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Influence of Excess Lysine and Methionine on Cholesterol, Fat and Performance of Broiler Chicks

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Abstract: This study was carried out to determine the effects of excess dietary Lysine (Lys) and Methionine (Met) on some blood parameters, cholesterol and fat content of carcass and the performance of broiler chicks. In a completely randomized design three hundred male Ross 308 broilers were allotted to five group, each of which included four replicates (15 birds per replicate). The groups received the same basal diet supplemented with Lys and Met (as TSAA) in 0, 10, 20, 30 or 40% more than NRC recommendation. The collected data were analyzed by SPSS software and Duncan's test was used to compare the means on a value of $p<0.05$. The results indicated that the two highest levels of Lys and Met treatments (30 and 40%> NRC recommendation) led to significant increase in carcass efficiency, breast muscle yield, heart and liver weight and also plasma cholesterol ($p<0.05$) whereas Feed Conversion Ratio (FCR), crude fat contents of breast and thigh muscles and plasma triglyceride were the least in these two treatment groups ($p<0.05$). Addition Lys and Met 40% more than NRC tend to significant decrease in body weight gain but there was no significant effect of treatment on cholesterol content of the breast and thigh muscle and also thigh and leg yield. The finding of this experiment showed that increasing Lys and Met to diets of today's broiler in excess of NRC recommendations can improve FCR, abdominal fat deposition, breast meat yield, fat content of the breast and thigh muscles and carcass efficiency and also results reported here support the hypothesis that it is possible to produce poultry meat with different fat content by supplementation excess Lys and Met to diet.

Key words: Lysine, methionine, cholesterol, fat, broiler, poultry meat

INTRODUCTION

There are a number of studies have been conducted to determine requirements of methionine and lysine as the first two limiting amino acids in practical corn-soybean based diets for broiler chicks. Recent researches have suggested that levels of lysine and methionine in excess of NRC (1994) recommendations may result in enhanced performance especially in regard to breast meat yield (Si *et al*., 2001; Schutte and Pack, 1995; Hickling *et al*., 1990; Moran and Bilgili, 1990), weight gain and feed conversion ratio (Si *et al*., 2001; Gorman and Balnave, 1995). Some studies else that have been conducted to evaluate the effects of these amino acids in excess of NRC recommendations on laying hens performance, confirmed it effect on egg production, feed conversion ratio, egg weight, egg mass and livability specially in low protein diets (Bouyeh and Gevorgian, 2011). Murray *et al*. (1998) found that addition of synthetic amino acids like lysine and methionine at high levels to the diet can stimulate insuline secretion from pancreas by aggregating in plasma which in turn releases amino acids and fatty acids (Sturkie, 1986) from the bodily saved sources and leads to protein synthesis, moreover, some reports have shown the positive effect of adding more lysine to the diet than required on the chickens suffering different stresses (Ayupov, 1985; Merch and MacMillan, 1987).

On the other hand, lysine and methionine as precursors of L-carnitine (Borum, 1983) can play important roles in lipid and energy metabolism in poultry. L-carnitine is a natural, vitamin-like substance that acts in the cells as a receptor molecule for activated fatty acids. The major metabolic role of it appears to be the transport of long-chain fatty acids into the mitochondria for B-oxidation (Coulter, 1995). A shortage of this substance results primary in impaired energy metabolism and membrane function (Harmeyer, 2002). In this regard, some researches indicated that carnitine supplementation of diets can be used to augment carnitine supply for use in metabolism, thereby facilitating fatty acid oxidation and reducing the amount of long-chain fatty acids available for storage in adipose tissue (Golzar Adabi *et al*., 2006).

Improvement in weight gain, feed conversion ratio, carcass characteristics or decrease in serum triglyceride in

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birds fed supplemented L-carnitine reported by researchers such as Lettner *et al*. (1992), Xu *et al*. (2003). The objective of the present study was to investigate the effects of dietary lysine and methionine in excess of usual levels on triglyceride and cholesterol of serum and carcass content and also productive performance of broiler chicks.

MATERIALS AND METHODS

This study was conducted at the broiler farm belonged to Islamic Azad univesity, Rasht branch using three hundred, 1 day old male broiler chickens (Ross, 308) that were selected very carefully in aspect of uniformity in body weight, appearance, motivity, etc. so that the body weight deviation of mean (46 g) was only 0.5 g. The chicks allotted to five treatment groups, each of which included four replicates of 15 birds, performed in a completely randomized design. Same basal diet was supplemented with 5 levels of synthetic lysine (as Lys-HCl) and methionine (D-L-methionine) in amount of 0 (control), 10, 20, 30 or 40% higher than NRC (1994) recommendation, regarding with lysine and Total Sulfur Amino Acids (TSAA) for broilers. Diets were fed from 1-42 days and included starter (1-21 days) and grower (21-42 days). Nutrient levels of the basal diets were based on the NRC (1994) recommendations. In order to buffer the excess chloride provided by L-Lys HCl, there was added 0.1% $NaHCO_3$ to both basal diets including starter and grower that were supplied in mash physical form (Table 1).

The broiler chickens were maintained in 2×1 m pens, equipped with bell drinkers and hanging tube feeders, feed and water were available *ad libitum*, light schedule, temperature and general management were performed according to Broiler Nutrition Specifications (2007). During 42 days experimental period, body weight gain, feed consumption, mortality and feed conversion ratio were recorded weekly, birds were checked twice a day for mortality; dead birds were weighed and the weight was used to adjust Feed Conversion Ratio (FCR: total feed consumed divided by weight of live birds plus dead birds). At 21 and 42 days of age three birds from each pen that were within one-half standard deviation of the overall pen mean and free from visible defects were randomly chosen for blood sampling which collected into a syringe from wing vein (1 mL from each bird) and placed in heparinized tubes. These blood samples were urgently sent to laboratory to determine triglyceride, cholesterol, Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), uric acid and glucose by especial kits (Pars Azemoon Co, Tehran, Iran).

At the end of the experiment, after blood sampling, feed but not water was withheld 6 h prior to slaughter and then that three birds of each replicate were processed for carcass characteristics. After weighing the carcass pieces, thigh (Biceps femoris) and breast (Pectoralis major) muscles without skin were taken, chopped, ground and frozen at -20°C until further analyses. After thawing, tissues were extracted with 2:1 chloroform: methanol. Total lipids were extracted as described by Folch *et al*. (1957) and cholesterol content of these tissues was determined enzymatically by the method of Allain *et al.* (1974) as modified by Sale *et al*. (1984). For evaluation of performance the weight of breast and thigh (with leg) muscles calculated as carcass weight percentage. At the end, data were analyzed by variance analysis method and Duncan's test was used to comparison the means based on a value of $p<0.05$ (SPSS, 2001).

Table 1: Composition (g kg^{-1}) of basal diets

Ingredient	Starter (0-21 days)	Grower (21-42 days)
Yellow corn	550.00	620.00
Dehulled soybean meal	380.00	320.00
Corn oil	21.50	14.00
Dicalcium phosphate	22.00	18.00
Oyster shell	14.00	15.80
Sodium chloride	2.00	2.00
Vitamin premix[1]	3.00	3.00
Trace mineral mix[2]	3.00	3.00
L-Lysine-HCl	1.20	1.10
DL-methionine (98%)	2.30	2.10
Sodium bicarbonate	1.00	1.00
Total	1000.00	1000.00
Nutrient		
ME (kcal kg^{-1})	3000.00	3000.00
CP (%)	21.90	19.80
Lysine (%)	1.10	1.00
Methionine (%)	0.50	0.40
TSAA (%)	0.90	0.75

[1]Provides per kg of diets: vit A 17500 IU, cholecalciferol 5000 IU, vit E 25 IU, B_{12} 0.03 mg, Riboflavin 15 mg, Niacin 75 mg, choline 700 mg, Folic acid 1.5 mg, Pyridoxine 6.25 mg, Biotin 0.127 mg, Thiamine 3.05 mg. [2]Provides per kg of diet: zinc 100 mg, manganese 120 mg, copper 10 mg, iron 75 mg, iodine 2.5 mg, selenium 0.15 mg, calcium 130 mg

RESULTS AND DISCUSSION

Data for some traits (body weight gain, feed conversion ratio, plasma triglyceride, cholesterol, LDL and HDL) belonged to 21 days of age are shown in Table 2. Analysis of variance indicated that the effect of supplemented lysine and methionine only on body gain was not significant and there was significant effect of treatments in terms of feed conversion ratio ($p<0.05$), plasma triglyceride, plasma cholesterol, LDL and HDL ($p<0.01$).

Linear reduction of feed conversion ratio was observed as lysine and methionine supplementation increased also the least plasma triglyceride belonged to the highest level of lysine and methionine (1.4 NRC) whereas the highest plasma cholesterol, LDL and HDL

Table 2: Effect of lysine and methionine on body weight, feed conversion and some blood parameters of broiler at 21 days of age

	Amount of lysine and methionine (based on TSAA) relative to NRC recommendation						
Variables	NRC (control)	1.1 NRC	1.2 NRC	1.3 NRC	1.4 NRC	SEM	p
Body weight gain (g)	863.00	831.00	811.0	849.00	773.00	11.14	NS
Feed conversion ratio	1.35^{ab}	1.34^{a}	1.3^{ab}	1.29^{b}	1.28^{b}	0.01	*
Plasma triglyceride (mg dL^{-1})	139.50^{a}	131.00^{a}	149.5^{a}	145.50^{a}	79.00^{b}	6.33	**
Plasma cholesterol (mg dL^{-1})	134.50^{b}	107.50^{c}	136.5^{b}	169.00^{a}	169.50^{a}	5.62	**
LDL (mg dL^{-1})	40.92^{b}	23.50^{c}	41.5^{b}	48.50^{b}	70.00^{a}	3.62	**
HDL (mg dL^{-1})	64.00^{b}	67.00^{b}	47.0^{c}	56.50^{c}	80.00^{a}	2.89	**

In each row means do not have the same letters, their differences are significant ($p<0.05$); *,** and NS are significant at 0.05 level, 0.01 and non-significant, respectively

Table 3: Effect of lysine and methionine on performance and carcass processing parameters of broilers at 42 days of age

	Amount of dietary lysine and methionine (based on TSAA) relative to NRC recommendation						
Variables	NRC (control)	1.1 NRC	1.2 NRC	1.3 NRC	1.4 NRC	SEM	p
Body weight gain (g)	2960.000^{a}	2920.000^{a}	2850.000^{ab}	2970.000^{a}	2730.000^{b}	28.590	*
Feed conversion ratio	1.830^{a}	1.820^{a}	1.890^{a}	1.620^{b}	1.690^{b}	0.025	**
Carcass efficiency (%)[1]	71.500^{c}	73.000^{b}	74.000^{b}	78.000^{a}	77.300^{a}	0.598	**
Breast muscle yield (%)[2]	34.670^{b}	36.650^{b}	35.600^{b}	38.120^{a}	39.100^{a}	0.454	*
Thigh and leg percentage (%)[2]	37.220	40.820	36.950	37.650	33.670	0.944	NS
Abdominal fat pad (%)	0.910^{a}	0.850^{ab}	0.840^{b}	0.670^{c}	0.440^{d}	0.041	**
Liver weight (%)	2.170^{c}	2.210^{c}	2.670^{b}	2.750^{ab}	2.940^{a}	0.740	**
Heart weight (%)	0.605^{c}	0.617^{c}	0.710^{b}	0.810^{a}	0.827^{a}	0.023	**

[1]Carcass weight: live body weigh just before slaughter; [2]with bone *,** and NS are significant at 0.05 level, 0.01 and non-significant, respectively in each row the means that do not have the same letters, their differences are significant ($p<0.05$)

($p<0.05$) in this treatment group. The second level of lysine and methionine (1.1 NRC) in term of cholesterol and LDL was the least. Table 3 shows the effect of levels of lysine and methionine on some performance traits at the end of experiment (42 days). No significant effect of trial diets was observed in term of thigh and leg percentage of carcass weight but significant effect relate to body gain, breast meat yield ($p<0.05$) and feed conversion ratio, carcass efficiency, abdominal fat pad, liver and heart weight ($p<0.01$) was observed. Comparison between treatment means showed that based on a value of $p<0.05$, in regard with body gain and abdominal fat, the least value belonged to the highest treatment (1.4 NRC). There was a linear reduction of abdominal fat parallel with increase dietary lysine and methionine whereas means of carcass efficiency, breast muscle and heart weight of this group and lower level (1.3 NRC) was significantly higher than others. The weight of liver and heart (as percentage of carcass weight) was linearly increased in response to addition of dietary lysine and methionine. Feed conversion ratio in 1.3 and 1.4 NRC treatments was significantly ($p<0.05$) lower than the treatment groups with lower levels of lysine and methionine (Table 3).

The effect of trial diets on some blood parameters and also breast and thigh muscle cholesterol and crude fat at the end of experiment (42 days) are shown in Table 4. Analysis of variance showed that excess lysine and methionine had no significant effect on the cholesterol content of breast and thigh muscle although two highest level treatment groups had the most numeric amount.

Supplemented with the excess lysine and methionine diet had significant effect on all of measured blood parameters include plasma triglyceride, total cholesterol LDL, HDL, uric acid and glucose (mg dL^{-1}) and also cholesterol and crude fat of breast and thigh muscle ($p<0.01$).

The highest level of lysine and methionine treatment significantly ($p<0.05$) had least plasma triglyceride (about 45% decreasing in comparison with control), breast and thigh crude fat level whereas the highest in plasma LDL, HDL and uric acid. In term of plasma total cholesterol, two upper levels of lysine and methionine treatments (1.3 and 1.4 NRC) showed the highest amount ($p<0.05$). Blood glucose only in control treatment (NRC) was significantly lower than others (Table 4). Mortality was very low (only 4 birds) that belonged to 4 different groups and so its data was not enough to analysis.

In this study plasma triglyceride, abdominal fat pad and the fat content of breast and thigh muscles were affected by supplemented lysine and methionine in excess of NRC (1994) recommendation, so that reduce about 45% in plasma triglyceride, 50% in abdominal fat, 35% in breast fat content and 27% in thigh muscle fat in the highest level of lysine and methionine group in comparison with control group (NRC) was observed (Table 3 and 4). This result can be caused by two separate effects of lysine and methionine in high level: as two amino acids tend to stimulate pancreas for further secretion insulin into blood. Insulin in poultry versus mammals is not an antilipolytic

Table 4: Effect of lysine and methionine on some blood parameters and fat and cholesterol content of breast and thigh of broilers at 42 days of age

	Amount of dietary lysine and methionine (based on TSAA) relative to NRC recommendation						
Variables	NRC (control)	1.1NRC	1.2 NRC	1.3 NRC	1.4 NRC	SEM	p
Plasma triglyceride (mg dL^{-1})	142.00^{a}	130.00^{b}	143.50^{a}	119.00^{c}	77.50^{d}	5.55	**
Plasma cholesterol (mg dL^{-1})	147.00^{b}	116.00^{c}	108.00^{d}	161.50^{a}	165.00^{a}	5.42	**
LDL (mg dL^{-1})	44.00^{b}	23.50^{c}	23.00^{c}	46.50^{b}	64.50^{a}	3.70	**
HDL (mg dL^{-1})	61.00^{b}	62.50^{b}	48.50^{c}	58.50^{b}	80.00^{a}	2.50	**
Blood uric acid (mg dL^{-1})	3.00^{c}	3.70^{b}	3.10^{c}	3.60^{b}	5.70^{a}	0.23	**
Blood glucose (mg dL^{-1})	159.00^{b}	214.00^{a}	233.00^{a}	237.00^{a}	227.00^{a}	7.10	**
Breast muscle cholesterol (mg/100 g wet tissue)	44.70	43.00	41.00	47.50	48.50	1.16	NS
Thigh cholesterol (mg/100 g wet tissue)	88.00	84.00	80.00	89.00	92.00	1.65	NS
Breast crude fat (mg/100 g wet tissue)	1.17^{b}	1.00^{c}	1.30^{a}	0.94^{c}	0.75^{d}	0.46	**
Thigh crude fat (mg/100 g wet tissue)	4.10^{ab}	3.97^{bc}	4.35^{a}	3.70^{c}	3.00^{d}	0.11	**

*,** and NS are significant at 0.05 level, 0.01 and non-significant, respectively; In each row the means that do not have the same letters, their differences are significant ($p<0.05$)

hormone, on the contrary, it can exert glucagon effect on release fatty acids and amino acids from the bodily saved sources and lead to protein synthesis (Sturkie, 1986) moreover, there is no question that breast meat yield as a major portion of the protein synthesis in the body is very sensitive to essential amino acids of the diets specially in todays broiler strains that are genetically emphasized on processing and breast meat yield and so, it is suggested that significant increasing breast muscle percentage and carcass efficiency in treatments with higher dietary levels of lysine and methionine (1.3 and 1.4 NRC) that is shown in Table 3 can be occurred from this aspects. This findings are in agree with that of Si *et al.* (2001), Schutte and Pack (1995) and Hickling *et al.* (1990). In contrast, some researchers such as Han and Baker (1993) failed to observe any favourable response to supplemented excess dietary lysine and methionine.

Supplementation of this two amino acids to diets as precursors of L-carnitine could be used to augment carnitine supply for use in metabolism, thereby facilitating fatty acid oxidation and so reducing the amount of long-chain fatty acids available for storage in adipose tissues. An increase in carnitine synthesis causes the increase of carnitine concentration in muscle and liver which leads to the increase activity of carnitine acetyltransferase and accelerate the transportation of acetyl-CoA from mitochondria to cytosol. Acetyl-CoA is the source of all the carbon atoms in cholesterol, on the other hand, some studies showed that administration of insulin increases activity of the effective enzyme for cholesterol synthesis means HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase (Murray *et al*., 1998). Significant increase in plasma cholesterol, LDL and HDL in the highest level of lysine and methionine (1.4 NRC) that observed in present study may be acceptable explanation from mentioned above aspects. This effect on breast and thigh muscle cholesterol was not significant (Table 4), the liver is the primary site for cholesterol synthesis in poultry and so make more change in plasma cholesterol in comparison with breast and thigh muscle, perhaps because plasma and liver cholesterol belong to the fast turnover cholesterol pool (Chobanian and Hollander, 1962). The muscle cholesterol pool comprises the slow turnover pool and equilibrates slowly with the plasma cholesterol pool. The muscle cholesterol pool is larger and perhaps less active and it may need a longer feeding period to show a significant increase or reduction of cholesterol levels. A part of excess Acetyl-CoA present in cytosol can go to gluconeogenesis pathway so, as expected, in this study supplemented lysine and methionine to basal diet (NRC), tend to significant ($p<0.05$) increase in blood glucose. A possible explanation about cholesterol concentrations, that were found to be much higher in the thigh than in breast muscle (Table 4) is that cholesterol usually associated with adipose tissue which is normally abundant in thigh than breast muscle.

Improvement in feed conversion ratio in the two highest levels of lysine and methionine treatments (Table 2 and 3) represents a more feed efficiency due to enhanced performance in metabolism of energy and protein which is in agree with some studies (Xu *et al.*, 2003; Si *et al.*, 2001; Gorman and Balnave, 1995).

Increase of liver weight (as percentage of carcass weight) in a linear manner as lysine and methionine increased (Table 3) may be due to a positive response to addition rate of metabolism for synthesis L-carnitine, glucose, cholesterol, protein and even degrade the excess lysine and methionine so that significant increase in blood uric acid was observed specially in the highest level of lysine and methionine treatment group (Table 4). In regard with heart weight percentage, a linear increase similar to liver weight was observed. Corresponding to some finding, L-carnitine is necessary for correct activity of heart muscle which itself made from lysine and methionine (Harmeyer, 2002). The present result from an aspect is in agree with Buyse *et al.* (2001) who found that addition L-carnitine to the broilers' diet caused increase in heart weight. This increase was related to improvement in heart efficiency and didn't cause by hypertrophy of right ventricle.

CONCLUSION

The results obtained from this study implicate that excess lysine and methionine could decrease abdominal fat content, feed conversion ratio, breast and thigh crude fat and plasma triglyceride and increase breast muscle yield and carcass efficiency. So, it is suggested that levels of lysine and methionine in excess of NRC (1994) recommendations may result in enhanced economical performance and processing yield especially in regard to the above-mentioned traits in today's high performance broiler strains like Ross 308 and also results reported here support the hypothesis that it is possible to produce poultry meat with different fat content by supplementation lysine and methionine in excess of usual.

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