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The Combination of Attapulgite, Betaine, and Chromium with Curcumin on Lipid Metabolism in Laying Hens under Tropical Conditions

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ABSTRACT

Liver health in laying hen is associated with lipid synthesis and metabolism. This study focused on oxidative parameters to maintain liver health and lipid metabolism in laying hens. The efficacy of curcumin as a herbal compound, combined with attapulgite, betaine, and organic chromium in a feed additive called Citrus XL, was evaluated in terms of its impact on lipid metabolism. This study involved 2000 ISA Brown strain laying hens aged 82-87 weeks. The heat stress index was calculated based on a temperature of 26.71 ± 1.11 ^{o}C and humidity of 83.21 ± 6.86 % in open-house cages with an 18-hour lighting period. This experiment included four treatments with five replications, including a basal diet with no Citrus XL (Control), a basal diet plus 0.5 kg/ton of Citrus XL, a basal diet plus 1.0 kg/ton of Citrus XL, and a basal diet plus 1.5 kg/ton of Citrus XL. To do so, blood biochemistry, fat content, liver score, malondialdehyde (MDA), and superoxide dismutase (SOD) levels were measured. The results indicated a significant increase in HDL levels as well as a reduction in LDL and MDA levels, liver scores, and egg yolk fat content. In conclusion, the treatment with 1.0 kg/ton of Citrus XL yielded the best results in terms of HDL, liver score, and liver MDA while Citrus XL treatment with 0.5 kg/ton produced the best results for LDL and yolk fat content.

Keywords: Curcumin, Heat stress, Laying hens, Lipid metabolism, Liver

INTRODUCTION

Curcumin is a phytobiotic herbal ingredient known for its essential role in the lipid metabolism rate. It can be used in animal feed, due to its anti-inflammatory, antioxidant, and anti-cancer properties, as well as its ability to prevent liver disease (Perrone et al., 2015). Betaine has potential benefits for laying hens, functioning as an osmolytic agent to mitigate heat stress and positively influencing tonic immobility (Abd El-Ghany and Babazadeh, 2022). Attapulgite is a mineral utilized as a supplement in chicken feed, characterized by its octahedral structure, which enhances viscosity, improves absorption capacity, and binds toxins within the digestive system of chickens (Kanoulas et al., 2023). Chromium, though not required in poultry feed, is naturally present in trace amounts and has been shown to improve the physiological, immunological, growth, and lipid profiles of chickens (Fraz et al., 2023). Curcumin has an efficient therapeutic role and protects against the effects of oxidative disorders in the liver through molecular and cellular mechanisms as hepatoprotective (Farzaei et al., 2018). This mechanism can improve cellular responses to oxidative stress disorders by expressing catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), and superoxide dismutase (SOD). The indication of oxidative stress can be excessive reactive oxygen species and an imbalance between the oxidants and antioxidants in the body, leading to the degradation of lipids, cell proteins, and DNA (Cichoz-Lach and Michalak, 2014). In layer poultry, the use of curcumin can specifically prevent a liver disease

called fatty liver hemorrhagic syndrome (FLHS), which is caused by a metabolic disorder that causes a very significant decrease in egg production, causing mass mortality.

The curcumin combined with attapulgite, betaine, and chromium is marketed as Citrus XL. As an industrial feed additive product for poultry, it potentially improves the lipid metabolism process, reduces heat stress, protects the liver function from fatty liver disease, and prolongs egg production in aged laying hens. Curcumin has a role in regulating lipid metabolism and inhibits hepatic steatosis (Feng et al., 2019). The mineral type of attapulgite can increase the absorption capacity of feed in the digestive tract and exchange ions. It can also help maintain intestinal health in chickens by preventing diarrhea in laying hens (Zhou et al., 2014; Tzora et al., 2017). The use of organic chromium (Cr) is also beneficial in protecting livestock from metabolic disorders and heat stress, as it helps prevent oxidative stress in cells and tissues (Youssef et al., 2022). The inclusion of betaine in the diet can optimize feed conversion ratios, increase chest muscle circumference, and enhance egg production. The osmolytic property of betaine reduces heat stress and has good potential for tonic immobility, thereby reducing stress in chicks (Abd El-Ghany and Babazadeh, 2022). Information regarding the combination of attapulgite, betaine, and chromium with curcumin has not yet been extensively studied or published.

Lipid metabolism refers to the utilization of fat digested from diet and absorbed from body fat to be used by body tissues. According to Attia et al. (2022), lipid compounds primarily form lipid metabolites involved in egg formation. In addition, the emergence of oxidative stress is caused by free radicals which break down into malondialdehyde (MDA) as an oxidative degradation product. Heat stress is an indicator of disturbances in livestock caused by high temperatures arising from environmental conditions (Thornton et al., 2021). Heat stress, triggered by environmental factors, can disrupt lipid metabolism in laying hens by promoting the formation of free radicals, thereby increasing MDA levels and reducing superoxide dismutase (SOD) activity (Emami et al., 2021). The enzymatic reaction in the layer liver to external stressors can disrupt the lipid metabolism process and the balance of nutritional requirements due to oxidative stress from hot temperatures (Bacou et al., 2021). The effects of heat stress from high body temperature will influence immunological biomarkers, blood chemistry, brain function, and other physical parameters, leading to oxidative damage of membranes and DNA (He et al., 2018). A score indicator is obtained by measuring the Heat Stress Index (HSI), which involves environmental temperature and humidity with various tolerance limits (Esnaola-Gonzalez et al., 2020). In chickens, heat stress can decrease feed intake efficiency (Killic and Simsek, 2013), reduce growth rates (Gholamreza et al., 2019), lower egg production, slow body weight gain, increase feed consumption ratio and raise mortality rates (Wasti et al., 2020). These effects are often influenced by a combination of air temperature, environmental heat, wind speed, and humidity.

The present study aimed to evaluate the effects of Citrus XL supplementation at specific doses on lipid metabolism and liver health in ISA Brown laying hens aged 82-87 weeks.

MATERIALS AND METHODS

Ethical approval

All experimental procedures were approved by the Animal Ethics Committee of IPB University following the guidelines for the use and care of animals in research (No. 053-2023 IPB).

Animal diets and research design

This experiment was carried out at Cisadane Pradana Farm in Semplak, Bogor City, Indonesia. A total of 2000 laying hens were used, housed individually in battery cages with dimensions of 50 cm (length) x 38 cm (width) x 45 cm (height). The feed additive, Citrus XL, produced by PT. Nutricell Pacific (Perseroan Terbatas) was used for the experiment. Citrus XL was a combination of organic chromium, attapulgite, phytobiotic curcumin, and betaine (Table 1).

Table 1. Citrus XL feed additive ingredient on laying hen lipid metabolism under tropical conditions

Material[1]	Amount	Unit
Chromium Organic	100.00	mg
Attapulgite	200.00	g
Phytobiotic	350.00	g
Betaine	200.00	g

[1)] PT: Nutricell Pacific.

The product was developed in collaboration between PT. Nutricell Pacific and IPB University, based on local farming conditions, and was provided by the factory for the trials. The laying hen used were the ISA Brown strain, aged 82-87 weeks. After an adaptation period, the

chickens were randomly assigned to four groups with five replications (n = 20), resulting in 100 hens per replication. The hens were fed complete diets supplemented with Citrus XL at 0, 0.5, 1.0, and 1.5 kg/ton of feed from 82 to 87 weeks of age. The diet used was complete rations provided by PT. Sreeya Sewu Indonesia, Tbk. and consisted of corn, bran, soybean meal, meat, and bone meal, olein, palm oil meal, wheat bran, distillers dried grains with solubles (DDGS), feather meal, amino acids, minerals, vitamins, phytase enzyme, non-starch polysaccharide (NSP) enzyme, antifungals, antioxidants, and organic acids. The complete nutritional components of the feed can be seen in Table 2. The feed was provided through restricted feeding at a rate of 115 g/day based on diet guidance daily feeding requirements (ISA Brown Product Guide, 2022), while water was provided *ad libitum* through nipple drinkers. Lighting was regulated for 18 hours per day, with 6 hours of darkness. Temperature and humidity were recorded to determine the Heat Stress Index (HSI), with an index score of < 150 indicating comfort for hens and >160 indicating heat stress (Pakpahan et al., 2023). The HSI during the study ranged from a minimum of 154 to a maximum of 172, with an average of 163.43 ± 5.01. The HSI results are shown in Figure 1. At the end of the study, five hens from each treatment group (one per replicate) were randomly selected for blood sampling. Approximately 3 ml of blood was drawn from the brachial vein in the left wing using a syringe and collected in vacuum tubes. The samples were transported to the Faculty of Animal Science laboratory at IPB University, where they were prepared under cold conditions and centrifuged at 1500 rpm for 30 minutes. The liver condition was assessed using a Nutricell Eggspert color scan tool to detect the color, which was reviewed with the L a b color coordinates (L = lightness; a = red/green; b = yellow/green). The color data were processed in Microsoft Excel and sent to the laboratory for testing fat, MDA, and SOD levels.

Table 2. Nutrient composition of experimental diet on the lipid metabolism of laying hens in tropical conditions

Nutrient Content[1] \ Treatments[2]	T1	T2	T3	T4
Water (max%)	13.00	13.00	13.00	13.00
Ash (max%)	14.00	14.00	14.00	14.00
Crude protein (min%)	17.00	17.00	17.00	17.00
Crude fat (min%)	3.00	3.00	3.00	3.00
Crude fiber (max%)	7.00	7.00	7.00	7.00
Calcium (%)	3.25-4.25	3.25-4.25	3.25-4.25	3.25-4.25
Phytase ≥ 400 FTU kg	0.45	0.45	0.45	0.45
Aflatoxin totally (max µ kg^{-1})	50	50	50	50
Lysine (min%)	0.84	0.84	0.84	0.84
Methionine (min%)	0.42	0.42	0.42	0.42
Methionine+cysistin (min%)	0.70	0.70	0.70	0.70
Threonine (min%)	0.58	0.58	0.58	0.58
Tryptophan (min%)	0.19	0.19	0.19	0.19

[1]PT. Sreeya Sewu Indonesia Tbk. [2]T1: diet without Citrus XL supplementation (Control), T2: Control diet supplemented with Citrus XL 0.5 kg/ton, T3: Control diet supplemented with Citrus XL 1.0 kg/ton, T4: Control diet supplemented with Citrus XL 1.5 kg/ton.

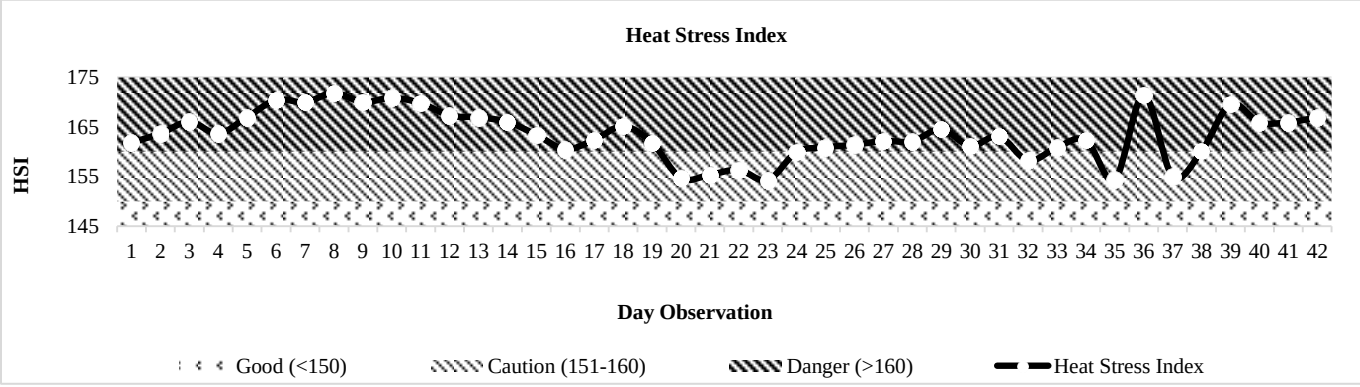

Figure 1. Heat stress index condition during the observation of a feeding Citrus XL in the diet of laying hen aged 82-87 weeks.

Blood cholesterol and triglyceride

The analysis of total blood cholesterol and triglyceride levels was conducted following the method of Sharma et al. (1987). Samples containing 0.5 ml of blood were randomly drawn from each treatment and replication group. Enzyme reagents and standard solutions from Liquidcolor, Human Diagnostics Worldwide (Germany), were prepared for the analysis. The preparation steps included filling the blank tube with 1000 µL of enzyme reagent. The standard tube was filled with 10 µL of standard cholesterol solution (for cholesterol analysis), 1000 µL of enzyme reagent, and 10 µL of standard triglyceride solution (for triglyceride analysis). Then, 1000 µL of enzyme reagent was added to the plasma preparation. The sample tube was filled with 10 µL of blood plasma and 1000 µL of enzyme reagent cholesterol and triglyceride. The mixture was then homogenized with vortex and then incubated at 20°C – 25°C for 20 minutes. Absorbance was measured using a spectrophotometer at 500 nm in wavelength, resulting in the blood cholesterol (mg/dL) and triglyceride (mg/dL) levels.

Blood high-density lipoprotein level

The analysis of blood high-density lipoprotein (HDL) levels was performed using the method of Wieland and Seidel (1983). A 3 µL blood sample was randomly taken for each treatment. Reagent 1 was prepared in a volume of 225 µL, consisting of 4-Aminoantipyrin (1.4 mmol/L), cholesterol esterase (1.6 KU/L), cholesterol oxidase (1 KU/L), and peroxidase (5 KU/L) as the component to detect the concentration of HDL level. Blood serum was homogenized with reagent 1 and incubated for 5 minutes at 37°C. The absorbance at 600nm was measured using a spectrophotometer as A1, representing the standard and initial value of HDL level. Reagent 2, consisting of 75 µL of HEPES buffer (25 mmol/L) and TOOS (N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline, sodium salt, dihydrate; 1.3 mmol/L), was then prepared. The blood serum was homogenized with reagent 2 and incubated for 5 minutes at 37°C. The absorbance was measured again at 600 nm using a spectrophotometer as A2, focusing on the red and purple coloration of the HDL levels. The change in absorbance (ΔA) was calculated using the following formula to determine the final HDL level (mg/dL).

$\Delta A = A2-A1$ (Formula 1)

Blood low-density lipoprotein level

The analysis of blood low-density lipoprotein (LDL) levels was carried out following the method of Wieland and Seidel (1983) using blood samples taken randomly in 3 µL volumes. Reagent 1, consisting of 225 µL HEPES buffer (23 mmol/L), 4-Aminoantipyrin (1.5 mmol/L), cholesterol esterase (1.6 KU/L), cholesterol oxidase (1 KU/L), and peroxidase (5.5 KU/L), was prepared. The blood serum was homogenized with reagent 1 and incubated for 5 minutes at 37°C, and the absorbance was measured at 600nm using a spectrophotometer as A1. Next, 75 µL of reagent 2 was prepared using HEPES buffer (25 mmol/L) and TOOS (1.3 mmol/L). The blood serum was homogenized with reagent 2, then incubated for 5 minutes at 37°C. The absorbance was read again at 600 nm with a spectrophotometer as A2. The change in absorbance (ΔA) was calculated using the following formula to determine the LDL level (mg/dL).

$\Delta A = A2-A1$ (Formula 2)

Egg yolk and liver fat analysis

The Soxhlet method was used to analyze the fat content of liver and egg yolk (AOAC, 2005). The sample sizes were 10 ml of egg yolk and 7 g of liver. After being dried for 30 minutes at 105^{o}C in the oven, the fat flask was cooled for 15 minutes in a desiccator. Next, the chilled pumpkin was weighed (A). A syringe was used to extract 5 cc of egg yolk (S) and 5 g of liver (S), which were placed on filter paper and tied with fat-free cotton wool. After being placed inside a paper thimble and extracted with hexane for 3-4 hours at 80^{o}C, the filter paper was chilled and weighed (B). The fat content was calculated using the following formula.

$$\text{Fat content (\%)} = \frac{B-A}{S} \times 100\% \quad \text{(Formula 3)}$$

Superoxide dismutase analysis

The analysis of SOD was conducted via the method of Ulhusna et al. (2019). A 0.5 g sample of liver tissue was chopped until fine and homogenized in 2.5 ml of phosphate buffer (PB, pH 7). The homogenate was placed in a tube and centrifuged at 3000 rpm for ten minutes at 4^{o}C. The supernatant was mixed with 8 ml of a chloroform-ethanol (3:5) solution and vortexed before being centrifuged again at 3000 rpm for 10 minutes at 4°C. The supernatant was then measured for absorbance at a wavelength of 480 nm using a spectrophotometer. Absorbance was measured at the first, second, third, and fourth minutes, and the SOD activity in the liver was expressed in U/mL.

Malondialdehyde analysis

The analysis of malondialdehyde (MDA) levels in the liver followed the thiobarbiturate acid reactive substance (TBARS) method as described by Ulhusna et al. (2019). A

0.5 g sample of liver tissue was finely chopped and homogenized in 5 ml of phosphate-buffered saline (PBS), with the remaining liver organs saved for further research. The homogenate was centrifuged at 3500 rpm for 10 minutes at 4°C, and the supernatant was extracted and transferred to an Eppendorf tube carried out in cold conditions. The amount of 1 ml of the supernatant was extracted from the Eppendorf and transferred into a test tube. A 4-milliliter test tube was filled with a cold 0.25 N HCl mixture that contained 0.5% butylated hydroxytoluene (BHT), 15% trichloroacetic acid (TCA), and 0.38% thiobarbituric acid (TBA). The oven was set to 80^{o}C for one hour, heating the test tube. The liquid tube was then allowed to cool for ten minutes in a water-filled bath. The tube was centrifuged for 10 minutes at 40° C at 3500 rpm. The supernatant was read at a wavelength of 532 nm with a UV-Vis spectrophotometer (Thermo Scientific Genesys 10S UV-Vis, USA). A standard curve was prepared using 1,1,3,3-tetraethoxypropane (TEP) at concentrations of 0, 1, 2, 3, 4, 5, 10, 20, 40, 80 µ, where Y is the MDA level (nmol/mg), and the liver MDA levels were calculated using the following formula.

$$Y = aX + b \quad \text{(Formula 4)}$$

Liver color score

The liver color indicator was measured using a colorimeter calibrated with standard white ceramic tiles. Samples were taken from each replicate by comparing the color of the chicken liver and measuring its brightness, identified by the Nutricell Eggspert Digital Colorimeter LS175 (China 2022).The lightness (L), redness (a), and yellowness (b) values of each liver sample were assessed in triplicate, and these values were translated into red (R), green (G), and blue (B). Identification of liver disease was based on the method described by Zhu et al., (2020), which assigns a score of 1-4 by evaluating the color of the liver and the number of hemorrhage points. A score of 1 indicates a normal, dark red liver with no hemorrhage. A score of 2 shows a mild, slightly yellow liver with 1-5 hemorrhages. A score of 3 indicated a moderate, yellow liver with 6-15 hemorrhages. Finally, a score of 4 signifies extreme, showing a brittle, dark yellow liver with 16-25 hemorrhages.

Statistical analysis

Data collected from this study were statistically analyzed using analysis of variance (ANOVA) with SPSS (2017) software version 25. If significant differences were found between treatment groups, Duncan's multiple range test (Duncan, 1955) was used for further analysis. Polynomial contrasts, including Linear, Quadratic, and Cubic models, were also applied. P values less than 0.05 were considered significant, and the mean data were expressed with standard deviations.

RESULTS

Blood biochemistry, liver malondialdehyde, and superoxide dismutase

Citrus XL supplementation at 0.5 to 1.5 kg/ton of feed did not have a significant effect on blood triglyceride levels in all groups. The best result was observed at 1.0 kg/ton, with 1355.4 ± 445.13 mg/dL, compared to the control treatment with 1502.6 ± 300.53 mg/dL ($p = 0.897$, $p > 0.05$). Blood cholesterol levels showed the best result at 0.5 kg/ton of Citrus XL, with 124.8 ± 36.09 mg/dL, compared to 151.6 ± 28.99 mg/dL in the control group ($p = 0.811$, $p > 0.05$). Supplementation of Citrus XL at 1.0 kg/ton had a significant effect (35.4 ± 5.85 mg/dL) on increased blood HDL levels compared to the control group (27.4 ± 4.39 mg/dL) with a p-value of 0.038, and quadratic polynomial contrast results of 0.031 ($p < 0.05$). Supplementation of Citrus XL at 0.5 kg/ton significantly reduced blood LDL levels (58.0 ± 15.24 mg/dL) compared to the control group (85.4 ± 16.50 mg/dL) with a p-value of 0.038 and a quadratic polynomial contrast result of 0.013 ($p < 0.05$). Supplementation of 1.0 kg/ton of Citrus XL had a significantly reduced liver MDA level (0.235 ± 0.013 U/mL) compared to the control group (0.264 ± 0.015 U/mL) with a p-value of 0.038 and a quadratic polynomial contrast result of 0.015 ($p < 0.05$). Although no significant effects on liver SOD levels were observed, polynomial contrast analysis showed a quadratic result with a p-value of 0.047, with the optimal dose identified as 1.0 kg/ton (Tables 3 and 4).

Fat level and liver score

Supplementation of Citrus XL at 0.5 to 1.5 kg/ton did not have any significant effect on the abdominal fat, which ranged between 2.98% and 4.26% of body weight. However, the control group had the lowest percentage, with 2.98 ± 1.19% ($p = 0.172$). Liver fat levels ranged from around 18.93% to 21.25% of liver weight, with the best result observed in the Citrus XL 1.0 kg/ton group, which presented 18.93 ± 9.13% ($p = 0.136$). Citrus XL supplementation at 0.5 kg/ton had a significant effect (47.93 ± 9.78%) in reducing egg yolk fat compared to the control group (63.39 ± 4.72%), with a p-value of 0.004 and a quadratic polynomial contrast of 0.001 ($p < 0.05$). Additionally, supplementation of Citrus XL at 1.0 kg/ton

had a significant effect (2.0 ± 0.70) in reducing the liver score compared to the control group (3.4 ± 0.89) with a p-value of 0.012 and a quadratic polynomial contrast of 0.002 ($p < 0.05$), as shown in Tables 5 and 6.

Table 3. Effects of dietary inclusion of Citrus XL on blood biochemistry, liver malondialdehyde, and superoxide dismutase of laying hen aged 82-87 weeks under tropical conditions

Variables \ Treatments[1]	T1	T2	T3	T4	P value[2]
Triglyceride (mg/dL)	1502.6 ± 300.53	1389.6 ± 455.97	1355.4 ± 445.13	1490.0 ± 216.42	0.897
Cholesterol (mg/dL)	151.6 ± 28.99	124.8 ± 36.09	133.2 ± 65.17	137.2 ± 37.93	0.811
HDL (mg/dL)	27.4 ± 4.39^{b}	35.4 ± 5.85^{a}	37.2 ± 5.80^{a}	34.4 ± 4.03^{a}	0.038
LDL (mg/dL)	85.4 ± 16.50^{b}	58.0 ± 15.24^{a}	62.2 ± 16.21^{a}	70.6 ± 7.36^{ab}	0.038
Liver SOD (U/mL)	83.64 ± 20.72	105.45 ± 15.21	112.73 ± 19.91	101.82 ± 9.95	0.084
Liver MDA (nmol/mg)	0.264 ± 0.015^{b}	0.249 ± 0.018^{ab}	0.235 ± 0.013^{a}	0.255 ± 0.011^{b}	0.038

[1]T1:diet without Citrus XL supplementation (Control), T2: Control diet supplemented with Citrus XL 0.5 kg/ton, T3: Control diet supplemented with Citrus XL 1.0 kg/ton, T4: Control diet supplemented with Citrus XL 1.5 kg/ton. [2]Probability Value. [a-b]Means in the same row with superscripts differ significantly ($p < 0.05$).

Table 4. Effects of dietary inclusion of polynomial contrast of Citrus XL on blood biochemistry, liver malondialdehyde, and superoxide of laying hen aged 82-87 weeks under tropical conditions

Variables	Polynomial Contrast[1]				X-Optimum[3]	R-Square[4]
	Linear	Quadratic	Cubic	Model[2]		R^2
Triglyceride (mg/dL)	0.923	0.463	0.904	Ns	-	-
Cholesterol (mg/dL)	0.699	0.448	0.661	Ns	-	-
HDL (mg/dL)	0.040	0.031	0.877	Qd	0.96	0.997
LDL (mg/dL)	0.180	0.013	0.353	Qd	0.86	0.914
Liver SOD (U/mL)	0.088	0.047	0.916	Qd	0.94	-
Liver MDA (nmol/mg)	0.184	0.015	0.253	Qd	0.87	0.878

[1]If there were significant differences ($p < 0.05$), the most appropriate equation model is selected. [2]Ns: no structure ($p > 0.05$), Ln: Linear; Qd: Quadratic; Cu: Cubic. [3]Maximum value of dose (x) to variable (y). [4]Variable variation rate (0-1).

Table 5. Effects of dietary inclusion of Citrus XL on fat levels and liver health of laying hen aged 82-87 weeks under tropical conditions

Variables \ Treatments[1]	T1	T2	T3	T4	P value[2]
Liver Score	3.4 ± 0.89^{bc}	2.4 ± 1.14^{ab}	2.0 ± 0.70^{a}	3.8± 0.44^{c}	0.012
Red[3]	114.6 ± 8.04	114.0 ± 16.53	114.4 ± 6.42	120.0 ± 7.00	0.770
Green[3]	84.8 ± 10.03	86.0 ± 12.7	81.4 ± 9.28	88.4 ± 2.88	0.703
Blue[3]	62.8 ± 4.86	61.4 ± 12.48	66.6 ± 6.58	62.2 ± 7.12	0.763
Liver Fat (%)	21.25 ± 4.27	19.47 ± 3.90	18.93 ± 9.13	19.56 5.48	0.136
Yolk Fat (%)	63.39 ± 4.72^{c}	47.93 ± 9.78^{a}	51.93 ± 5.63^{ab}	59.79 ± 2.09^{ac}	0.004
Abdominal Fat (%)	2.98 ± 1.19	3.97 ± 0.98	4.26 ± 0.94	4.07 ± 0.41	0.172

[1]T1:diet without Citrus XL supplementation (Control), T2: Control diet supplemented with Citrus XL 0.5 kg/ton, T3: Control diet supplemented with Citrus XL 1.0 kg/ton, T4: Control diet supplemented with Citrus XL 1.5 kg/ton. [2]Probability Value. [3]Liver color was calculated using a Nutricell Eggspert digital colorimeter. [a-c] Means in the same row with different superscript letters significantly ($p < 0.05$).

Table 6. Effects of dietary inclusion of polynomial contrast of Citrus XL on fat levels and liver health of laying hen aged 82-87 weeks of age under tropical conditions

Variables	Polynomial Contrast[1]				X-Optimum[3]	R-Square[4]
	Linear	Quadratic	Cubic	Model[2]		R^2
Liver Score	0.639	0.002	0.353	Qd	0.72	0.939
Red[5]	0.434	0.513	0.842	Ns	-	-
Green[5]	0.747	0.502	0.371	Ns	-	-
Blue[5]	0.840	0.690	0.342	Ns	-	-
Liver Fat (%)	0.650	0.664	0.994	Ns	-	-
Yolk Fat (%)	0.592	0.001	0.227	Qd	0.78	0.919
Abdominal Fat (%)	0.074	0.178	0.903	Ns	-	-

[1]If there were significant differences ($p < 0.05$), the most appropriate equation model is selected. [2]Ns: no structure ($p > 0.05$), Ln: Linear; Qd: Quadratic; Cu: Cubic. [3]Maximum value of dose (x) to variable (y). [4]Variable variation rate (0-1). [5]Liver color was calculated using a Nutricell Eggspert digital colorimeter.

DISCUSSION

The results showed that using Citrus XL as a feed additive at a level of 1.0 kg/ton significantly increased HDL levels in the blood and significantly reduced liver MDA levels. The optimum level of Citrus XL for increasing HDL was estimated to be 0.96 kg/ton, with a quadratic polynomial contrast. The reduction of MDA levels was optimal at an estimated level of 0.87 kg/ton. Curcumin has been shown to positively influence metabolism and inhibit excess fat accumulation (Aderemi and Alabi, 2023). It contains beneficial therapeutic properties due to antioxidants and herbal ingredients that help improve blood circulation throughout the organism (Mughal, 2019). In the study by Deng et al. (2023), curcumin supplementation in feed was found to increase HDL levels in human metabolism. The antioxidant content in curcumin had a positive effect on the health of old chickens by maintaining the stability of excessive fat growth and facilitating lipid metabolism in the blood. This was achieved by modulating HDL activity and regulating the activity and levels of biomarkers such as apolipoprotein A1, cholesterol acyltransferase, paraoxonase -1, and myeloperoxidase (Xie et al., 2012; Saberi-Karimian et al., 2021). A meta-analysis study by Ilyas et al. (2023), highlighted the crucial role of mineral supplementation in the activity of GPx enzymes in the liver, which contributes to increasing blood HDL levels, and reducing LDL and cholesterol levels. Citrus XL supplementation in the diet reduced liver MDA levels compared to the control treatment, where it was known that the liver was a precursor in the formation of egg yolk (Cui et al., 2020). The liver also plays a role in detoxifying toxins in the body as well as metabolic waste substances, contributing to lipid metabolism in poultry (Zaefarian et al., 2019). Low MDA levels indicated a form of antioxidant status in livestock and its response in organs, reflecting the final product of lipid peroxidation as a biomarker of oxidative stress (Respati et al., 2023). The inclusion of betaine in Citrus XL likely protected the liver from oxidative stress due to decreased liver function in old chickens. Previous studies have shown that optimizing betaine enhanced antioxidant activity by increasing liver detoxification, thereby alleviating the development of liver fibrosis and preventing radiation-induced liver damage (Shedid et al., 2018).

Providing Citrus XL in the diet at a level of 0.5 kg/ton had a significant effect on reducing LDL levels in the blood, with the optimum levels estimated at 0.86 kg/ton. Older layers typically have elevated LDL levels, which contribute to higher amounts of bad cholesterol circulating in their bodies. The increased amount of LDL in the blood causes endothelial damage and increased lines of fat formation, leading to coronary artery disease, which attacks medium and large arteries characterized by endothelial cells and lipids in the middle, making it essential to maintain normal LDL levels to preserved cellular function and structure (Rafieian-Kopaei et al., 2014; Bandyopadhyay et al., 2018). The meta-analysis by Qin et al. (2017) indicated that curcumin reduces LDL levels, thereby supporting lipid metabolism and promoting overall health.

Other results regarding the use of Citrus XL at various doses did not show a significant effect on triglyceride levels. However, it was noted that a dose of 1.0 kg/ton resulted in better triglyceride levels than other treatments. This improvement can be attributed to the combination of organic chromium, betaine, and curcumin, which exhibit good antioxidant properties and help maintain cellular integrity in the blood. Additionally, older chickens face complexities in lipid metabolism; as they age, triglyceride levels tend to increase which will, in turn, bring about a decrease in egg production (Liu et al., 2018). Chromium (Cr) is vital for proper insulin action and is necessary for normal protein, fat, and carbohydrate metabolism when included in feed. Its mineral use can help manage heat stress in chickens, maintain a healthy metabolic rate (Chowdhury et al., 2003; Haq et al., 2016), and decrease triglyceride levels in the blood chemistry profile (Wardani et al., 2020a). Han et al. (2020) showed that the use of Cr was beneficial to the health of chickens in limited quantities and significantly reduced triglyceride levels in the blood with various other additions compared to the control group. Citrus XL at 0.5-1.5 kg/ton doses had no significant effect on liver SOD in laying hens aged 82-87 weeks under tropical conditions; however, the highest superoxide dismutase (SOD) levels were observed with the 1.0 kg/ton treatment, which outperformed the control. This indicated that SOD was an antioxidant enzyme which was vital for the body and was produced for the defense against oxidative stress (Thorpe et al., 2013). It was central in scavenging superoxide anions from cell oxygen molecules (Hwang et al., 2020). Higher oxidative stress levels were associated with older chickens; this was demonstrated by the fact that higher stress levels were correlated with lower SOD levels, indicating that this product provided effective protection against lipid peroxidase (Surai, 2016). Oxidative stress in cells was caused by intracellular disruptions in the equilibrium

between the generation of reactive oxygen species (ROS) and antioxidant defenses (Kakkar et al., 2017), with free radicals damaging DNA, protein, and lipid structures (Schieber and Chandel, 2014) as well as SOD (Wardani et al., 2020b; Kemal et al., 2023) and MDA (Rehman et al., 2018). The use of Citrus XL may beneficially affect liver SOD levels in chickens, aiding in the management of heat stress conditions and accelerating the lipid metabolism cycle in the liver. The betaine content improved mitochondrial function and prevented fatty liver disease to maintain cells (Zhang et al., 2019) while Cr enhanced chicken by boosting antioxidant capacity, reducing stress, and enhancing immunity (Xin et al., 2022). There was no significant effect on total cholesterol levels in the blood, with the lowest cholesterol results observed at the 0.5 kg dose, which was better than the other treatments compared to the control. The potential of Citrus XL was partly due to Cr. According to the study by Sarai et al. (2022), the use of Cr can reduce total cholesterol levels in the blood during heat stress in chickens. Chromium can boost egg production while maintaining egg quality, making it beneficial for older laying hens (Chen et al., 2021). Controlling cholesterol in the blood is very beneficial for maintaining health and has the potential to extend the life of egg production in aged laying hens by incorporating components with high nutritional value. The total amount of cholesterol in the blood also provided information that healthy chickens can produce eggs, which is healthy for humans (El-Sabrout et al., 2022; Myers and Ruxton, 2023). Controlling lipid metabolism in blood-circulated tissues by reducing cholesterol can stabilize lipid accumulation and improve the health status of poultry (Tan et al., 2022). Elevated total cholesterol levels were a concern as they were associated with increased age and the risk of cardiovascular disease (Van Vliet et al., 2009). Using Citrus XL at a dose of 1.0 kg/ton showed better cholesterol levels compared to other treatments.

The findings indicated that the use of Citrus XL significantly improved liver condition to prevent fatty liver hemorrhagic syndrome with a dose of 1.0 kg/ton and resulted in a score of 2.0 compared to the control having a score of 3.4. This improvement was accompanied by a reduction in liver fat levels across various treatments, with the lowest fat levels observed at the 1.0 kg/ton dose. In the control treatment, the liver condition was fragile and easily damaged, exhibiting multiple hemorrhagic spots indicative of fatty liver disease. Liver damage in turkeys and broilers can be assessed by measuring the concentration of metabolites as biomarkers in the blood and observing any decrease in protein levels (Beyoğlu and Idle, 2020; Tardieu et al., 2021). According to Heeren and Scheja (2021), a fragile liver is caused by liver lipid metabolism disorders or lipotoxicity problems, which trigger stress and reduce liver function in old chickens (You et al., 2023). A healthy avian liver, characterized by a fresh brownish-red color, suggests optimal liver function in detoxifying toxins and synthesizing fats (Zaefarian et al., 2019). In the present observation, liver color in the treatments was evaluated using a digital colorimeter, assessing scores on Lightness (L), red/green coordinate points (a), and yellow/blue coordinate points (b), which were converted to Red, Green, and Blue. A deep and fresh liver color indicates a healthy liver and can potentially prolong egg production in old age. The use of curcumin to improve liver function in laying hens, complemented by betaine and organic chromium, was a beneficial combination for re-energizing liver function and extending the productive period in laying hens. Furthermore, curcumin aids chicken metabolism in preventing heat stress conditions, particularly during extreme temperatures (Hu et al., 2019; Liu et al., 2020; Wasti et al., 2020). Curcumin has also been optimized as a hepatoprotective agent to protect against injury and the effects of aging (Afrin et al., 2017; Li et al., 2021). Chickens exposed to heat stress can experience cell damage and disrupt egg production, necessitating proper management to prevent declines in egg yield, which could lead to financial losses (Li et al., 2020; Ahmad et al., 2022). A yellowish liver color indicated a health problem in the body. Anatomically, hepatocytes are microscopic cells, if they become filled with pigment or accumulate fat deposits, their color will change.

The results of this study did not show a significant effect on abdominal fat; however, there was a notable impact on reducing yolk fat. There was a lower fat content in the egg yolk in each treatment but the lowest fat content was observed at 0.5 kg/ton. The optimum levels of Citrus XL in reducing the fat content of the yolk was estimated at 0.78 kg/ton. High fat levels in the liver can inhibit the egg formation process, and if left uncontrolled, may lead to fatty liver disease, consequently affecting the fat concentration in egg yolk. Given that egg yolks contain high levels of fat, it was essential to limit their content to maintain overall health by managing fat intake. The age of the chickens also affected laying performance, egg yolk fat content, and their health status (Keum et al., 2018). The decrease in egg yolk fat content observed with various treatments of Citrus XL shows its potential to mitigate heat stress at the metabolic level. It improved internal egg quality due to optimal fat mobilization in adipose tissue

(Chen et al., 2023), which was an important consideration for consumers seeking low-fat eggs. Based on research from Yusuf et al. (2023), egg quality can be improved for consumer's health by including vitamin D3 supplements in their diet. However, the results of this study indicated that the abdominal fat in aged laying hens during 82 to 87 weeks of age, when using various doses, could not reduce the abdominal fat content of laying hens in hot environmental conditions. An indicator of heat stress exposure was improper food absorption, leading to fat deposits in the chicken's stomach (Brugaletta et al., 2022). The accumulation of fat in poultry, especially in broilers and laying hens, often results from their early life exposure to environmental stressors, including both heat and cold stress. In addition, older chickens tend to accumulate more abdominal fat, which restricts nutrient absorption, necessitating the implementation of effective feeding strategies (Fouad and El-Senousey, 2014).

CONCLUSION

This study indicated that supplementation at a level of 1.0 kg/ton of Citrus XL provided the best results in treating heat stress and preventing FLHS in chickens aged 82-87 weeks. It significantly improved lipid metabolism and liver health scores, increased blood HDL levels, and reduced egg yolk fat and MDA in ISA Brown laying hens. Future research should be carried out to evaluate Citrus XL during the first laying period until peak egg production.

DECLARATIONS

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Author's contributions

Hafidz Muhammad Muhshi contributed to the conception design of the research, data analysis, writing, review, and drafting of the manuscript. Rita Mutia, Sumiati Sumiati, and Wira Wisnu Wardani were involved in each step of the research and manuscript revision. Ilham Akbar and Nofitra Dewi Suparno Putri were involved in data collection, critical review, and research analysis. All authors confirmed the last edition of the manuscript for submission and publication.

Competing interests

The authors declare no competing interest in the publication of this study.

Ethical considerations

All the authors had checked and confirmed the article through ethical issues such as plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy before the submission.

Availability of data and materials

The availability of data and supplemental materials contained the original contributions that were presented in the study. To inquire, kindly get in touch with the corresponding author.

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REFERENCES

Aderemi FA and Alabi OM (2023). Turmeric (*Curcuma longa*): An alternative to antibiotics in poultry nutrition. Translational Animal Science, 7(1): 1-6. DOI: https://www.doi.org/10.1093/tas/txad133

Abd El-Ghany WA and Babazadeh D (2022). Betaine: A potential nutritional metabolite in the poultry industry. Animals, 12(19): 2624. DOI: https://www.doi.org/10.3390/ani12192624

Afrin R, Arumugam S, Rahman A, Wahed MII, Karuppagounder V, Harima M, Suzuki H, Miyashita S, Suzuki K, Yoneyama H et al. (2017). Curcumin ameliorates liver damage and progression of NASH in NASH-HCC mouse model possibly by modulating HMGB1-NF-κB translocation. International Immunopharmacology, 44: 174-182. DOI: https://www.doi.org/10.1016/j.intimp.2017.01.016

Ahmad R, Yu YH, Hsia FSH, Su CH, Liu HC, Tobin I, Zhang G, and Cheng YH (2022). Influence of heat stress on poultry growth performance, intestinal inflammation, and immune function, and potential mitigation by probiotics. Animals, 12: 1-17. DOI: https://www.doi.org/10.3390/ani12172297

Association of official analytical chemists (AOAC) (2005). Official methods of analysis of aoac international, 18th Edition. Gaithersburg. Available at: https://books.google.co.id/books/about/Official_Methods_of_Analysis_of_AOAC_Int.html?id=AEExAQAAIAAJ&redir_esc=y

Attia YA, Al-Harth MA, Al Sagan AA, Abdulsalam NM, Hussein EOS, and Olal MJ (2022). Egg production and quality, lipid metabolites, antioxidant status and immune response of laying hen fed diets with various levels of soaked flax seed meal. Agriculture, 12: 1-16. DOI: https://www.doi.org/10.3390/agriculture12091402

Bacou E, Walk C, Rider S, Litta G and Perez-Calvo E (2021). Dietary oxidative distress: A review of nutritional challenges as models for poultry, swine and fish. Antioxidants, 10(4): 1-41. DOI: https://www.doi.org/10.3390/antiox10040525

Bandyopadhyay D, Qureshi A, Ghosh A, Ashish K, Heise LR, Hajra A, and Ghosh RK (2018). Safety and efficacy of extremely low ldl-cholestrerol levels and its prospects in hyperlipidemia management. Journal of Lipids, 2018: 1-8. DOI: https://www.doi.org/10.1155/2018/8598054

Beyoğlu D and Idle JR (2020). Metabolomic and lipidomics biomarkers for premalignant liver disease diagnosis and therapy. Metabolites, 10(2): 1-58. DOI: https://www.doi.org/10.3390/metabo10020050

Brugaletta G, Teyssier J, Rochell SJ, Dridi S, and Sirri F (2022). A review of heat stress in chickens. part I: insight into physiology and

gut health. Frontiers in Physiology, 13: 1-15. DOI: https://www.doi.org/10.3389/fphys.2022.934381

Chen XL, Zeng YB, Liu LX, Song QL, Zou ZH, Wei QP, and Song WJ (2021). Effects of dietary chromium propionate on laying performance, egg quality, serum biochemical parameters, and antioxidant status of laying ducks under heat stress. Animals, 15: 1-6. DOI: https://www.doi.org/10.1016/j.animal.2020.100081

Chen Y, Yu S, Zhang L, Xiao M, and An L (2023). Effects and mechanisms investigation of yolk quality in huaixiang chickens. Animals, 13: 1-13. DOI: https://www.doi.org/10.3390/ani13223513

Cichoz-Lach H and Michalak A (2014). Oxidative stress as a crucial factor in liver diseases. World Journal of Gastroenterology, 20: 8082-8091. DOI: https://www.doi.org/10.3748/wjg.v20.i25.8082

Chowdhury S, Pandit K, Roychowdury P, and Bhattacharya B (2003). Role of chromium in human metabolism, with special reference to type 2 diabetes. Journal of Association of Physicians of India, 51: 701-705. Available at: https://www.pubmed.ncbi.nlm.nih.gov/14621042/

Cui Z, Amevor FK, Feng Q, Kang X, Song W, Zhu Q, Wang Y, Li D, and Zhao X (2020). Sexual maturity promotes yolk precursor synthesis and follicle development in hen via liver-blood-ovary signal axis. Animals. 10(12): 2348. DOI: https://www.doi.org/10.3390/ani10122348

Deng Z, Yang K, Cai X, Mei H, Xiao H, and Gao W (2023). The beneficial effects of curcumin supplementation on blood lipid levels among patients with metabolic related in diseases in asia area: A systematic review and meta-analysis of randomized controlled trials. Frontiers in Sustainable Food Systems, 7: 1167913. DOI: https://www.doi.org/10.3389/fsufs.2023.1167913

Duncan DB (1955). Multiple range and multiple F tests. Biometrics, 11(1): 1-42. DOI: https://www.doi.org/10.2307/3001478

Fraz A, Parker NB, Löhr CV, and Cherian G (2023). Evaluating the impact of organic chromium with flax seed in broiler diets: Effects of production performance, breast muscle pathology, and meat quality aspects. Poultry Science, 102: 102331. DOI: https://www.doi.org/10.1016/j.psj.2022.102331

El-Sabrout K, Aggag S, and Mishra B (2022). Advanced practical strategies to enhance table egg production. Scientifica, 2022: 1-17. DOI: https://www.doi.org/10.1155/2022/1393392

Emami NK, Jung U, Voy B, and Dridi S (2021). Radical response: Effects of heat stress-induced oxidative stress on lipid metabolism in the avian liver. Antioxidants, 10: 1-15. DOI: https://www.doi.org/10.3390/antiox10010035

Esnaola-Gonzalez I, Gómez-Omella M, Ferreiro S, Fernandez I, Lázaro I, and García E (2020). An IoT platform towards the enhancement of poultry production chains. Sensors, 20(6): 1-20. DOI: https://www.doi.org/10.3390/s20061549

Farzaei MH, Zobeiri M, Parvizi F, El-Senduny FF, Marmouzi I, Coy-Barrera E, Naseri R, Nabavi SM, Rahimi R, and Abdollahi M (2018). Curcumin in liver diseases: A systematic review of the cellular mechanisms of oxidative stress and clinical perspective. Nutrients, 10(7): 855. DOI: https://www.doi.org/10.3390/nu10070855

Feng D, Zou J, Su D, Mai H, Zhang S, Li P, and Zheng X (2019). Curcumin prevents high-fat diet-induced hepatic steatosis in ApoE$^{-/-}$ mice by improving intestinal barrier function and reducing endotoxin and liver TLR4/NF-κB inflammation. Nutrition & Metabolism, 16: 79. DOI: https://www.doi.org/10.1186/s12986-019-0410-3

Fouad AM and El-Senousey HK (2014). Nutritional factors affecting abdominal fat deposition in poultry: A review. Asian-Australasian Journal of Animal Sciences, 7: 1057-1068. DOI: http://www.doi.org./10.5713/ajas.2013.13702

Gholamreza Z, Xi H, Xi F, and U AD (2019). How can heat stress affect chicken meat quality – A review. Poultry Science, 98: 1551-1556. DOI: http://www.doi.org/10.3382/ps/pey399

Han M, Chen Y, Li J, Dong Y, Zhiqiang M, Li J, and Zhang L (2020). Effects of organic chromium sources on growth performance, lipid metabolism, antioxidant status, breast amino acid and fatty acid profiles in broilers. Journal of the Science of Food Agriculture, 101: 3917-3926. DOI: https://www.doi.org/10.1002/jsfa.11053

Haq Z, Jain RK, Khan N, Dar MY, Ali S, Gupta M, and Varun TK (2016). Recent advances in role of chromium and its antioxidant combinations in poultry: A review. Veterinary World, 9: 1392-1399. DOI: https://www.doi.org/10.14202/vetworld.2016.1392-1399

He SP, Arowolo MA, Medrano RF, Li S, Yu QF, Chen JY, and He JH (2018). Impact of heat stress and nutritional interventions on poultry production. World's Poultry Science Journal, 74(4): 647-664. DOI: https://www.doi.org/10.1017/S0043933918000727

Heeren J and Scheja L (2021). Metabolic-associated fatty liver disease and lipoprotein metabolism. Molecular Metabolism, 50: 1-17. DOI: https://www.doi.org/10.1016/j.molmet.2021.101238

Hu R, He Y, Arowolo MA, Wu S, and He J (2019). Polyphenols as potential attenuators of heat stress in poultry production. Antioxidants, 8: 1-11. https://www.doi.org/10.3390/antiox8030067

Hwang J, Jin J, Jeon S, Moon SH, Park MY, Yum D, Kim JH, Kang J, Parko MH, Kim E et al. (2020). SOD1 suppresses pro-inflammatory immune responses by protecting against oxidative stress in colitis. Redox Biology, 37: 101760. DOI: https://www.doi.org/10.1016/j.redox.2020.101760

IBM Corp (2017). IBM SPSS Statistics for Windows version 25. Armonk, NY. Available at: https://www.ibm.com/spss

Ilyas A, Fauzi IA, Wardani WW, and Suprayudi MA (2023). Meta analysis of growth performance and immune parameters in freshwater fish fed with dietary supplementation of selenium. Journal of Applied Aquaculture, 36(2): 514-533. DOI: https://www.doi.org/10.1080/10454438.2023.2278687

Kakkar SS, Banga HS, and Brar RS (2017). Oxidative stress biomarkers in evaluation of therapeutic potential of ascorbic acidin poultry under hot climate. Journal of Agriculture and Veterinary Science, 10: 41-44. DOI: https://www.doi.org/10.9790/2380-1007024144

Kanoulas V, Papadopoulos GA, Tassis P, Koutouzidou G, Arsenos G, and Fortomaris P (2023). Dietary supplementation of attapulgite improves growth performance in pigs from weaning to slaughter. Veterinary Sciences, 9: 557. DOI: https://www.doi.org/10.3390/vetsci9100557

Killic I and Simsek E (2013). The effects of heat stress on egg production and quality of laying hen. Journal of Animal and Veterinary Advances. 12: 42-47. DOI: https://www.doi.org/10.3923/javaa.2013.42.47

Kemal R, Fauzi IA, Nuryati S, Wardani WW, and Suprayudi MA (2023). Evaluation of selenoprotein supplementation on digestibility, growth, and health performance of pacific white shrimp *Litopenaeus vannamei*. Aquaculture Nutrition, 2023: 1-12. https://www.doi.org/10.1155/2023/2008517

Keum MC, An B, Shin K, and Lee K (2018). Influence of dietary fat sources and conjugated fatty acid on egg quality, yolk cholesterol, and yolk fatty acid composition of laying hen. Brazilian Journal of Animal Science, 47: e20170303. DOI: https://www.doi.org/10.1590/rbz4720170303

Li G, Liu L, Yin B, Liu Y, Dong W, Gong S, Zhang J, and Tan J (2020). Heat stress decreases egg production of laying hen by inducing apoptosis of follicular cells via activating the FasL/Fas and TNF-α systems. Poultry Science, 99: 6084-6093. DOI: https://www.doi.org/10.1016/j.psj.2020.07.024

Li S, Liu R, Wei G, Guo G, Yu H, Zhang Y, Ishfaq M, Fazilani SA, and Zhang X (2021). Curcumin protects against aflatoxin B1-induced

liver injury in broilers via the modulation of long non-coding RNA expression. Ecotoxicology and Environmental Safety, 208: 111725. DOI: https://www.doi.org/10.1016/j.ecoenv.2020.111725

Liu M, Lu Y, Gao P, Xie X, Li D, Yu D, and Yu M (2020). Effect of curcumin on laying performance, egg quality, endocrine hormones, and immune activity in heat-stressed hen. Poultry Science, 99: 2196-2202. DOI: https://www.doi.org/10.1016/j.psj.2019.12.001

Liu X, Lin X, Mi Y, Zeng W, and Zhang C (2018). Age-related changes of yolk precursor formation in the liver of laying hen. Journal of Zhejiang University Science, 19: 390-399. DOI: https://www.doi.org/10.1631/jzus.B1700054

Mughal MH (2019). Turmeric polyphenol: A comprehensive review. Integrative Food, Nutrition, and Metabolism, 6: 1-6. DOI: https://www.doi.org/10.15761/IFNM.1000269

Myers M and Ruxton CHS (2023). Eggs: Healthy or risky? A review of evidence from high quality studies on hen's eggs. Nutrients, 15(12): 2657. DOI: https://www.doi.org/10.3390/nu15122657

Pakpahan AMB, Retnati Y, Mutia R, and Wardani WW (2023). Evaluation of the physical quality of supplement wafer and application of supplement layer performance. Buletin Peternakan, 43: 190-197. https://www.doi.org/10.21059/buletinpeternak.v47i3.84653

Perrone D, Ardito F, Giannatempo G, Dioguardi M, Troiano G, Russo LL, Lillo AD, Laino L, and Muzio LL (2015). Biological and therapeutic activities, and anticancer properties of curcumin (review). Experimental and Therapeutic Medicine, 10: 1616-1623. DOI: https://www.doi.org/10.3892/etm.2015.2749

Qin S, Huang L, Gong J, Shen S, Huang J, Ren H, and Hu H (2017). Efficacy and safety of turmeric and curcumin in lowering blood lipid levels in patients with cardiovascular risk factors: A meta-analysis of randomized controlled trials. Nutrition Journal, 16: 68. DOI: https://www.doi.org/10.1186/s12937-017-0293-y

Rafieian-Kopaei M, Setorki M, Doudi M, Baradaran A, and Nasri H (2014). Atherosclerosis: process, indicators, risk factors, and new hopes. International Journal of Preventive Medicine, 5: 927-946. Available at: https://www.pubmed.ncbi.nlm.nih.gov/25489440/

Rehman ZU, Meng C, Sun Y, Safdar A, Pasha RH, Munir M, and Ding C (2018). Oxidative stress in poultry: Lessons from the viral infections. Oxidative Medicine and Cellular Longevity, 2018: 1-15. DOI: https://www.doi.org/10.1155/2018/5123147

Respati AN, Yanza YR, Yano AA, Astuti D, Ningsih N, Triswanto, Purnamayanti L, Gading BMWT, Wardani WW, Jayanegara A et al. (2023). Meta-analysis of the effects of dietary sources of selenium on lactational performance and oxidative stress. Animal Feed Science Technology, 305: 115782. DOI: https://www.doi.org/10.1016/j.anifeedsci.2023.115782

Saberi-Karimian M, Nosrati M, Orooji A, Kazemi T, Javandoost A, Mohammadi A, Ferns GA, Aghasizadeh M, and Ghayour-Mobarhan M (2021). The effect of curcumin on hdl cholesterol uptake capacity in obese individuals: Pilot study. Journal of Cardio Thoracic Medicine, 9: 833-838. DOI: https://www.doi.org/10.22038/jctm.2021.58306.1336

Sarai FJS, Shakouri MD, and Seidavi A (2022). Effect of chromium picolinate, alone of in combination with vitamin c or formic acid on growth, carcass traits, immune, and blood parameters of broiler under heat stress. Pakistan Journal of Zoology, 1: 1-13. DOI: https://www.doi.org/10.17582/journal.pjz/20220605060642

Schieber M and Chandel NS (2014). Ros function in redox signaling and oxidative stress. Current Biology, 24: 453-462. DOI: https://www.doi.org/10.1016/j.cub.2014.03.034

Sharma A, Artiss JD, and Zak BA (1987). A method for the sequential colorimetric determination of serum triglycerides and cholesterol. Clinical Biochemistry, 20(3): 167-72. DOI: https://www.doi.org/10.1016/s0009-9120(87)80115-7

Shedid SM, Abdel-Magied N, and Saada HN (2018). Role of betaine in liver injury induces by the exposure to ionizing radiation. Environmental Toxicology, 2: 123-130. DOI: https://www.doi.org/10.1002/tox.22664

Surai PF (2016). Antioxidant systems in poultry biology: Superoxide dismutase. Journal of Animal Research and Nutrition, 1: 1-17. DOI: https://www.doi.org/10.21767/2572-5459.100008

Tan Z, Halter B, Liu D, Gilbert ER, and Cline MA (2022). Dietary flavonoids as modulators of lipid metabolism in poultry. Frontiers in Physiology, 13: 863860. DOI: https://www.doi.org/10.3389/fphys.2022.863860

Tardieu D, Matard-Mann M, Collén PN, and Guerre P (2021). Strong alterations in the sphingolipid profile of chicken fed a dose of fumonisins considered safe. Toxins, 13: 1-16. DOI: https://www.doi.org/10.3390/toxins13110770

Thornton P, Nelson G, Mayberry D and Herrero M (2021). Increases in extreme heat stress in domesticated livestock species during the twenty-first century. Global Change Biology, 27: 5762-5772. DOI: https://www.doi.org/10.1111%2Fgcb.15825

Thorpe GW, Reodica M, Davies MJ, Heeren G, Jarolim S, Pillay B, Breitenbach M, Higgins VJ, and Dawes IW (2013). Superoxide radicals have a protective role during H2O2 stress. Molecular Biology of the Cell, 18: 2876-2884. DOI: https://www.doi.org/10.1091/mbc.E13-01-0052

Tzora A, Giannenas I, Karamoutsios A, Papaionnou N, Papanastasiou D, Bonos E, Skoufos S, Bartzanas T, and Skoufos I (2017). Effects of oregano, attapulgite, benzoic acid and their blend on chicken performance, intestinal microbiology and intestinal morphology. Journal of Poultry Science, 54: 218-227. DOI: https://www.doi.org/10.2141/jpsa.0160071

Ulhusna FA, Winarto A, and Wresdiyanti T (2019). The profile of superoxida dismutase and malondialdeyde level in the liver tissue of hypercholesterolemic rats treated with Holothuria nobilis polysaccharide. Jurnal Kedokteran Hewan, 13: 37-44. DOI: https://www.doi.org/10.21157/j.ked.hewan.v13i2.13189

Van Vliet P, van de Water W, de Craen AJM, and Westendorp RGJ (2009). The influence of age on the association between cholesterol and cognitive function. Experimental Gerontology, 44: 112-122. DOI: https://www.doi.org/10.1016/j.exger.2008.05.004

Wardani WW, Alimuddin A, Junior MZ, Setiawati M, Nuryati S, and Suprayudi MA (2020a). Growth performance, fillet quality, and immunity after challenge with streptococcus agalactiae of red tilapia (oreochromis sp.) fed diet containing cysteamine and creatine. Aquaculture Research, 52: 4237-4247. DOI: https://www.doi.org/10.1111/are.15262

Wardani WW, Alimuddin A, Junior MZ, Setiawati M, Nuryati S, and Suprayudi MA (2020b). Evaluation of cysteamine supplementation in red tilapian (*Oreochromis* sp.) diet: Serum insulin and somatostatin, IGF-1 and GLUT4 genes expression, growth performance, and robustness against stress. Aquaculture, 528: 1-9. DOI: https://www.doi.org/10.1016/j.aquaculture.2020.735514

Wasti S, Sah N, and Mishra B (2020). Impact of heat stress on poultry health and performance, and potential mitigation strategies. Animals, 10(8): 1266. DOI: https://www.doi.org/10.3390/ani10081266

Wieland H and Seidel D (1983). A simple specific method for precipitation of low density lipoprotein. Journal of Lipid Research, 24: 904-909. Available at: https://www.pubmed.ncbi.nlm.nih.gov/6631224/

Xie K, Jia X, Xu P, Huang X, Gu W, Zhang F, Yang S, and Tang S (2012). The addition of modified attapulgite reduces the emission of nitrous oxide and ammonia from aerobically composted chicken manure. Journal of the Air & Waste Management Association, 62: 1174-1181. DOI: https://www.doi.org/10.1080/10962247.2012.699442

Xin X, Han M, Wu Y, Dong Y, Miao Z, Zhang J, Song X, Jia R, Su Y, Liu C et al. (2022). Dietary supplemental chromium yeast improved the antioxidant capacity, immunity and liver health in broilers under high stocking density. Animals, 12: 1-12. DOI: https://www.doi.org/10.3390/ani12172216

You M, Zhang S, Shen Y, Zhao X, Chen L, Liu J, and Ma N (2023). Quantitative lipidomics reveals lipid perturbation in the liver of fatty liver hemorraghic syndrome in laying hen. Poultry Science, 102(2): 102352. DOI: https://www.doi.org/10.1016/j.psj.2022.102352

Youssef IMI, Abdo IMI, Elsukkary HFA, El-Kady MF, and Elsayed M (2022). Effects of dietary supplementation of chromium methionine chelate on growth performance, oxidative stress, hematological indices, and carcass traits of broiler chickens. Tropical Animal Health and Production, 54: 1-10. DOI: https://www.doi.org/10.1007/s11250-022-03260-1

Yusuf GM, Sumiati, Mutia R, Wardani WW, Akbar I, and Putri NDS (2023). Performance, egg quality, bone health, and immunity assessments of lohmann laying hen supplemented with vitamin d3 in the diet. Tropical Animal Science Journal, 46: 461-470. DOI: https://www.doi.org/10.5398/tasj.2023.46.4.461

Zaefarian F, Abdollahi MR, Cowieson A, and Ravindran V (2019). Avian liver: The forgotten organ. Animals, 9: 1-23. DOI: https://www.doi.org/10.3390/ani9020063

Zhang L, Qi Y, Alui Z, Liu S, Zhang Z, and Zhou L (2019). Betaine increases mitochondrial content and improves hepatic lipid metabolism. Food & Function, 10: 216-223. DOI: https://www.doi.org/10.1039/C8FO02004C

Zhou P, Tan YQ, Zhang L, Zhou YM, Gao F, and Zhou GH (2014). Effects of dietary supplementation with the combination of zeolite and attapulgite on growth performance, nutrient digestibility, secretion of digestive enzymes and intestinal health in broiler chickens. Asian-Australasian Journal of Animal Sciences, 27: 1311-1318. DOI: https://www.doi.org/10.5713/ajas.2014.14241

Zhu L, Liao R, Xiao C, Zhu G, Wu N, Tu Y, and Yang C (2020). Potential biomarker for fatty liver hemorrhagic syndrome in laying hen. Journal World's Poultry Research, 10: 545-555. DOI: https://www.doi.org/10.36380/jwpr.2020.62