g. Broiler chicks were individually wing-banded. The samples were placed into individual cages, measuring 40x30x35 cm in an environmentally controlled house, where the broiler chicks were allowed to acclimatize for 14 days. The experimental period continued for 30 days (four weeks).

The room temperature was exposed to the upper critical temperature zone for broilers, i.e., 35-36°C during the experimental period and the relative humidity was maintained between 65 and 75%. All broiler chicks were fed isoenergetic and isonitrogenous mash as the basal diet. Feed and water were presented ad libitum. The broiler samples were randomly allocated to 7 treatment groups, with 5 replicates. Each group was assigned 1 to 7 dietary treatments.

The dietary treatments consisted of Javenese ginger powder (JGP) and garlic extract (GE): Control group = D0 (without JGP and GE), JGP of 10 g kg^{-1} basal diet (D1), JGP of 15 g kg^{-1} basal diet (D2), GE of 5 mL kg^{-1} basal diet (D3), GE of 10 mL kg^{-1} basal diet (D4), JGP of 10 g: GE of 5 mL (D5) and JGP of 10 g: GE of 10 mL (D6).

Preparation of javanese ginger powder and garlic extract: Fresh Javenese ginger (JG) bulbs were harvested in January and obtained from the Al Mustofa Sukabumi vegetable garden. Fresh JG bulbs with husks were thinly spread and dried under sunlight at 31-35°C for 1 day[17-19]. The air-dried JG was further dried under 50°C in drying oven and, finely ground to a powder. The dried JGP used in this experiment contained 99.4 g moisture kg^{-1}.

Garlic juice was prepared as described previously[20,21] and the fresh garlic bulbs were purchased from a modern local market. Fresh garlic bulbs were peeled and cut into slices. Briefly, peeled garlic cloves were homogenized with an equivalent weight of distilled water in a food blender for 1 min. The mixture was then allowed to stand for 30 min at room temperature. Garlic juice was collected by filtering the mixture through cheesecloth.

Sample collection and analytical determination: Blood samples were collected from each broiler using the wing vein (vena cava superior) and were obtained beginning at when the samples were 21 days old, using a sterilized syringe and

vacuum tube containing K_3EDTA. Haematological parameters were analyzed by a haematology analyzer. The collected blood samples were also centrifuged to separate the plasma. The plasma was used to determine the concentration of biochemical parameters by an automatic biochemical analyzer using a commercial kit.

Statistical analysis: Data of biochemical and haematological parameters were analyzed using one-way analysis of variance (ANOVA). The means for treatment showed significant differences when compared by Duncan's multiple range test procedure, with significance based on the 0.05 level of probability[22].

RESULTS AND DISCUSSION

Haematological responses: The effects of JGP and GE supplementation with different levels on haematological conditions of broilers are shown in Table 1. Diet supplemented with JGP and GE individually or in combination had significant effects ($p<0.05$) on all of haematological parameters (RBC, Hb, PVC, WBC, Lymphocytes, Neutrophil, N/L, pH and ESR). The results of the current study showed that a combination of JGP and GE can improve the blood values to maintain the metabolism rate. Some studies have suggested that natural substances significantly increased hematological values. This is in agreement with the results of studies[20] reporting that natural extract increased the differential leukocytes count. The use of natural substances on haematological status has also been reported[21], showing a significant effect on blood profile.

The results of this study also demonstrated that the concentrations of Hb, PCV, RBC and WBC were increased significantly ($p<0.05$) with combination JGE and GE (D5 and D6). These results indicated that interaction of chemical compounds in the JGP with GE effectively increased erythropoiesis via specific signalling factors. Mushawwir *et al.*[5,23] suggested that all blood cells are derived from a single type of stem cell through a process called haematopoiesis. The specific signalling is one main factor that supports this process in the haematopoietic organs. Cortes-Coronado *et al.*[24] reported that specific signalling factors were involved in triggering the production of different types of blood cells. Erythropoietin is a hormone released from the kidney that triggers the differentiation of stem cells into erythrocytes. Although a previous study demonstrated heterogeneous results on the effect garlic on haematological profile, the simultaneous effect with JGP in this experiment led to increased blood cell formation of heat-stressed broilers.

In contrast, the number of neutrophils and N/L ratio were both, declined significantly ($p<0.05$). The level of neutrophil and N/L ratio were lowest in the treatment groups of broilers supplemented with the combination of JGP and GE. The results in the current study associated heat stress with a decline in immunity status. Holt *et al.*[25] reported that neutrophils are the most common leukocytes in vertebrate blood. These immune cells engulf damaged cells, microorganisms and other foreign pathogens via phagocytosis. A rise in the neutrophils concentration without additional natural substances in heat stress, was reported by Mushawwir *et al.*[23], in ruminant and heat-stressed Peking duck[26].

Conversely, the lymphocytes level in heat-stressed broilers without addition of JGP and GE significantly declined ($p<0.05$) compared to the broiler group supplemented with JGP and GE individually or in combination. This decrease resulted in a significant increase in the ratio of neutrophils to lymphocytes (N/L ratio) ($p<0.05$) in heat-stressed broilers without supplementation with JGP and GE (D0). Meanwhile, N/L ratio dropped dramatically with JGP and GE individual treatments or with combination treatment (D1-D6). The results of this study indicated that the bioactive compounds in JGP and GE can reduce the heat stress effects in broilers. Odore *et al.*[27] also have reported declined lymphocytes and increased N/L ratio in animals that were transportation stressed as well as in

Table 1: The effect of JGP and GE levels on haematological parameters of heat-stressed broilers

Level of JGP and GE	Haematological						
	D0	D1	D2	D3	D4	D5	D6
RBC ($x10^6$ mm^{-3})	2.78±0.15^a	3.12±0.11^b	3.14±0.13^b	3.12±0.08^b	3.24±0.04^b	3.29±0.14^b	3.31±0.08^b
Hb (g%)	8.5±0.01^a	10.5±0.11^b	10.4±0.15^b	10.3±0.04^b	10.8±0.15^b	11.3±0.10^{bc}	11.5±0.05^c
PCV (%)	29.87±0.13^a	33.38±0.13^b	32.63±0.11^b	33.47±0.17^b	33.46±0.12^b	35.09±0.09^c	35.17±0.18^c
WBC ($x10^4$ mm^{-3})	96.13±0.05^a	82.27±0.13^b	83.89±0.11^c	82.24±0.07^b	83.23±0.25^c	81.13±0.17^d	81.21±0.32^d
Lymphocytes (L) ($x10^2$ mm^{-3})	5.28±0.16^a	5.78±0.24^a	6.67±0.28^b	6.79±0.18^b	7.67±0.17^b	7.05±0.23^b	7.11±0.21^b
Neutrophil (N) ($x10^2$ mm^{-3})	21.05±0.18^a	18.57±0.31^b	18.27±0.21^b	18.51±0.57^b	18.66±0.17^b	17.11±0.33^c	15.37±0.12^d
N/L Ratio	3.98±0.11^a	3.21±0.13^a	2.73±0.28^b	2.72±0.31^b	2.43±0.16^{bc}	2.42±0.14^{bc}	2.16±0.13^c
pH	7.96±0.09^a	7.27±0.02^b	7.50±0.01^b	7.45±0.02^b	7.55±0.03^b	7.10±0.08^{bc}	7.05±0.07^c
ESR (mL h^{-1})	6.13±0.21^a	4.87±0.21^b	4.75±0.19^b	4.88±0.62^b	4.43±0.14^b	4.62±0.35^b	4.46±0.24^b

RBC: Red blood cells, Hb: Haemoglobin, PCV: Packed cell volume, WBC: White blood cells, ESR: Erythrocyte sedimentation rate, ^{a,b,c}Means in each column with different superscripts are significantly different ($p<0.05$); Values are given in Means±SD

Table 2: The effect of JGP and GE levels on some biochemical parameters of blood plasma in heat-stressed broilers

	Level of JGP and GE						
Indices	D0	D1	D2	D3	D4	D5	D6
Cholesterol (mg dL^{-1})	285.56±3.47[a]	217.67±3.72[b]	219.48±4.72[b]	221.73±4.25[b]	218.56±1.47[b]	218.56±4.27[b]	217.56±4.17[b]
LDL (mg dL^{-1})	28.22±5.03[a]	16.65±5.07[b]	19.73±4.01[b]	14.53±3.06[c]	13.53±2.24[c]	13.38±2.03[c]	13.34±2.05[c]
HDL (mg dL^{-1})	92.31±1.26[a]	85.93±1.05[b]	82.84±1.92[c]	87.93±1.02[b]	86.91±1.11[b]	76.91±1.26[d]	75.45±0.32[d]
Triglycerides (mg dL^{-1})	53.83±1.84[a]	54.74±1.58[b]	53.69±1.74[a]	41.83±0.28[c]	43.73±1.75[c]	43.23±1.95[c]	41.34±1.28[c]
Glucose (mg dL^{-1})	23.85±4.43[a]	24.62±3.56[a]	29.04±3.52[b]	28.39±4.19[b]	29.17±3.58[b]	29.87±4.14[b]	30.05±4.63[b]
Malondialdehyde (MDA)	3.22±0.46[a]	1.79±0.71[b]	1.97±0.27[b]	1.98±0.53[c]	1.49±0.43[d]	1.58±0.19[d]	1.05±0.41[e]
Lactic dehydrogenase (IU L^{-1})	0.97±0.03[a]	0.53±0.02[b]	0.51±0.01[b]	0.38±0.02[c]	0.39±0.01[c]	0.24±0.03[d]	0.21±0.03[d]
Lactate (mg dL^{-1})	2.18±0.16[a]	1.35±0.30[b]	1.36±0.18[b]	1.36 ±0.10[b]	1.35±0.08[b]	0.73±021[c]	0.74±0.21[c]
Albumin (mg dL^{-1})	2.78±1.13[a]	2.31±1.24[a]	3.06±0.14[b]	3.14±1.17[b]	3.07±0.13[b]	3.33±0.13[b]	3.41±0.13[b]
Globulin (mg dL^{-1})	1.21±0.02[a]	1.23±0.05[a]	1.13±0.11[a]	2.15±0.08[b]	2.24±0.02[b]	2.30±0.02[b]	2.27±0.02[b]
Protein Total (mg dL^{-1})	4.99±1.11[a]	3.54±1.08[a]	4.19±1.09[a]	5.29±0.10[b]	5.31±0.11[b]	5.63±0.11[b]	5.68±0.11[b]
Uric acid (mg dL^{-1})	3.27±0,04[a]	2.69±0,03[b]	2.85±0,04[b]	2.72±0.02[b]	2.60±0.74[b]	2.78±0.07[b]	1.36±0.06[c]
Creatine Kinase (CK) (mg dL^{-1})	0.96±0.01[a]	0.35±0.01[b]	0.36±0.01[b]	0.32±0.02[b]	0.31±0.02[b]	0.22±0.02[bc]	0.17±0.01[c]
Creatinine (mg dL^{-1})	4.72±0.22[a]	3.62±0.11[a]	2.13±0.20[b]	2.03±0.73[c]	1.82±0.12[c]	1.54±0.11[d]	1.32±0.05[d]

[a,b,c]Means in each row with different superscripts are significantly different ($p<0.05$); Values are given in Mean±SD

broiler. Chowdhury *et al.*[17], Yalcin *et al.*[28,29], Ao *et al.*[30], Lee *et al.*[31], Peinado *et al.*[32] and Pearce *et al.*[33] reported the effect of garlic and natural substances on stress which were similar to those found in the current study.

Blood biochemistry: The levels of biochemical parameters in broiler blood plasma are shown in Table 2. Dietary JGP, GE and their combination significantly reduced ($p<0.05$) cholesterol, LDL, HDL and triglyceride contents. However, the contents of plasma triglycerides were not significantly reduced ($p>0.05$) with supplementation of 15 g of JGP. Moreover, addition of 10 g JGP significantly increased ($p<0.05$) plasma triglycerides compared to the broiler group without supplemented JGP and GE (D0). The concentrations of LDL, HDL and triglycerides in this study were reduced more with supplementation with GE or the combination of JGP with GE.

Based on the observations in the current study regarding addition of GE or combination JGP and GE, the combination of natural substances was more effective in inhibiting lipid synthesis. These results indicated that garlic has hypolipidemic activities. Some previous reports demonstrated the hypolipidemic effects of garlic in a wide range of animal species[17,30,33]. Yalcin *et al.*[28,29] demonstrated the effect of garlic compounds in inhibiting cholesterol 7α-hydroxylase, hepatic fatty acid synthase and also the gene of hydroxy- β-methylglutaryl coenzyme A (HMG-CoA) reductase. Qiuetal.[34], Singh and Gupta[35] and Cayan and Erener[36] have reported that ginger is a potent inhibitor of lipid synthesis.

The concentrations of glucose, MDA and others markers were significantly affected ($p>0.05$) by supplementation of JGP, GE and their combination. The results of the current study indicated that addition of JGP and GE can improve the metabolism rate in heat-stressed broilers. Physiological stresses, such as heat and transportation stress, commonly lead to metabolism reduction in animals. Reactive oxygen species (ROS) are a result of this stress and increased ROS gives rise to lipid oxidation. MDA is the end product of lipid oxidation by ROS. Decreased MDA with JGP and GE showed that these natural substances could decrease the ROS production in heat-stressed broilers. Similarly, the same results were found in this study, regarding the levels of lactate dehydrogenase and lactate. Nonetheless, the greatest decrease in lactate dehydrogenase and lactate levels occurred in the broiler group supplemented with a combination of JGP and GE.

The relationship of ROS and lactate dehydrogenase is related to hypoxia-inducible factor (HIF). This correlation can be explained by the fact that one of the molecules that play a role in cellular metabolism is hypoxia-inducible Factor (HIF)-1[37]. Nonetheless, research on HIF signal mostly focused on oxygen pressure and has developed an understanding that HIF is governed by stressors such as hyperthermia and ROS[38,39]. Protein of HIF induces the enzyme activity of lactate dehydrogenase[40]. The results of this study confirmed that chemical compounds in JGP and GE were able to act as antioxidants to render ROS unreactive.

Meanwhile, energy requirement in the respiration muscles is increased to support heavy muscle contraction in heat evaporation of heat-stressed broilers. In Table 2, it is shown that CK and creatinine were both significantly increased ($p<0.05$) in broilers exposed to heat stress without supplementation of JGP and/or GE (D0). The CK and creatinine levels dropped dramatically ($p<0.05$) in the blood plasma with dietary levels of JGP, GE and the combination of both.

Some investigators have reported that in maximally active fast-twitch muscles, the demand for ATP is so great that the

blood flow cannot provide oxygen and fuels fast enough to sufficiently supply ATP by aerobic respiration alone. Under these conditions, stored muscle glycogen is broken down to lactate[41,42]. The accumulation of lactate and the consequent decrease in pH reduce the efficiency of maximally active muscles. Previously, studies demonstrated that skeletal muscle, contains another source of ATP, known as phosphocreatine, which can rapidly regenerate ATP from ADP by CK[43].

CONCLUSION

Based on the results of this study, supplementation with the combination of JGP with GE significantly enhanced haematological and biochemical profile, therefore, we conclude that JGP and GE can be used to avoid heat-stress in broilers.

SIGNIFICANCE STATEMENTS

This study demonstrated that JGP with GE in broilers positively influenced the metabolism and performance after heat-stress. The results of this experiment will help researchers and farmers uncover the critical areas of physiological broiler stress related to temperature, which many researchers and farmer were previously unable to investigate. The results of this study also contribute to effective strategies of broiler producers in tropical regions.

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