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Effect of Choline Chloride Supplement and Canola Oil on the Broiler Chick's Liver Cholesterol and Triglyceride Contents

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Abstract: This experiment was carried out to evaluation usage different levels of Canola Oil (CO) (0, 2 and 4%) and Choline Chloride Supplement (CCS) (0, 500 and 1000 mg kg^{-1}) in the basal diet (corn and soybean meal) and their effects on the broiler chicks liver chemical parameters include cholesterol and triglyceride contents. This trial was conducted in 3×3 factorial experiment with Completely Randomized Design (CRD) with 270 male chicks (Ross 308). All diets were isoenergetic and isonitrogenous and balanced with NRC recommendation. Two male birds selected with each pen and slaughtered. Result for liver chemical analyses show that interaction effects of canola oil and choline chloride supplement could affected liver cholesterol and triglyceride contents. Canola oil and choline chloride supplement in 6 and 9 treatment (T6 = 2% CO+1000 mg kg^{-1} CCS and T9 = 4% CO+1000 mg kg^{-1} CCS) could significantly decrease the liver cholesterol and triglyceride contents respectively ($p<0.0001$). Result shown the levels of 1000 and 500 mg kg^{-1} choline chloride supplement is highest effect on the liver cholesterol content respectively. Afterward of 6 and 9 treatment, 8 and 5 treatment (4% CO, 500 mg kg^{-1} CCS and 2% CO, 500 mg kg^{-1} CCS, respectively), could significantly decrease the liver cholesterol and triglyceride contents ($p<0.0001$). Generally choline chloride in all diet treatment can decrease the levels of liver triglyceride and cholesterol content significantly ($p<0.0001$).

Key words: Broiler, canola oil, choline chloride, liver, cholesterol and triglyceride

INTRODUCTION

Broiler industry is increasing dramatically throughout the developing countries. There have been a notable increase in growth rate and feed efficiency in commercial broiler chickens in last 30 years. Nowadays, human need a foremost food for a attain the best peace. Hereof, advert to alimentation of human is very important for a nutrition critic.

Current commercial hybrids with high performance require high energy diets which would enable the maximum exploitation of those genetic potential. The number 3 refers to the place on the molecule where the first unsaturated double bond is found. The consumption of Polyunsaturated Fatty Acids (PUFA) and in particular, Eicosapentaenoic Acid (EPA) and Docosahexanoic Acid (DHA), has been shown to have beneficial effects on human health (Kinsella *et al*., 1990; Mensink and Katan, 1989). Canola oil provides varying quantities of the essential nutrient good fatty acids. Canola oil is anexcellent source of good fats. It is very high in monounsaturated fat, contains intermediate amounts of the precursor omega-6 and omega-3 polyunsaturated fatty acids Linoleic Acid (LA) and Alpha-Linolenic Acid (ALA) respectively and is very low in saturated fat. Canola oil as a good contains significant amounts of vitamin E and phytosterols.

Choline has 3 chemically reactive methyl groups attached to the nitrogen atom of the glycine molecule. Therefore, it can be used as a methyl group donor partially to replace methionine in poultry and pig (Schrama and Gerrits, 2000). In poultry, choline's methyl group is available after the conversion to betaine in the liver. Recent research suggests that betaine and choline has an energy sparing role by reducing maintenance requirement poultry and pig (Scharma and Gerrits, 2000). The results obtained from the experiment were analyzed by analyses of variance using the General Linear Model (GLM) procedure of SAS and means were compared by Duncan's Multiple Range Test (SAS Institute, 2000).

MATERIALS AND METHODS

Animals and diets: Experimental was conducted of the Ross 308 strain were obtained from a commercial hatchery (270, 1 day old male broiler chicks) and were placed in floor pens of 1.65×0.671 m with 10 birds per pen.

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Feed and water were provided ad libitum. The experiment arrangement consisted of a 3×3 factorial design (3 CO levels and 3 CCS level) with 3 replicate per each treatment. Canola oil was used at 0, 2 and 4% in diets and choline chloride was used at 0, 500 and 1000 mg kg^{-1} in diet. Crude protein levels and metabolisable energy were NRC recommendation and few lower than recommendation as NRC recommendation. Metabolisable energy of canola oil was 7450 kcal kg^{-1} that used for diet formulation. This diet (Table 1-3) were formulated to meet nutrient requirements according to NRC. Diets were containing the same level of methionine, lysine, vitamins and minerals. The treatment diets of were isoenergetic and isonitrogenous:

T1 = Control (Soybean+ Corn)
T2 = 0% CO + 500 mg kg^{-1} CCS
T3 = 0% CO + 1000 mg kg^{-1} CCS
T4 = 2% CO + 0 mg kg^{-1} CCS
T5 = 2% CO + 500 mg kg^{-1} CCS
T6 = 2% CO + 1000 mg kg^{-1} CCS
T7 = 4% CO + 0 mg kg^{-1} CCS
T8 = 4% CO + 500 mg kg^{-1} CCS
T9 = 4% CO + 1000 mg kg^{-1} CCS

The chickens were weighed at the start of the experiment and during the experiment, live weight and total feed consumption per pen were recorded and feed conversion ratio was calculated at 21, 42 and 56 days of the experiment. Mortality was also recorded for each treatment. Two birds from each replicate were slaughtered after bleeding at days 56 and liver frozen in -21°C and translated to lab for analyses a cholesterol and triglyceride content. One gram the liver tissue is cut and homogenized in the TRISS dilution and determined cholesterol and triglyceride content in autoanalyser system.

Table 1: Percentage composition of experiment diet in starter period

Ingredients	(%)
Corn	53.50
Soybean	34.00
Canola oil	0.50
Starch	8.00
Wheat bran	0.00
DL-Methionine	0.54
Lysine	0.00
Choline (60%)	0.00
DCP	1.38
Oyster	1.33
Vitamin	0.25
Mineral	0.25
Salt	0.25
Coccidiostat	0.00
Sand	0.00
	100.00
Calculated nutrient content	
ME kcal kg^{-1}	2919.594
Crude protein (%)	20.901
Calcium (%)	0.942
Available P (%)	0.434
ME/CP	139.658
Ca/P	2.169

Vitamin content of diets provided per kilogram of diet: vitamin A, D, E and K; Composition of mineral premix provided as follows per kilogram of premix: Mn,120,000 mg; Zn, 80,000 mg; Fe, 90,000 mg; Cu, 15,000 mg; I, 1,600 mg; Se, 500 mg; Co, 600 mg

Table 2: Percentage composition of experimental diet in grower period

	Experimental diets (%)								
Ingredients	T1	T2	T3	T4	T5	T6	T7	T8	T9
Corn	64	64	64	60	60	60	55.0	55	55
Soybean	27.4	27.4	27.4	28	28	28	27.1	27.1	27.1
Canola fat	0	0	0	2	2	2	4.00	4	4
Starch	3.74	3.74	3.74	2.06	2.06	2.06	1.22	1.22	1.22
Wheat bran	1	1	1	2	2	2	5.50	5.5	5.5
Methionine	0	0	0	0	0	0	0.00	0	0
Lysine	0	0	0	0	0	0	0.00	0	0
Choline (60%)	0	0.000084	0.000168	0	0.000084	0.000168	0.00	0.000084	0.000168
DCP	0.89	0.89	0.89	0.92	0.92	0.92	0.89	0.89	0.89
Oyster	1.5	1.5	1.5	1.48	1.48	1.48	1.46	1.46	1.46
Mineral	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Coccidiostat	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Sand	0.33	0.33	0.33	2.42	2.42	2.42	3.66	3.66	3.66
	100	100	100	100	100	100	100.0	100	100
Calculated nutrient content									
ME kcal/kg	2920.238	2920.238	2920.238	2920.242	2920.242	2920.242	2919.984	2919.984	2919.984
CP	18.236	18.236	18.236	18.160	18.160	18.160	18.170	18.170	18.170
Calcium	0.903	0.903	0.903	0.898	0.898	0.898	0.899	0.899	0.899
Available P	0.351	0.351	0.351	0.350	0.350	0.350	0.358	0.358	0.358
ME / CP	160.136	160.136	160.136	160.806	160.806	160.806	160.708	160.708	160.708
Ca /P	2.574	2.574	2.574	2.565	2.565	2.565	2.515	2.515	2.515

Vitamin content of diets provided per kilogram of diet: vitamin A, D, E and K; Composition of mineral premix provided as follows per kilogram of premix: Mn,120,000 mg; Zn, 80,000 mg; Fe, 90,000 mg; Cu, 15,000 mg; I, 1,600 mg; Se, 500 mg; Co, 600 mg

Table 3: Percentage composition of experimental diet in finisher period

	Experimental diets (%)								
Ingredients	T1	T2	T3	T4	T5	T6	T7	T8	T9
Corn	66.5	66.5	66.5	57.5	57.5	57.5	56	56	56
Soybean	24.1	24.1	24.1	25.85	25.85	25.85	24	24	24
Canola fat	0	0	0	2	2	2	4	4	4
Starch	3.81	3.81	3.81	4.34	4.34	4.34	1.94	1.94	1.94
Wheat bran	0	0	0	5	5	5	6	6	6
Methionine	0.44	0.44	0.44	0.45	0.45	0.45	0.45	0.45	0.45
Lysine	0.043	0.043	0.043	0.015	0.015	0.015	0.08	0.08	0.08
Choline (60%)	0	0.000084	0.000168	0	0.000084	0.000168	0	0.000084	0.000168
DCP	0.89	0.89	0.89	0.92	0.92	0.92	0.89	0.89	0.89
Oyster	1.38	1.38	1.38	1.36	1.36	1.36	1.31	1.31	1.31
Mineral	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Coccidiostat	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Sand	1.937	1.937	1.937	1.665	1.665	1.665	4.43	4.43	4.43
	100	100	100	100	100	100	100	100	100
Calculated nutrient content									
ME kcal/kg	2920	2920	2920	2920	2920	2920	2920	2920	2920
CP	16.5	16.5	16.5	16.4	16.4	16.4	16.5	16.5	16.5
Calcium	0.79	0.79	0.79	0.79	0.79	0.79	0.74	0.74	0.74
Available P	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
ME / CP	176.8	176.8	176.8	177.4	177.4	177.4	176.6	176.6	176.6
Ca /P	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6

Vitamin content of diets provided per kilogram of diet: Vitamin A, D, E and K; Composition of mineral premix provided as follows per kilogram of premix: Mn,120,000 mg; Zn, 80,000 mg; Fe, 90,000 mg; Cu, 15,000 mg; I, 1,600 mg; Se, 500 mg; Co, 15,000 mg

Statistical analyses: Data were analyzed in a complete randomized design using the GLM procedure of SAS version 12 (SAS Inst. Inc. NC):

$$y_{ijk} = \mu + a_i + b_j + (a \times b)_{ij} + \varepsilon_{ijk}$$

where,

y_{ijk} = All dependent variable
μ = Overall mean
a_i = The fixed effect of CO levels (i = 1, 2, 3)
b_j = The fixed effect of CCS levels (j = 1, 2, 3)
ε_{ijk} = The random effect of residual

The 3 oil levels (0, 2 and 4% canola oil) and 3 choline chloride supplement levels (0, 500 and 1000 mg kg^{-1}) were analyzed as a 3×3 factorial. When interactions occurred ($p<0.05$), interaction means were separated using Duncan multiple range test to compare different treatment means.

RESULTS AND DISCUSSION

Result for liver chemical analyses show that in Table 4. Result show that interaction effects of canola oil and choline chloride supplement could affected liver cholesterol and triglyceride content. Canola oil and choline chloride supplement in 6 and 9 treatments (T6 = 2% CO + 1000 mg kg^{-1} CCS and T9 = 4% CO + 1000 mg kg^{-1} CCS) significantly decrease the liver cholesterol and triglyceride contents relationship in the other treatment ($p<0.0001$). Whereas choline chloride supplement independently in 2 treatment (500 mg kg^{-1} CCS without canola oil levels) could decrease significantly cholesterol contents in liver tissue ($p<0.0001$). Whither ward independently effects of canola oil couldn't affected cholesterol and triglyceride content in the liver tissue. Result in Table 4 shown the levels of 1000 and 500 mg kg^{-1} choline chloride supplement is highest effect on the liver cholesterol content respectively. Afterward of 6 and 9 treatment, 8 and 5 treatment (T8 = 4% CO, 500 mg kg^{-1} CCS and T5= 2% CO, 500 mg kg^{-1} CCS), could significantly decrease the liver cholesterol and triglyceride contents ($p<0.0001$). Some study suggest choline chloride supplement, decrease the triglyceride and cholesterol contents in the different of body organs such as liver tissue (Pesti *et al*., 1980; Workel *et al*., 1999; Kettunene *et al*., 2001a, b; Attia *et al*., 2005). It has seem choline chloride supplement have an effectively role in decrease the fatty liver syndrome because choline chloride with a methyl group donor has a important role in the fat metabolism (Kettunen *et al*., 2001a). Choline has 3 chemically reactive methyl groups attached to the nitrogen atom of the glycine molecule. Therefore, it can be used as a methyl group donor partially to replace methionine in poultry and pig (Schrama and Gerrits, 2000). The role of choline in the prevention of conditions such as perosis and liver enlargement in chicks is already well known. Pesti *et al*. (1980) has recently summarized the nutritional

Table 4: Least square means for liver triglyceride and cholesterol content

	Treatment										
	1	2	3	4	5	6	7	8	9	SEM	p>F
Triglyceride	3.07de	3.35cd	3.35cd	3.03de	2.54c	2.13a	3.00de	2.54c	2.90b	0.321	<0.0001
Cholesterol	0.63de	0.51d	0.84cd	0.60de	0.91c	1.48a	0.58de	1.08b	1.37ab	0.243	<0.0001

roles of methionine, choline and betaine and their inter-relationships. Choline and betaine besides methionine are all sources of labile methyl groups metabolism of these 2 compounds is interrelated (Pesti *et al*., 1980; Kettunen *et al*., 2001a, b). He clearly demonstrated that choline has essential metabolic function for which neither betaine nor methionine can substitute. One of these essential functions is in fat metabolism in the liver, i.e. utilization and outward transport of fat, so preventing abnormal accumulation of fat within hepatocytes-so called fatty liver.

In several research's it has showed canola oil under various decrease the cholesterol and triglyceride contents in the liver tissue and decrease the fatty liver syndrome (Janice and Hermann, 2005; Carneira *et al*., 2005). Canola oil is an excellent source of monounsaturated fat, contains intermediate amounts of the precursor omega-6 and omega-3 Polyunsaturated Fatty Acids (PUFA), Linoleic Acid (LA) and Alfa-Linoleic Acid (ALA) respectively and is very low saturated fat. Canola oil as a source of phytosterols. Phytosterols (plant sterols) are structural analogs of the cholesterol found in animals and humans. The consumption of phitosterols has been shown in numerous studies to lower blood cholesterol levels and may therefore help reduce the risk of cardiovascular disease (Awed and Fink, 2000). Resent research suggest that phitosterols may also provide protection against cancers of the colon, breast (Awed and Fink, 2000).

Since, saturated fat increase amount of Harmful Low-Density Lipoprotein (LDL) cholesterol in the blood and liver. Trans fat intake should also be limited, as it increase harmful LDL cholesterol levels and decreases beneficial High-Density Lipoprotein (HDL) cholesterol levels in blood and liver (Mensink and Katan, 1989; Katan, 1995; Ascherio and Willett, 1997). Monounsaturated fat has also been shown to lower cholesterol (Grundy, 1997; Mensink and Katan, 1995). It has seem canola oil with attend choline chloride supplement have a positive role in decrease a fatty liver syndrome, because the different levels of choline chloride supplement decrease the liver fatty content (cholesterol and triglyceride) independently.

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