

Effects of Amino Acid Supplementation on Nutritional Values and L-Carnitin of Broiler Meat

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Abstract. Fat is the source of energy and fatty acid in poultry nutrition but palm oil addition will increase fat and lower protein meat content. Proper diet manipulation can modify broiler meat nutrient content and increase its nutritional value. Essential and nonessential amino acid supplementation was reported to affect meat nutrient composition. This research was aimed to manipulate meat nutrient content by amino acid supplementation. Completely Randomized Design was assigned to six treatment and four replicates. Broilers were fed with treatment feed consisted of R0 (basal feed with 5% palm oil); R1 (basal feed + 0.25% glycine); R2 (basal feed + 0.25% taurine); R3 (basal feed + 15% total lysine and methionine basal feed); R4 (basal feed + 0.25% glycine + 0.25% taurine); R5 (basal feed + 0.25% glycine + 0.25% taurine + 15% total lysine and methionine of basal feed). Parameters observed were dry matter content, crude protein, crude fat and L-carnitine of meat. Meat nutrient content was subjected to anova followed by Tukey test for changes in variables, while descriptive statistics were used to analyzed L-carnitine. Result showed that amino acid supplementation significantly increased broiler dry matter and fat content but did not significantly affect protein content. L-carnitine decreased after amino acid supplementation except broilers fed with R5 ration. It can be concluded that broilers fed with R5 ration was the most nutritious due to high L-carnitine and meat protein but relatively low fat.

Key words: amino acid, broiler, L-carnitine, meat nutrient content

Abstrak. Lemak merupakan sumber energi dan sumber asam lemak pada nutrisi unggas, tetapi penambahan minyak sawit akan meningkatkan proporsi lemak dan menurunkan protein. Manipulasi yang tepat pada pakan ayam broiler dapat memodifikasi kandungan nutrisi daging dan meningkatkan nilai nutrisinya. Suplemenasi asam amino esensial dan nonesensial dilaporkan dapat berpengaruh terhadap komposisi nutrisi daging. Penelitian ini bertujuan untuk memanipulasi kandungan nutrisi daging dengan suplementasi asam amino. Rancangan percobaan menggunakan rancangan acak lengkap (RAL) dengan enam perlakuan dan empat ulangan. Ayam broiler diberi pakan perlakuan yang terdiri dari R0 (ransum basal mengandung 5% minyak sawit); R1 (ransum basal + 0,25% glisin); R2 (ransum basal + 0,25% taurin); R3 (ransum basal + 15% total lisin dan methionin ransum basal); R4 (ransum basal + 0,25% glisin + 0,25% taurin); R5 (ransum basal + 0,25% glisin + 0,25% taurin + 15% total lisin dan methionin ransum basal). Parameter yang diamati pada penelitian ini meliputi bahan kering, protein kasar, lemak kasar dan L-karnitin pada daging. Kandungan nutrisi daging dianalisis menggunakan analisis variansi kemudian dilanjutkan uji Tukey apabila ada perbedaan yang muncul dalam peubah, sedangkan L-karnitin dianalisis menggunakan deskriptif statistik. Hasil penelitian menunjukkan suplementasi asam amino meningkatkan kandungan bahan kering dan lemak daging, tetapi tidak berpengaruh pada kandungan protein daging. Kandungan L-karnitin daging sebagian besar turun dengan suplementasi asam amino, kecuali ayam yang diberi pakan R5. Disimpulkan bahwa kandungan daging ayam broiler yang diberi pakan R5 paling nutrisi karena mengandung L-karnitin daging dan protein tinggi dan relatif rendah lemak.

Kata kunci : asam amino, ayam broiler, L-karnitin, kandungan nutrisi daging

Introduction

Broiler meat are the source of animal protein to meet human protein need. Broiler consumers recently prefer low fat meat or

“leaner” and they dislike the texture and taste of highly fatty meat for its harm to health (Leeson and Summer, 2005). Broiler meat quality depends on genetic, sex, slaughtering,

and environmental condition, particularly feed (Gropper et al., 2009; Barteczko and Lasek 2008; Cavani et al., 2009). Proper manipulation of broiler feed can modify meat nutrient and increase nutritional value (Barteczko and Lasek, 2008). Panja et al. (1995) reported that broiler fed with ration containing 6% palm oil showed fat increase and protein decrease. Essential and nonessential amino acid supplementation was reported to affect meat nutrient content (Lilly, 2010; Namroud et al., 2010; Zeweil et al., 2011).

Nonessential amino acid supplementation in this research were glycine and taurine, while essential amino acids were lysine and methionine. Supplementation of glycine in low protein diet was reported to lower carcass crude fat (Namroud et al., 2010). Glycine also works in protein biosynthesis and is one of protein building blocks that supplies precursor for cysteine synthesis leading to the use of other amino acids for protein synthesis (Powell et al., 2009). Taurine supplementation in ration with protein source significantly increased protein content and lowered carcass crude fat. Plant protein source deficient to taurine and taurine biosynthesis in body was insufficient for daily taurine need. Accordingly, taurine supplementation increased meat protein. Taurine is hypolipidemic so it lowers meat fat (Zeweil et al., 2011).

Lysine and methionine addition to ration containing sufficient lysine and methionine did not affect carcass nutrients (Mukhtar et al., 2007), but amino acid deficiency can lead to protein decrease and crude fat increase in meat (Lilly 2010). Lysine and methionine also work as L-carnitine precursor essential in fowl fat and energy metabolism (Arslan, 2006). Krajčovičova-Kudláčková et al. (1999) reported positive correlation between blood free carnitine and consumption of lysine and methionine, showing that carnitine need was partly supplied by carnitine synthesis in body. The main substrates in carnitine biosynthesis

were protein-bound lysine and methionine, besides vitamin C, vitamin B6, and Fe^{2+} as the cofactors (Harmeyer, 2002). L-carnitine biosynthesis from γ -butyrobetaine took place in kidney (human), testis (rat), bone muscle (sheep), brain (human) and liver in all mammals. During biosynthesis process, L-lysine supplies carbon atom chains and nitrogen atom in carnitine, and methionine supplies methyl cluster (Arslan, 2006).

This research was aimed to manipulate meat nutrient by lowering meat fat content, increasing protein and L-carnitine in meat by amino acid supplementation given to basal feeds-containing 5% palm oil and sufficient protein and amino acid. Amino acid levels administered in the treatments were based on prior research to optimize broiler performance namely 0.25% glycine (Alzawqari et al., 2010), 0.25% taurine (Lee et al., 2003), and 30% lysine and methionine from NRC standard or equal to 15% basal ration (Bouyeh and Gevorgyan, 2011).

Materials and Methods

Experimental design. Basal diets were corn and soybean meal based and formulated to meet or exceed the nutrient requirements recommended by Leeson and Summer (2005) and NRC (1994). Basal diet contained 5% palm oil in starter and finisher period (Table 1). Basal diet was then supplemented with essential and non-essential amino acid, based on previous study for the best performance. Experimental design used was completely randomized design (CRD) with six treatments and four replicates consisting of ten chicks per replicate. Meat nutrient content included dry matter content, crude protein, and crude fat analyzed using AOAC (1990) method. Meat nutrient content was analyzed using anova and Tukey's test to determine treatment differences (Steel and Torrie 1980). Meat L-carnitine content was measured from four meat of each replicates,

Table 1. Ingredients and nutrient composition on the starter and finisher basal diet (as feed basis)

Ingredient (%)	Starter (0-21)	Finisher (22-42)
Corn	50.20	58.00
Rice bran	6.17	3.50
Soybean meal 46%	35.00	30.00
Crude palm oil (CPO)	5.00	5.00
DL-Methionine	0.25	0.24
L-Lysine	0.08	0.08
Salt	0.44	0.42
Dicalcium phosphate	1.58	1.60
Limestone	1.18	1.06
Premix Min-Vit	0.10	0.10
Nutrient composition		
Dry matter (%)	87.77	88.16
Crude protein (%)	21.03	19.36
Crude fat (%)	7.49	10.66
Crude fiber (%)	2.75	2.29
Ash (%)	5.03	4.40
ME (kkal/kg)*	3076.33	3155.50
Calcium (%)*	0.93	0.90
P Available (%)*	0.47	0.41
Sodium (%)*	0.22	0.21
Methionine (%)*	0.62	0.58
Lysine (%)*	1.34	1.18
Cystein (%)*	0.34	0.30
Met + Cys (%)*	0.96	0.89
Tryptophane (%)*	0.31	0.28
Glycine (%)**	0.93	0.83
Taurine (%)***	0.00	0.00

Source: Calculated from (*) Leeson and Summers (2005), (**) NRC (1994) and (***) Spitze et al. (2003)

and data were analyzed using descriptive statistics. Meat L-carnitine was measured using high performance liquid chromatography with tipe of HPLC Waters Alliance e2695 and detektor UV-VIS 2489.

Chicks and experimental facility. Two hundred and forty Ross 308 unsex broiler chicks were obtained from a commercial hatchery after being vaccinated against New-castle disease and infectious bronchitis (via coarse spray at hatch). Broiler chicks were weighed, wing-banded, and sorted by weight to obtain uniform distribution. Chicks were randomly distributed across 24 pens (10 chicks/m²). Each pen contained one feeder and drinker bell, and

the concrete pad floor was covered with rice hulls. During the first week, 100 watt bulb lamps inside the pen were switched on for 24 hrs, and the rest of the weeks only used fluorescent light at night. The lighting program were 24L:0D at starter period and 23L:1D at finisher period. Broilers were fed with crumble feed and water was given ad libitum.

Measurement. The variables measured were meat proximate and L-carnitine content. On 21st and 42nd days, one chick from each pen was randomly-selected, fasted for 6 hours then slaughtered with kosher style method. Sample was obtained from right and left upper legs, then stored at -20°C for further analysis.

Results and Discussion

Dry Matter

The effect of amino acid supplementation on thigh meat is presented on Tabel 2. Dry matter content of 21 and 42 days-old broiler was 22.20-23.53%, and 23.17-24.78%, respectively. It indicated that dry matter content of 42-days-old broiler was higher than that of 21 days old. Leeson and Summer (2005) also stated that the higher broiler body weight, the higher the dry matter contained. Amino acid supplementation to broiler feed significantly affected ($P<0.05$) 21-days-old broiler's dry matter content and highly significantly affected ($P<0.01$) that of 42-days-old. The increase of dry matter was due to increase in meat crude fat. The previous study reported that the increase of meat dry matter paralleled to crude fat (Namroud et al., 2010; Lilly, 2010). Amino acid supplementation caused amino acid excess in feed, leading to crude fat increase in meat. In this instance, amino acid would be eliminated or transmitted to produce carbon skeleton for fatty acid synthesis. The result of this research and the previous one showed that amino acid supplementation to amino acid sufficient feed only affected the increase of meat crude protein (Namroud et al., 2010; Lilly, 2010). In contrast, Zeweil et al. (2011) showed that taurine supplementation could increase dry matter of breast meat due to protein increase. Dry matter content of R4 was the highest of all

because of the simultaneous increase of fat and protein.

Crude Protein

Protein content of 21-days-old broiler was 18.22 - 18.69% while 42-days-old was 17.02-17.37%. Amino acid supplementation insignificantly affected ($P>0.05$) on protein content of 21 and 42-days-old broiler. This was in accordance with Leeson and Summer (2005) that carcass protein was slightly affected by nutrition, assuming that there was no protein deficiency and meat resulted was in accordance with genetics. Some findings also demonstrated that broiler fed with ration containing different protein and energy levels did not significantly affect crude protein of breast meat (Barteczko and Lasek, 2008; Horniakova and Abas, 2009; Namroud et al., 2010). Amino acid supplementation was reported to increase meat protein when supplemented to amino acid-depleted feed or phytoprotein source. Zeweil et al. (2011) stated that taurine supplementation to phytofeed significantly increased crude protein content in meat. Taurine supplementation increased protein content was assumed due to the increase of taurine concentrate in thigh meat. Supplementation of 0.5% synthesized taurine increased taurine content in thigh meat as much as 246% compared to that of control, and taurine content in breast muscle was 778 $\mu\text{g/g}$ (Lee et al., 2004). Other study showed increase of amino acid density in amino acid deficient-

Table 2. Effect of supplementation amino acid thigh meat

Parameter	R0	R1	R2	R3	R4	R5
Meat proximate (%) 21 age						
Dry matter	22.20 \pm 0.35 ^a	23.20 \pm 0.78 ^{ab}	23.24 \pm 0.42 ^{ab}	22.49 \pm 0.37 ^{ab}	23.61 \pm 0.65 ^b	23.53 \pm 0.69 ^b
Crude protein	18.23 \pm 0.18	18.25 \pm 0.22	18.69 \pm 0.29	18.22 \pm 0.15	18.44 \pm 0.33	18.47 \pm 0.19
Crude fat	1.47 \pm 0.09 ^a	2.11 \pm 0.14 ^b	1.67 \pm 0.14 ^{ab}	1.77 \pm 0.19 ^{ab}	1.57 \pm 0.16 ^{ab}	1.88 \pm 0.10 ^b
Meat proximate (%) 42 age						
Dry matter	23.17 \pm 0.21 ^a	23.90 \pm 0.66 ^{ab}	24.42 \pm 0.43 ^b	23.77 \pm 0.65 ^{ab}	24.78 \pm 0.64 ^b	23.93 \pm 0.25 ^{ab}
Crude protein	17.19 \pm 0.17	17.02 \pm 0.11	17.37 \pm 0.24	17.23 \pm 0.60	17.36 \pm 0.25	17.13 \pm 0.19
Crude fat	3.7 \pm 0.36 ^a	4.99 \pm 0.27 ^b	5.56 \pm 0.23 ^b	4.17 \pm 0.28 ^a	5.71 \pm 0.17 ^c	5.09 \pm 0.19 ^b

R0 (basal feed with 5% palm oil); R1 (basal feed + 0.25% glycine); R2 (basal feed + 0.25% taurine); R3 (basal feed + 15% total lysine and methionine basal feed); R4 (basal feed + 0.25% glycine + 0.25% taurine); R5 (basal feed + 0.25% glycine + 0.25% taurine + 15% total lysine and methionine of basal feed)

feed-significantly increased crude protein in meat (Lily, 2010). Amino acid deficiency and imbalance would distract protein synthesis that caused decrease in protein deposit (Kidd et al., 1998).

Crude Fat

Crude fat in breast meat of 21-day-old was 1.47-2.11% and that of 42-days-old was 3.70-5.71%. Amino acid significantly affected ($P<0.01$) crude fat in thigh meat of 21 and 42-days-old broiler. Research result also demonstrated that –crude fat average of 42-days-old broiler was higher than that of 21-old-day. This was in line with Leeson and Summer (2005) that fat content in carcass was significantly affected by nutrition and the age of broiler. The more energy consumed and the older is the broiler, the more was the fat deposit (Leeson and Summer, 2005). Amino acid in adipose tissue depends on type of fat consumed (Crespo et al., 2001; Viveros et al., 2009). Meat fat of broiler fed with ration containing 6% palm oil mostly comprised of monosaturated fatty acid and saturated fatty acid namely oleic acid (18:1), palmitic acid (16:0), stearic acid (18:0) (Panja et al., 1995)

Increase of fat content was also due to amino acid that caused imbalance and excessive amino acid in feed that it could not be used as protein synthesis. Unused amino acid for protein synthesis or compound containing nitrogen would be released from amino acid cluster through deamination or transmission process. This process released amino cluster from amino acid, and the rest compounds were called carbon skeleton of keto- α acid. Carbon cluster from amino acid can be metabolized to produce glucose and fatty acid as energy source. Simultaneous excess of energy, protein and carbohydrate intake made carbon skeleton from amino acid be used for fatty acid synthesis (Gropper et al., 2009). Therefore, amino acid supplementation increased triglyceride serum and meat crude fat. The previous study

demonstrated that meat fat positively correlated with blood triglyceride (Lilly, 2010; Zeweil et al., 2011; Bouyeh and Gevorgyan, 2011). Imbalanced and deficient amino acid also caused decrease in thyroxin hormone secretion that increased fat deposit (Namroud et al., 2010). The highest crude fat in broiler was R1 for 21-days-old and R4 for 42-days-old. The high value of crude fat was due to the highest triglyceride serum which in R1 at 21 days old was 71.39 mg/dl and in R4 at 42 days old was 82.08 mg/dl.

Carnitine Content in Broiler Thigh Meat

L-carnitine in meat of 21 and 42-days-old broilers as presented in Figure 1 averaged on 7.49 to 15.28 mg/100g. Xu et al. (2003) however showed that free carnitine in breast meat was 0.96 to 1.15 $\mu\text{mol/g}$, or equal to 15.36 to 18.40 mg/100 g. The different value was due to different samples used and different carnitine forms observed. Arslan (2006) reported that carnitine could be found in free form or bound form (as acylcarnitine) with long or short fatty acid chain. L-carnitine concentration highly depended on species, tissue types, and nutrition status. L-carnitine in meat of 21-days-old broiler was 8.83 to 15.28 mg/100 g higher than that of 42-days-old namely 7.49 to 12.70 mg/100 g, except R0.

L-carnitine of meat fed with R3 was the lowest of all treatments because lysine and methionine supplementation caused imbalanced and excessive amino acid. Lysine is standard amino acid used in ideal concept of amino acid (Baker, 2009), while methionine is the most toxic than the other amino acids (Adeyemo, 2010). Accordingly, lysine and methionine supplementation without considering amino acid balance may cause imbalanced and excessive amino acid. It could induce distress in fowl that caused meat L-carnitine decrease. L-carnitine of 21 and 24 days old broilers in R5 tended to increase, indicating the combination of lysine,

methionine, glycine and taurine supplementation could increase amino acid balance that could increase L-carnitine of broiler meat.

Biosynthesis carnitine was determined by feed, age and hormonal status of fowl. L-carnitine biosynthesis in body can meet the need of normal L-carnitine in all mammals and birds when precursor and cofactors are sufficient in feed. It is however not the case with newborn animal (underdeveloped biosynthesis process), distress condition, high performance cattle, physical fatigue and highly fatty diet (Arslan, 2006; Rezeai et al., 2008). Sufficient lysine and methionine enable fowl to meet daily L-carnitine including broiler breeder. Therefore, different supply of precursor carnitine did not significantly affect tissue carnitine (Arslan, 2006). Previous study demonstrated that lysine and methionine supplementation failed to increase plasma carnitine in rats fed with alcoholic feed. Lysine supplementation can increase carnitine content in liver, breast meat and male broiler serum. The response of L-carnitine supplementation and precursor was also affected by L-carnitine

and precursor in feed, supplementation period, metabolic energy supply, sex, genotype status and fowl physiology, and environmental circumstances (Arslan, 2006).

Correlation of Lysine and Methionine Consumption with Carnitine-Content in Meat

Figure 2 and 3 presents the correlation of lysine and methionine consumption with carnitine content in 21 and 42 days old broilers namely 0.386 and 0.122, respectively. It indicated that lysine and methionine consumption had low correlation with L-carnitine in thigh meat, similar to the relation between lysine and methionine consumption with free carnitine content in omnivorous human (Krajčovičova-Kudláčková et al., 1999). Omnivorous diet enables L-carnitine to derive from exogenous and biosynthesis, resulting in insignificant correlation. Lysine and methionine consumption positively correlating with plasma carnitine was when feed contained very low L-carnitine. Krajčovičova-Kudláčková et al. (1999) reported a high correlation between lysine and methionine consumption with serum carnitine in vegetarian because fruit and vegetables contain a very little carnitine.

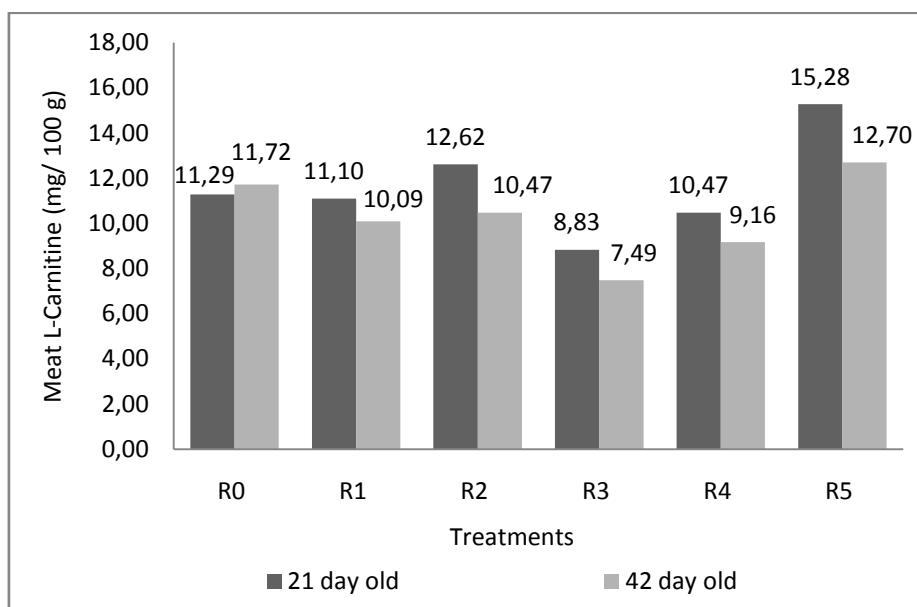


Figure 1. L-carnitine content of 21 and 42 days old broilers

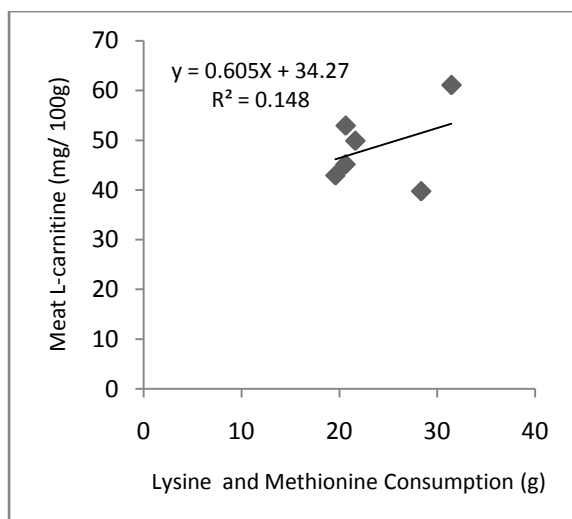


Figure 2. Correlation between lysine and methionine consumption with meat L-carnitine of 21-days-old broiler

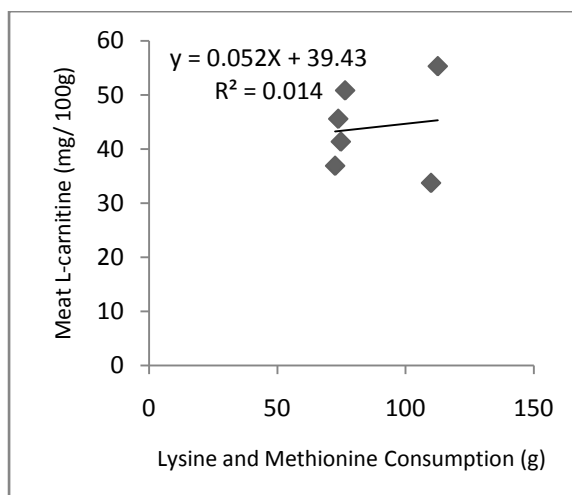


Figure 2. Correlation of lysine and methionine consumption with meat L-carnitine of 42 day old broiler

Low correlation of lysine and methionine consumption with meat L-carnitine could be because basal feed already contained L-carnitine, so L-carnitine source did not depend on biosynthesis in body. Carnitine content in some materials of basal feed was 5mg/kg corn, 12 mg/kg soybean meal, 25 mg/kg rice bran, and 0 mg/kg palm oil (Arslan, 2006). Accordingly, starter and finisher basal feed contained 12.00 and 10.82 mg/kg respectively. L-carnitine in basal feed enabled fowl to meet the need of L-carnitine from feed and

biosynthesis so that the correlation of lysine and methionine consumption with meat L-carnitine was relatively low. The other factor of this low correlation was the different tissue and form of the observed carnitine and the used basal feed. The previous study correlated lysine and methionine consumption with free carnitine in serum while this study correlated L-carnitine in thigh meat. Xu et al. (2003) reported that L-carnitine supplementation had a more significant effect on free carnitine content in serum than in breast meat.

There was positive correlation of L-carnitine content with meat fat of 21 day old broiler. Result showed that L-carnitine increase could not lower meat fat by turning fatty oxidation into energy. The previous study also demonstrated that carnitine increase in breast meat positively correlated with fat content increase in breast meat of male broiler. Supplementation of 50 or 75 mg L-carnitine/kg feed significantly increased carnitine content in liver, breast meat, male broiler serum and increased fat content of male broiler's breast meat (Xu et al., 2003). Arslan (2006) furthermore explained that L-carnitine supplementation ≥ 50 mg/kg feed depressed glucose-6-phosphate dehydrogenase, malic dehydrogenase, isocitrate dehydrogenase, and lipoprotein lipase in subcutaneous fat and carnitine palmitoyltransferase I in chest muscle so that L-carnitine supplementation would lower subcutaneous fat deposit and increased intramuscular fat.

Conclusion

Amino acid supplementation increased dry matter content, crude fat and L-carnitine but not significantly affected crude protein of broiler. Broiler fed with combination of glycine, taurine, lysine and methionine supplementation was the most nutritious because it contained high L-carnitine and protein but relatively low fat.

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