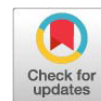


Research Article



Choline Supplementation in Diets with Different Levels of Methionine on Fat Metabolism in Quail during the Production Period

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Abstract | This study aimed to assess the effectiveness of choline chloride supplementation in the diet to reduce DL-Methionine use and evaluate the fat metabolism of laying quail. This study involved 180 quails, 42 days old, which were kept for eight weeks. A 2 × 3 factorial completely randomized design (CRD) with three replicates was used. Different amounts of choline chloride were added (A1: no choline chloride and A2: 1500 ppm choline chloride supplementation). Methionine was added to the feed in three different ways (B1: low methionine (0.19%), B2: standard methionine (0.79%), and B3: high methionine (1.05%). The observed variables were liver fat, kidney fat, abdominal fat, blood lipids (total cholesterol, triglyceride, and serum HDL), and egg chemical quality (cholesterol and yolk fat). The results showed that choline chloride and methionine supplementation significantly ($P < 0.05$) decreased liver fat and significantly ($P < 0.01$) decreased abdominal fat in laying quail. Kidney fat, blood lipids (total cholesterol, triglyceride, and serum HDL), and quail's egg chemical quality (cholesterol and yellow fat) were unaffected by choline chloride and methionine supplementation. This study concludes that choline chloride supplementation of 1500 ppm in quail rations containing sufficient methionine (0.79%) can reduce liver fat and abdominal fat.

Keywords | Blood lipids, Choline chloride, Fat liver, Methionine, Quail, Yolk

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INTRODUCTION

Fatty liver and kidney syndrome (FLKS) is a group of metabolic problems and abnormalities that appear as fatty liver and kidneys. These problems are caused by the body not making enough very low-density lipoprotein (VLDL) and fat being made too quickly. Lipogenesis is the primary source of body fat in poultry. The lipogenesis capacity of birds during the laying period is higher than that of growing birds. Follicles in laying period birds se-

crete estrogen, stimulating lipogenesis and producing high triacylglycerol levels in plasma and liver. Due to intensive estrogen metabolism, high egg-laying capacity stimulates fatty liver. Efforts to prevent FLKS include adding lipotropic factors such as choline and methionine (Leeson and Summers, 2005).

Choline is an essential substance for poultry. The main functions of choline are (i) a staple element of phospholipids in building and maintaining cell structure, (ii) methyl

metabolism, (iii) metabolism and transportation of lipids and cholesterol, and (iv) responsible for the transmission of nerve impulses that cannot be formed without choline in the body (Al Rajabi *et al.*, 2014). Choline deficiency in laying birds can cause impaired growth and decreased lipotropic activity, resulting in fatty liver (Selvam *et al.*, 2018). Adding choline to the diet improved feed efficiency in broilers by reducing feed intake without altering body weight gain.

Gregg *et al.* (2023) raise the production of quail eggs (Khairani *et al.*, 2016), improve the egg antioxidant capacity of quail (Olgun *et al.*, 2022), and lower the levels of total lipids and triglycerides in the liver of laying hens (Dong *et al.*, 2019).

Methionine is a limiting amino acid in poultry feed and functions as a methyl group transfer (Savaram *et al.*, 2022). Several studies have shown a relationship between methionine and choline in methylation reactions (Bekdash, 2023). Methionine becomes a methyl donor for choline formation through transmethylation. In order to make up for methionine's lack of methyl donation, choline can also donate its methyl to homocysteine and vice versa (Khairani *et al.*, 2016). Adding (high) methionine in broiler rations has been shown to increase lipid catabolism in the liver and reduce lipid accumulation. This study aimed to evaluate fat metabolism in quail (*Coturnix coturnix japonica*) laying period due to choline chloride supplementation in rations containing different levels of methionine: low, standard, and high.

MATERIALS AND METHODS

ETHICS STATEMENT

This research was conducted in accordance with the ethical guidelines for the study of experimental animals as stipulated by Indonesian Law Number 18 of 2019 about Animal Livestock and Animal Science.

ANIMAL, FEED AND HOUSING

This study involved 180 quails, 42 days old, which were kept for eight weeks. The animals were divided into six treatments and three replicates, each replicate consisting of 10 quails. Based on recommendations, the treatment quail rations were isocaloric and isoprotein, with energy and protein requirements of 2950 kcal/kg and 18%, respectively (Leeson and Summers, 2005). The composition and nutrient content of the treatment ration based on (Khairani *et al.*, 2016) are presented in Table 1. The cages were battery system cages measuring 60 cm x 60 cm x 40 cm.

EXPERIMENTAL DESIGN

This study used a randomized complete block design

(CRD) factorial of 2 x 3 and 3 replications. There were six treatments, which were a combination of factor A, namely two levels of choline chloride supplementation (A1: without choline chloride, A2: 1500 ppm choline chloride supplementation), and factor B, namely three levels of methionine in the ration ((B1: low methionine ration (0.19%), B2: standard methionine ration (0.79%), B3: high methionine ration (1.05%)).

VARIABLES MEASURED

Blood sampling was done at the end of the study. Blood was taken from the jugular vein in as much as 1 ml using a syringe. Blood samples were put in heparinized tubes. Furthermore, blood samples were used for blood lipid analysis. Quail slaughter was conducted at the end of the study (14 weeks old). The purpose of slaughtering quail was to obtain liver, kidney, and abdominal fat. Samples were taken randomly as many as 2 quails from each replicate to take the liver and kidneys for fat analysis. The slaughter method used in this study was to cut the carotid artery, jugular vein and oesophagus.

The observed variables were liver fat and kidney fat analyzed using the Soxhlet method (Association of Official Agricultural Chemists, 2019); blood lipids (total cholesterol, triglycerides, and serum HDL) analyzed by the Kit method, quail egg chemical quality consisting of: yolk cholesterol analyzed using the Liebermann Burchard Color Reaction method (Burke *et al.*, 1974) and yolk fat analyzed using the Soxhlet method (Association of Official Agricultural Chemists, 2019).

STATISTICAL ANALYSIS

Data were processed with analysis of variance (ANOVA). If there are significantly different results, it is continued with Duncan's multiple range test.

RESULTS AND DISCUSSION

LIVER FAT, KIDNEY FAT AND ABDOMINAL FAT

The mean liver, kidney and abdominal fat of quail fed the treatment diets are presented in Table 2. Feeding standard (B2) and high methionine (B3) diets significantly ($P < 0.05$) decreased the liver fat content of quail compared to low methionine (B1) diets. Supplementation of 1500 ppm choline chloride (A2) significantly ($P < 0.05$) reduced liver fat compared to no supplementation (A1). Rations containing standard (B2) and high methionine (B3) significantly ($P < 0.01$) reduced abdominal fat compared to rations containing low methionine (B1), from 1.04% to 0.61%. Supplementation of 1500 ppm choline chloride (A2) significantly ($P < 0.01$) reduced abdominal fat in quail compared to no supplementation (A1), from 0.9% to 0.63%. Kidney fat was not affected by ration treatment.

Table 1: Composition (%) of the experimental diets and calculated nutrient contents¹.

Composition	Treatment					
	A1B1	A1B2	A1B3	A2B1	A2B2	A2B3
Feed ingredients	Percentage (%)					
Yellow corn	58.6	58.4	58.33	58.6	58.4	58.33
Rice bran	1	0.3	0.2	1	0.3	0.2
CGM	3	3	3	3	3	3
Soybean meal	22.5	22.5	22.5	22.5	22.5	22.5
Fish meal	3.8	3.8	3.8	3.8	3.8	3.8
Palm oil	2.6	2.85	2.88	2.6	2.85	2.88
DCP	0.8	0.8	0.71	0.8	0.8	0.71
CaCO ₃	7.1	7.1	7	7.1	7.1	7
NaCl	0.1	0.1	0.1	0.1	0.1	0.1
Premix	0.5	0.4	0.4	0.5	0.4	0.4
DL-Methionine	0	0.75	1.075	0	0.75	1.075
Total	100	100	100	100	100	100
Choline chloride (ppm)				1500	1500	1500
Nutrient contents						
ME (kkal/kg)	2951.55	2951.65	2950.01	2951.55	2951.65	2950.01
Crude protein (%) [*]	18.14	18.03	18.01	18.14	18.03	18.01
Crude fat (%) [*]	5.14	5.35	5.37	5.14	5.35	5.37
Crude fiber (%) [*]	2.37	2.28	2.27	2.37	2.28	2.27
Methionine+Cystine (%) ^{**}	0.22	0.82	1.08	0.22	0.82	1.08
Methionine (%) ^{**}	0.19	0.79	1.05	0.19	0.79	1.05
Lysine (%)	1.07	1.07	1.07	1.07	1.07	1.07
Calcium (%)	3.18	3.18	3.12	3.18	3.18	3.12
Phosphorus available (%)	0.47	0.46	0.45	0.47	0.46	0.45
Choline mg /kg ^{**}	1498.3	1498.3	1498.3	2998.3	2998.3	2998.3

Note: ¹Khairani *et al.* (2016); A1B1: low methionine (0.19%) plus 0 ppm choline chloride containing diets; A1B2: standard methionine (0.79%) plus 0 ppm choline chloride containing diets; A1B3: high methionine (1.05%) plus 0 ppm choline chloride containing diets; A2B1: supplementation of choline chloride at 1500 ppm in low methionine diet (0.19%); A2B2: supplementation of choline chloride at 1500 ppm in standard methionine diet (0.79%); A2B3: supplementation of choline chloride at 1500 ppm in high methionine diet (1.05%); ^{*}analyzed at the Laboratory of Research Center for Bioresources and Biotechnology, Bogor Agricultural University, 2014; ^{**}analyzed at the PT. Saraswanti Indo Genetech Bogor, 2014.

Table 2: Average liver fat, kidney fat and abdominal fat of quails given research ration for eight weeks (age 6-14 weeks).

Variable	Choline (A)	Methionine (B)			Average	Normal ¹	FLKS
		B1	B2	B3			
Liver fat (%)	A1	18.03±6.68	6.09±1.60	11.01±3.53		4.70	9.00 - 22.60
	A2	10.37±1.45	6.86±1.70	5.09±2.68	11.71±6.48a		
	Average	15.09±6.25a	6.47±1.53b	8.05±4.29b	7.15±2.93b		
Kidney fat (%)	A1	1.94	1.35	1.27	1.52	5.00	9.00
	A2	1.18	1.17	1.13	1.16		
	Average	1.56	1.26	1.20			
Abdominal fat (%)	A1	1.24±0.20	0.73±0.15	0.74±0.07	0.90±0.28A		
	A2	0.85±0.01	0.49±0.11	0.53±0.38	0.63±0.26B		
	Average	1.04±0.25A	0.61±0.17B	0.64±0.27B			

Note: A1: no supplementation of choline chloride; A2: supplementation of choline chloride at 1500 ppm; B1: low methionine diet; B2: standard methionine diet; B3: high methionine diet. Different lowercase letters in a column/row indicate significant differences ($P < 0.05$), and different capital letters in a column/row indicate very significant differences ($P < 0.01$). Kidney fat samples were taken as a composite from each treatment; FLKS: fatty liver and kidney syndrome; ¹Riis (1983).

Table 3: Average blood lipids (total cholesterol, triglycerides, and HDL) of quails given research rations for eight weeks (age 6-14 weeks).

Variable	Choline (A)	Methionine (B)			Average
		B1	B2	B3	
Cholesterol total (mg dl ⁻¹)	A1	209.19±54.42	234.86±41.66	281.02±16.49	221.02±39.06
	A2	215.08±77.55	160.78±0.50	258.66±23.25	217.84±59.33
	Average	212.13±60.01	205.23±50.14	242.40±28.09	
Triglycerides (mg dl ⁻¹)	A1	1433.00±442.10	1886.04±152.97	1606.84±95.17	1641.96±310.14
	A2	1498.58±60.64	1635.33±341.92	1752.14±209.53	1628.68±230.65
	Average	1465.79±284.60	1760.68±273.82	1679.49±165.89	
HDL (mg dl ⁻¹)	A1	52.13±18.39	74.49±23.19	87.80±26.81	71.47±25.35
	A2	53.55±18.34	50.33±9.78	72.77±12.34	58.88±16.01
	Average	52.84±16.44	62.41±20.70	80.28±20.40	

Note: A1: no supplementation of choline chloride; A2: supplementation of choline chloride at 1500 ppm; B1: low methionine diet; B2: standard methionine diet; B3: high methionine diet.

This study's quail liver fat content ranged from 5.09-18.03%. The decrease in liver fat was 38.94% from 11.71% (without supplementation) to 7.15%. In the low methionine ration treatment, without choline chloride supplementation (A1B1), liver fat was classified as FLKS at 18.03%. Supplementation of 1500 ppm choline chloride in low methionine rations (A2B1) can reduce liver fat by 42.48%, from 18.03% (without supplementation) to 10.37%. This study shows that supplementing choline chloride 1500 ppm in rations containing low methionine can reduce quail liver fat. However, liver fat is still relatively high, but the effectiveness of choline chloride in reducing liver fat is seen.

The decrease in fat in the liver is because choline and methionine are lipotropic agents that assist in fat metabolism and prevent fatty liver. Through the phosphatidylethanolamine cycle, methionine can help make lecithin and help the liver make very low-density lipoprotein (VLDL) (Toohey, 2014). Meanwhile, choline can form phosphatidylcholine, which plays a role in synthesizing VLDL in the liver. This VLDL is important in triglyceride transport from the liver to the bloodstream and extrahepatic tissues. Supplementation of choline chloride in the diet helps prevent excess lipid accumulation and the onset of fatty liver in livestock (Wen *et al.*, 2014). The fat content of quail kidneys in this study ranged from 1.13-1.94%, respectively. The quail kidney fat was normal and still far from the FLKS category. Meanwhile, there were treatments for liver fat that were classified as FLKS.

Abdominal fat linearly decreased with increasing methionine in the diet (B2 and B3) compared to the B1 treatment due to the association of the amino acid methionine with carnitine and hormone-lipase sensitive activity. The biosynthesis of carnitine in the body from the amino acids lysine or methionine occurs in the liver and kidney. This carnitine is responsible for fatty acid transport from the cytosol to

the mitochondria. According to Zhan *et al.* (2006), taking extra methionine led to a significant decrease in belly fat, an increase in liver carnitine and free carnitine levels, hormone-sensitive lipase activity in belly fat, and a decrease in serum free fatty acid levels while a decrease in serum uric acid levels. Therefore, the decrease in abdominal fat was due to increased carnitine synthesis in the liver and hormone-sensitive lipase activity in abdominal fat.

Abdominal fat in this study was influenced not only by methionine supplementation but also by choline chloride supplementation in the diet. The reduction in abdominal fat can be attributed to the use of methyl groups from choline for carnitine synthesis. The increased production of carnitine due to the availability of methyl groups from choline leads to a decrease in abdominal fat. Various factors influence body fat deposition, including genetic diversity, environmental conditions, nutrition, and stress (Dridi *et al.*, 2022). According to Fouad and El-Senousey (2014), key strategies to reduce abdominal fat include optimizing poultry ration formulation, replacing saturated fatty acids with unsaturated fatty acids, implementing feed restrictions to prevent livestock obesity, and utilizing feed additives that help reduce the accumulation of abdominal fat.

BLOOD LIPIDS

Quail blood lipids consisting of: total cholesterol, triglycerides and serum HDL (Table 3). The average cholesterol levels produced during the study ranged from 160.78-258.66 mg dl⁻¹. Cholesterol levels in this study are still at normal levels. According to Thrall *et al.* (2012), the blood cholesterol of bird species, including quails, ranges from 100-250 mg dl⁻¹. The blood cholesterol results from the study were still in normal condition, meaning that the treatment from this study did not hurt blood cholesterol content. Choline supplementation can normalize cholesterol metabolism, improve liver health by maintaining cholesterol homeostasis, and prevent fatty liver (Al Rajabi

et al., 2014). No difference in total cholesterol, triglycerides, and HDL in the treatments is due to the homeostasis of blood cholesterol. The mechanisms of blood cholesterol homeostasis include regulating cholesterol synthesis via HMG-CoA reductase, resetting LDL receptors, and activating intracellular cholesterol esterification through ACAT. These processes collectively prevent excessive accumulation of intracellular cholesterol, maintaining overall cholesterol balance (Duan *et al.*, 2022).

The average triglycerides produced during the study ranged from 1433.00-1886.04 mg dl⁻¹. In the research results of (Kara and bulbul, 2021), the triglyceride content in quail serum and vegetable oil sources ranged from 624.14-873.33 mg dl⁻¹. The high triglycerides are due to the large amount of fatty acids converted into triglycerides for transport and storage. These fatty acids are obtained from the breakdown of food sourced from carbohydrates, fats, and proteins.

The average HDL levels produced during the study ranged from 50.33 to 87.80 mg dl⁻¹. HDL is cholesterol-carrying high-density lipoproteins (Cho, 2022). The primary function of HDL is to transport free cholesterol contained in blood vessels to HDL receptors in the liver to be excreted through bile. HDL is often called good cholesterol because it is a lipoprotein that transports lipids from the periphery to the liver (Dastmalchi *et al.*, 2023). HDL is a lipoprotein that maintains the cholesterol balance so that it does not accumulate in the cell; the balance is managed by removing sterols from the membrane at the same level as the amount of cholesterol synthesized in the liver (Thrall *et al.*, 2012). Furthermore Robert *et al.* (2021), reported that HDL molecules are relatively small compared to other lipoproteins. Additionally, HDL exerts protective functions, including the induction of nitric oxide production and inhibition of reactive oxygen species, which may prevent LDL oxidation.

CHEMICAL QUALITY OF EGGS

Egg chemical quality consisting of: cholesterol and yellow fat (Table 4) were not affected by choline chloride and

methionine treatments. The resulting cholesterol content tends to decrease in rations given choline chloride supplementation. This is because choline is the main component of phosphatidylcholine (lecithin), a phospholipid. This phospholipid can help metabolism in the liver by breaking down fat into smaller components and separating it into two between fats, in which there is good cholesterol and bad cholesterol. Bad cholesterol will be wasted with feces because these phospholipids can reduce the absorption and increase cholesterol excretion. Zeisel, (2012) states that choline has many roles, including in fat and cholesterol metabolism. Choline supplementation can normalize cholesterol metabolism, which is related to preventing fatty liver and improving liver function. According to Al Rajabi *et al.* (2014), choline can improve liver health by maintaining cholesterol homeostasis by maintaining normal cholesterol metabolism. According to Selvam *et al.* (2018) Choline chloride supplementation, whether synthetic or from herbal sources like the polyherbal formulation (PHF), has been shown to improve liver lipid metabolism, leading to decreased total cholesterol and triglycerides, thereby regulating fat metabolism in poultry diets.

The cholesterol content of egg yolk in this study ranged from 8.36-12.57 mg g⁻¹. Cholesterol in this study was higher than the Rhodes *et al.* (2015) standard of 8.80 mg g⁻¹, lower than the quail egg yolk cholesterol in the study of Putri *et al.* (2022), which is 21 mg g⁻¹ egg yolk. Different results in other studies are due to the rations given having different compositions and nutrient contents. The composition and content of egg yolk depend on the ratio given (Xia *et al.*, 2022). According to Nys *et al.* (2011), factors that affect the chemical quality of eggs are feed type, livestock breed, genetics, and hormones. Most cholesterol found in egg yolk is synthesized in the poultry liver, transferred through the blood in the form of lipoproteins, and then deposited into the follicles.

The egg yolk fat produced in this study has a relatively similar value. This is because the fat in the ration has a relatively similar fat content from each treatment. According to Göçmen *et al.* (2021) that different fat sources in quail

Table 4: Average chemical quality of eggs (cholesterol and yolk fat) of quail-fed research rations for eight weeks (age 6-14 weeks).

Variable	Choline (A)	Methionine (B)			Average
		B1	B2	B3	
Yolk cholesterol (mg g ⁻¹)	A1	9.39±2.55	10.72±3.25	12.57±3.51	10.89±3.04
	A2	8.78±1.45	9.10±2.86	8.36±0.38	8.75±1.65
	Average	9.09±1.88	9.91±2.87	10.46±3.21	
Yolk Fat (%)	A1	26.29±1.86	27.43±0.67	27.64±0.70	27.12±1.22
	A2	26.44±1.06	27.35±0.24	27.84±1.39	27.21±1.07
	Average	26.36±1.36	27.39±0.45	27.74±0.99	

Note: A1: no supplementation of choline chloride; A2: supplementation of choline chloride at 1500 ppm; B1: low methionine diet; B2: standard methionine diet; B3: high methionine diet.

diets significantly influence the fatty acid composition of egg yolk. Fat is an essential component needed in making yolk. The main composition in egg yolk is fat (65-70%) and protein (30%) (Li-Chan and Kim, 2008). The average egg yolk fat content in this study ranged from 26.290%-27.837%, lower than the study by Genchev, (2012) on quails, which ranged from 33.09-33.47%. Zerehdaran *et al.* (2004) stated that feed composition significantly influences fat formation in the body of livestock.

CONCLUSIONS AND RECOMMENDATIONS

Supplementation of 1500 ppm choline chloride in a ration containing sufficient methionine (0.79%) can reduce liver fat and abdominal fat.

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NOVELTY STATEMENT

We investigated the utilization of choline chloride supplementation in feed to reduce the use of DL-Methionine in laying quail.

AUTHOR'S CONTRIBUTIONS

Khairani: Conceptualization, Methodology, Formal Analysis, Data Curation, Writing-Original Draft, Writing-reviewing and editing.

Komang Gede Wiryawan: Conceptualization, Supervision, Writing-reviewing and editing.

Sumiati: Conceptualization, Supervision, Writing-reviewing and editing.

Ridho Kurniawan Rusli: Validation, Writing-reviewing and editing.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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