

# **Effect of Excess Lysine and Methionine on Immune system and Performance of Broilers**

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## **Abstract**

The present work was carried out to investigate the effects of excess dietary Lysine (Lys) and Methionine (Met) on some blood immune parameters and the performance of broiler chicks. Three hundred male Ross 308 broilers were allotted to five group, each of which included four replicates (15 birds per replicate) in a completely randomized design. The treatment groups received the same basal diet supplemented with Lys and Met (as TSAA) in 0, 10, 20, 30 or 40% more than NRC (1994) 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% more than NRC recommendation) led to significant increase in blood lymphocytes and decrease in heterophyles and ratio of heterophyles to lymphocytes as a stress index ( $P < 0.01$ ). There was a linear increase in Newcastle antibody parallel with increasing dietary Lys and Met in 42 days of age ( $P < 0.01$ ) but not 21d. Carcass efficiency, breast muscle yield, heart and liver weight were also increased by the two highest levels of Lys and Met ( $P < 0.05$ ), whereas feed conversion ratio (FCR) was 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 ( $P < 0.05$ ) but there was no significant effect of treatments on 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 immune system functions, FCR, abdominal fat deposition, breast meat yield and carcass efficiency. Results reported here support the hypothesis that it is possible to produce more healthy and economic poultry meat by supplementation excess Lys and Met to broiler diets.

**Key words:** Lysine, Methionine, Immune system, broiler.

## **Introduction**

There are a number of studies have been conducted to determine the 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, 2004; Schutte and Pack, 1999; Hicking 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 its effect on egg production, feed conversion ratio, egg weight, egg mass and livability specially in low protein diets (Bouyeh and Gevorgyan, 2011). Murray et al (1998) found that addition of synthetic amino acids like lysine and methionine at high levels to the diet can stimulate insulin 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, 1989; March and MacMillan, 1987).

On the other hand, lysine and methionine as precursors of L-carnitine (Barum, 1983; Feller and Rudman, 1988) 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  $\beta$ -oxidation (Coulter, 1995). A shortage of this substance results primarily 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; Kidd et al, 2004; Daskiran and Tetler, 2001).

Improvement in weight gain, feed conversion ratio, carcass characteristics or decrease in serum triglyceride in birds fed supplemented L-carnitine reported by researchers such as Lettner et al (1992), Xu et al (2003).

Several kinds of animal feed supplements of different origins have been recently developed with the general aim of enhance, either through an exogenous pathway or by stimulating the animal's immune response, a health status that in turn may allow a gradual decrease in antibiotics use. Among such compounds, there are some additives widely acknowledged and extensively used in animal nutrition, such as fatty acids, vitamins and minerals, as well as acidifiers, enzymes, flavoring and antioxidant agents, coccidiostats, and probiotics, which are usually employed at higher levels than strictly nutritional compounds. In addition, a more recent class of substances has been used to activate animal immune system, including oral immunoglobulins, intestinal flora stimulators, and oligosaccharides that block the adhesion of pathogenic bacteria. Also, the addition and improving the lysine and methionine in the poultry and fish diets, improve immunity of the birds against different diseases (Eduardo *et al.*, 2009. Rubin et al 2003).

Methionine, lysine (Swain & Johri, 2000; Rama Rao *et al.*, 2003; Shini & Brydeen, 2005) and arginine (Tayade *et al.*, 2006ab; Lee *et al.*, 2002) are three amino acids that have proven immune regulatory action. Methionine is an essential amino acid with at least four main roles that may be involved direct or indirectly in immune system responses. First, Met participates in protein synthesis. Second, Met is a glutathione precursor, a tripeptide that reduces reactive oxygen species (ROS) and thus protects cells from oxidative stress. Third, methionine is required for the synthesis of polyamines (spermine and spermidine), which take part in nucleus and cell division events. Fourth, methionine is the most important methyl group donor for methylation reactions of DNA and other molecules.

Several studies demonstrated that methionine constructively affects the immune system, improving both cellular and humoral immune response. It was reported that methionine requirements for optimal immunity are higher than for optimal growth (Tsiagbe *et al.*, 1987; Swain & Johri, 2000; Shini & Bryden, 2005), and that restriction of sulfur amino acids (SAA) results in severe lymphocyte depletion in intestinal tissues (Peyer's patches) and *lamina propria* (Swain & Johri, 2000). One of the mechanisms proposed to explain methionine interference in the immune system is the proliferation of T cells, which are sensitive to intracellular glutathione and cysteine levels, compounds also participate in methionine metabolism (Kinscherf *et al.*, 1994).

In a study, the effect of lysine deficiency on chicken immune function was evaluated using broiler chickens fed a diet with lysine at 67% of the control diet (1.24% lysine). The evaluation

of humoral immune function was conducted by measuring the antibody production to a live Newcastle disease virus (NDV) vaccination using the hemagglutination Inhibition (HI) test and enzyme-linked immunosorbent assay (ELISA). The cellular immune function was evaluated through the use of cutaneous basophil hypersensitivity test. The antibody response to NDV vaccination was reduced in broiler chickens fed a lysine-deficient diet when measured by The cell-mediated immune response was also reduced by lysine deficiency.

Rubin et al ( ) study aimed at assessing the effects of methionine and arginine on the immune response of broiler chickens submitted to immunological stimuli. Three methionine concentrations (0.31, 0.51, and 0.66% from 1 to 21 days of age; 0.29, 0.49, and 0.64% from 22 to 42 days of age) and 2 arginine concentrations (1.33 and 1.83%; 1.14 and 1.64% for the same life periods) were tested, Vaccines against Marek's disease, fowl pox, infectious bronchitis, Sheep red blood cells (SRBC), and avian tuberculin were administered to one group as immunological stimuli; Arginine levels did not influence either bird performance or immune response. Methionine concentrations higher or lower than usually adopted in broiler production (0.51 and 0.49%) equally failed to influence the birds' immune humoral response, but the best CIR was observed at the intermediate methionine level. Vaccines administered on the first day of age impaired bird performance up to the 21<sup>st</sup> day of age.

Also, the effects of methionine and lysine deficiency on freshwater was evaluated by Khalil et al (2010). During the study, the effects on different immunological parameters were assayed. The results revealed that, the lymphocytes, monocytes, basophils and eosinophils percentages decreased severely in deficiency of methionine and lysine. The level of Total protein, albumin and albumin / globulin ratio showed a higher level in control group. Meanwhile the lower level observed in the groups fed on methionine and lysine deficiency

The objective of the present study was to determine the effects of dietary lysine and methionine in excess of usual levels on some immune and productive performance of broiler chicks.

## **Materials and Methods**

This experiment was conducted at the broiler farm belonged to Islamic Azad univesity, Rasht branch, using three hundred one-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 (46gr) was only 0.5gr. 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 to 42 d and included starter (1 to 21 d) and grower (21 to 42 d). 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<sub>3</sub> to both basal diets including starter and grower that were supplied in mash physical form (Table 1).

*The broiler chickens were maintained in 2 x 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 Ross broiler manual (2009). During 42 d 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 day 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.*<sup>1</sup>

At the end of the experiment, after blood sampling, feed but not water was withheld 6 hr 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<sup>0</sup>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 Allan 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 inc., 2001).

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**Table 1. Composition (g/kg) of basal diets**

<b>Ingredient</b>	<b>Starter (0-21 d)</b>	<b>Grower (21-42 d)</b>
Yellow corn	550	620
Dehulled soybean meal	380	320
Corn oil	21.5	14
Dicalcium phosphate	22	18
Oyster shell	14	15.8
Sodium chloride	2	2
Vitamin premix <sup>1</sup>	3	3
Trace mineral mix <sup>2</sup>	3	3
L-Lysine-HCl	1.2	1.1
DL-methionine (98%)	2.3	2.1
Sodium bicarbonate	1	1
Total	1000	1000
<b>Nutrient:</b>		
ME (kcal/kg)	3000	3000
CP (%)	21.90	19.8
Lysine (%)	1.10	1.00
Methionine (%)	0.50	0.40
TSAA (%)	0.90	0.75

<sup>1</sup> Provides per kg of diets: vit A 17500 IU, cholecalciferol 5000 IU, vit E 25 IU, B<sub>12</sub> 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.

<sup>2</sup> Provides per kg of diet: zinc 100 mg, manganese 120 mg, copper 10 mg, iron 75 mg, iodine 2.5mg, selenium 0.15 mg, calcium 130 mg

## Results and Discussion:

### 1- Effect on immune system:

Table ... shows the data for some blood parameters relate to body immune system under effect of different levels of dietary lysine and methionine. As it is shown, analysis of variance indicated that the effect of supplemented lysine and methionine only on Newcastle antibody in 21 days of age of the broilers was not significant but this influence on other studied parameters including percentage of lymphocytes and heterophyles, heterophyle : lymphocyte ratio and also Newcastle antibody in 42 days of age of the broilers was significant ( $p < 0.01$ ).

In regard with percentage of lymphocyte, the highest amount belonged to the highest level of dietary lysine and methionine (+40% NRC) and the lowest, to the third treatment level (+20% NRC), there was no significant difference among the rest of treatment groups but they significantly differ with the highest and lowest data ( $p < 0.05$ ).

Some differences was observed between the effects of lysine and methionine on percentage of heterophyle, so that the treatment with lysine and methionine +20% NRC recommendation showed significantly highest mean ( $p < 0.05$ ) and the lowest belonged to +30% and +40% NRC treatment groups. When *heterophyle to lymphocyte* ratio, as a particular parameter, was calculated and statistically analyzed, it was revealed that the two highest levels of dietary lysine and methionine (+30% and +40% NRC recommendation) showed significantly the least ratio ( $p < 0.05$ ), although there was not observed any significant difference between the treatment +10% NRC and +30% NRC . In this parameter the highest mean belonged to the treatment +20% NRC (Table 5).

Effect of the treatments on Newcastle antibodies in 21 and 42 days of age of the broilers was different so that no significant difference was observed among treatment groups in 21 days of age. On the contrary it was observed a linear trend to increase Newcastle antibody parallel to increasing dietary lysine and methionine so that control treatment (NRC) showed the lowest and the highest level of dietary lysine and methionine (+40% NRC), the highest antibody ( $p < 0.05$ ).

**Table 2. Effect of lysine and methionine on some blood immune parameters of the broilers.**

Variable	NRC (control)	1.1 NRC	1.2 NRC	1.3 NRC	1.4 NRC	SEM	P
Lymphocytes (%)	64.70 <sup>b</sup>	65.50 <sup>b</sup>	56.70 <sup>c</sup>	64.00 <sup>b</sup>	71.00 <sup>a</sup>	1.28	**
Heterophyles (%)	34.75 <sup>b</sup>	27.50 <sup>c</sup>	41.50 <sup>a</sup>	28.75 <sup>c</sup>	25.75 <sup>c</sup>	1.41	**
Heterophyle/ Lymphocytes	0.53 <sup>b</sup>	0.42 <sup>c</sup>	0.73 <sup>a</sup>	0.44 <sup>c</sup>	0.36 <sup>d</sup>	0.30	**
Newcastle antibody (21 d) <sup>1</sup>	4.30	4.60	4.90	5.00	5.30	0.11	NS
Newcastle antibody (42 d) <sup>1</sup>	6.00 <sup>c</sup>	6.50 <sup>c</sup>	6.50 <sup>c</sup>	7.50 <sup>b</sup>	8.50 <sup>a</sup>	0.22	**

In each row means that do not have the same letters, their differences are significant ( $P < 0.05$ )

\*,\*\* and <sup>NS</sup> are significant at 0.05 level, 0.01 and non-significant, respectively.

<sup>1</sup>- Log<sub>2</sub> HI titer.

Methionine participates in protein synthesis as an essential amino acid and is also as a glutathione precursor that helps to protect cells from oxidative stress, and is required for the synthesis of polyamines (spermine and spermidine), which take part in nucleus and cell division processes and besides, methionine is the most important methyl group donor for methylation reaction of DNA and other molecules. On the other hand lysine is also essential amino acid that is necessary to produce proteins like antibodies, so adequate dietary levels of these amino acids are needed to support optimum performance of immune system. Some poultry nutritionists use the level recommended by the National Research Council (NRC) as a guideline in establishing their own amino acid requirements regardless of location, health or environmental conditions. There are few research works relate to the effect of lysine and methionine on immune system. Several studies demonstrated that methionine and lysine constructively affect the immune system improving both cellular and humoral immune response. It was reported that methionine and lysine requirements for optimal immunity are higher than for optimal growth (Tsiagbe et al, 1987; Swain and Johri, 2000; Shini and Bryden, 2005; Khalil et al, 2010). Also it is reported that restriction of sulfur amino acids (SAA) results in severe lymphocyte depletion in intestinal tissues (Swain and Johri, 2000).

As it is shown in Table 5, in addition, the parameters relate to immune system are improved in treatment groups with higher levels of lysine and methionine that corresponds to the above mentioned research works.

*Heterophyle to lymphocyte* ratio is usually calculated as a parameter relate with stress, so that the more ratio shows the more problem in body due to stress condition. In this study blood sampling was performed a short time after weighting in the end of experimental period (42 d of age) to study the effect of treatments on broilers under stress condition too (weighting always tends to cause stress in broilers). Data showed that the minimum *heterophyle to lymphocyte* ratio belong to the highest level of lysine and methionine (+40%NRC) which means that higher amount of lysine and methionine could modulate the negative effects of stress in this condition.

On the other hand lysine and methionine as precursors of carnitine could be effective on immune system because L-carnitine has been found to exhibit immune-modulatory effects so that increase the proliferative responses of both marine and human lymphocytes was observed following mitogenic stimulation at in vitro experiment (Cavazza, 1983; Franceschi et al, 1990) and in vivo (Kurth et al, 1994; Typit et al, 1991; Mast et al, 2000). So, the results of present work in regard with immune response are in agreement with those studies.

## **2- Effect on Performance and blood parameters in 21 and 42 days of age:**

Data for some traits (body weight gain, feed conversion ratio, plasma triglyceride, cholesterol, LDL and HDL) belonged to 21 d of age are shown in Table 2. Analysis of variance indicated that the effect of supplemented lysine and methionine only on body weight 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 which had the highest level in this treatment group ( $P < 0.01$ ). The second level of lysine and methionine (1.1 NRC) in term of cholesterol and LDL was the least.

**Table 3. Effect of lysine and methionine on body weight, feed conversion and some blood parameters of broiler at 21 day of age**

Variable	Amount of lysine and methionine(based on TSAA) relative to NRC recommendation					SEM	P
	NRC (control)	1.1 NRC	1.2 NRC	1.3 NRC	1.4 NRC		
Body weight gain (g)	863	831	811	849	773	11.14	NS
Feed conversion ratio	1.35 <sup>ab</sup>	1.34 <sup>a</sup>	1.3 <sup>ab</sup>	1.29 <sup>b</sup>	1.28 <sup>b</sup>	0.01	*
Plasma triglyceride (mg/dl)	139.5 <sup>a</sup>	131 <sup>a</sup>	149.5 <sup>a</sup>	145.5 <sup>a</sup>	79 <sup>b</sup>	6.33	**
Plasma cholesterol (mg/dl)	134.5 <sup>b</sup>	107.5 <sup>c</sup>	136.5 <sup>b</sup>	169 <sup>a</sup>	169.5 <sup>a</sup>	5.62	**
LDL (mg/dl)	40.92 <sup>b</sup>	23.5 <sup>c</sup>	41.5 <sup>b</sup>	48.5 <sup>b</sup>	70 <sup>a</sup>	3.62	**
HDL (mg/dl)	64 <sup>b</sup>	67 <sup>b</sup>	47 <sup>c</sup>	56.5 <sup>c</sup>	80 <sup>a</sup>	2.89	**

In each row means do not have the same letters, their differences are significant ( $P < 0.05$ )

\*\*\* and <sup>NS</sup> are significant at 0.05 level, 0.01 and non-significant, respectively.

Table 3 shows the effect of levels of lysine and methionine on some performance traits at the end of experiment (42 d). 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 (1.3NRC) 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).

**Table 4. Effect of lysine and methionine on performance and carcass processing parameters of broilers at 42 day of age**

Variable	Amount of dietary lysine and methionine (based on TSAA) relative to NRC recommendation					SEM	P
	NRC (control)	1.1 NRC	1.2 NRC	1.3 NRC	1.4 NRC		
Body weight gain (g)	2960 <sup>a</sup>	2920 <sup>a</sup>	2850 <sup>ab</sup>	2970 <sup>a</sup>	2730 <sup>b</sup>	28.59	*
Feed conversion ratio	1.83 <sup>a</sup>	1.82 <sup>a</sup>	1.89 <sup>a</sup>	1.62 <sup>b</sup>	1.69 <sup>b</sup>	0.025	**
Carcass efficiency (%) <sup>1</sup>	71.5 <sup>c</sup>	73 <sup>b</sup>	74 <sup>b</sup>	78 <sup>a</sup>	77.3 <sup>a</sup>	0.598	**
Breast muscle yield (%) <sup>2</sup>	34.67 <sup>b</sup>	36.65 <sup>b</sup>	35.60 <sup>b</sup>	38.12 <sup>a</sup>	39.10 <sup>a</sup>	0.454	*
Thigh and leg percentage (%) <sup>2</sup>	37.22	40.82	36.95	37.65	33.67	0.944	NS
Abdominal fat pad (%)	0.91 <sup>a</sup>	0.85 <sup>ab</sup>	0.84 <sup>b</sup>	0.67 <sup>c</sup>	0.44 <sup>d</sup>	0.041	**
Liver weight (%)	2.17 <sup>c</sup>	2.21 <sup>c</sup>	2.67 <sup>b</sup>	2.75 <sup>ab</sup>	2.94 <sup>a</sup>	0.74	**
Heart weight (%)	0.605 <sup>c</sup>	0.617 <sup>c</sup>	0.710 <sup>b</sup>	0.810 <sup>a</sup>	0.827 <sup>a</sup>	0.023	**

<sup>1</sup> Carcass weight: live body weight just before slaughter

<sup>2</sup> with bone

\*,\*\* and NS are significant at 0.05 level, 0.01 and non-significant their differences are significant (P<0.05)

In this study plasma triglyceride and abdominal fat pad were affected by supplemented lysine and methionine in excess of NRC (1994) recommendation, so that reduce about 45% in plasma triglyceride and 50% in abdominal fat was observed at the highest level of lysine and methionine group in comparison with control group (Table 3). This result can be caused by two separate effects of lysine and methionine in high level: 1), as two amino acids tend to stimulate pancreas for further secretion insulin into blood. Insulin in poultry versus mammals is not an anti-lipolytic hormone, on the contrary, it can exert the 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 today's broiler strains that are

genetically emphasized on high processing characteristic 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 Hicking et al (1990). In contrast, some researchers such as Han and Baker (1993) failed to observe any favorable response to supplemented excess dietary lysine and methionine.

2), supplementation of these two amino acids to diets as precursors of L-carnitine, could be tend 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 increase the 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.

Body weight gain in the highest level of lysine and methionine was the least among all of the treatment groups. A part of this difference can be due to less fat content in the carcass of this treatment group (+40%NRC) and a less part may be because of dietary amino acid imbalance due to adding only two amino acids without considering others. 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 Belnave, 1995) and also decrease in fat content of body.

Increasing 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. 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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