Effect of utilization of different types of lipids on amino acid profile in muscles and liver of Ross-308 broiler strain

Efecto de la utilización de diferentes tipos de lípidos sobre el perfil de aminoácidos en músculos e hígado de pollos de engorde Ross-308

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ABSTRACT

In this work, the effect of three types of fat based on utilization of saturated fat (SF) and unsaturated fat (USF) with mixing them in different level proportion in diet for Ross-308 broiler strain chickens was investigated. Amino acids profile in breast and thigh muscle and liver were investigated. The trial lasted 42 days and divided in pre-starter (7 d), starter (9 d), grower (17 d) and finisher (5 d) for testing groups were enhanced with C group included 5% packed fat (commercial name of animal fat) and T1 included 2.5 % packed fat +2.5% rapeseed oil. T2 included 2.5% packed fat +2.5% sunflower oil. T3 included 2.5% packed fat +1.25 rapeseed oil +1.25% sunflower oil. The result observed the trial which including mixing of USF improved of amino acid profile in breast muscle. No significant differences ($P>0.01$) among all groups were found for different type of total amino acids except Threonine acid was significant differences ($P<0.01$), and in the experimental groups when compared to control group had higher value (35.29g.kg^{-1}) versus T3 was lowest value (33.11g.kg^{-1}) group. No significant differences ($P>0.01$) among all groups were found for thigh muscle. In liver, there were no significant differences ($P>0.01$), whereas cysteine acid was significantly different ($P<0.01$) and high value was found in group control (15.42g.kg^{-1}).

Key words: Broiler, fat, amino acid profile, human health

RESUMEN

En este trabajo se investigó el efecto de tres tipos de sustancias grasas basado en el uso de grasa saturada (GS) y grasa no saturada (GNS) y la mezcla entre ellas con diferentes niveles de proporción en la dieta de pollos de engorde Ross-308. Se investigó el perfil de aminoácidos en la pechuga y el músculo del muslo, así como del hígado. El engorde fue de 42 días de duración y las dietas de pre-inicio (7 d), inicio (9 d), crecimiento (17 d) y acabado (5 d) para los grupos de pruebas se han mejorado con el grupo C al 5% de grasa y T1 2,5% de grasa + 2,5% de semillas de colza. T2, 2,5% de grasa + 2,5% de aceite de girasol. T3, 2,5% de grasa + 1,25% de semilla de colza + 1,25% de aceite de girasol. La inclusión de la mezcla de GNS mejoró el perfil de aminoácidos en el músculo de la pechuga. No se encontraron diferencias significativas entre todos los grupos para el tipo diferente del total de aminoácidos excepto para el ácido treonina con diferencias significativas ($p<0,01$). En los grupos experimentales en comparación con el control el cual tiene el mayor valor (35.29 g.kg^{-1}). T3 tuvo el valor menor (33.11g.kg^{-1}). No se encontraron diferencias significativas ($P>0,01$) entre todos los grupos para el músculo del muslo. En el hígado, no se encontraron diferencias significativas ($P>0,01$), mientras que el ácido cisteína fue significativamente diferente ($P<0,01$) y se encontró un alto valor en el grupo control (15.42g.kg^{-1}).

Palabras clave: pollos de engorde Ross-308, grasa, perfil de aminoácidos, salud humana

INTRODUCTION

Feeds are a major component of the total cost of broiler production. Broiler rations should be formulated to supply the correct balance of energy, protein and amino acids, minerals, vitamins and essential fatty acids to allow optimum growth and performance (Lilly *et al*., 2011; Laudadio *et al*., 2012). Broiler meat is one of the principal sources to fill the genuine gaps of the animal protein and can play leading role in providing balanced diet (Alam and Khan, 2000). Poultry industry is producing meat

and eggs under intensive husbandry, during the past half century; chicken meat production has changed from being a byproduct of the egg industry to an industry as an independent, with annual production of more than 7 billion broilers and roasters (Khan, 2009). Methionine and lysine are two important limiting amino acids obtained from poultry diet. The requirement of these two amino acids is substantial but variable for changes in genetic, nutrition and management of broiler chicks.

Feed consumption, growth rate and carcass composition are affected by individual amino acids that is receiving considerable attention for development of broiler industry. Methionine acts as lipotropic agent through its role as an amino acid in balancing crude protein (Hesabi *et al*., 2008). It is well known that crude protein and lysine interaction is considered to be an important factor which affects performance and carcass quality of growing chicks; so the dietary requirement of crude protein is actually a requirement for the lysine contained in the crude protein (Rezaei *et al*., 2004). Poultry diets that are composed of natural feed stuffs can therefore be supplemented with small amounts of synthetic amino acids to meet the bird's requirements for the most limiting amino acids (Aftab, 2007). Hussein *et al*. (1982) observed that the reduction of litter N and ammonia production may be accomplished by decreasing dietary crude protein to levels below the requirements. Some researchers have shown that reduced crude protein-amino acid supplemented diets support good growth and feed consumption of broilers (Aletor *et al*., 2000). Addition of methionine over and above the recommended requirement of broilers improves their performance in terms of body weight gain and food conversion efficiency (Simon *et al*., 1995).

The aim of this study was to find the influences of utilization different type of lipids and their proportion on amino acid profile in broiler muscles and liver, which reflected on human consumer's health.

MATERIALS AND METHODS

Experimental trial

The experiment was conducted in Vígľaš; Testing station with 800 broiler chickens of the Ross - 308 line. The objective of this study was to investigate the influence of utilization difference sector of fat (saturated and unsaturated) as a feed additive. This experiment was done at the basis of cooperation university test farm in Kolíňay in Slovakia republic. The fat added to complete feed mixtures manufacturer in different concentration at all the groups, according to the scheme and design of experiment shown in Table 1. All other feed ingredients, mineral feed premixes and additives used in the same batch in all groups in the production of each type of complete feed mixtures.

Management

Broiler chickens were kept under the Ross recommended procedure. Water, heating, conditions of hall, rations distributed *adlibitum* and uniform light provide 24 hours daily. Chickens were housed on the deep litter in the same technological conditions. Microclimate indicators in the range of temperature and humidity were measured and recorded three times per day, at 7.00 am, 12.00 and 17.00 pm.

Amino acid analysis

Cysteine and methionine oxidized to cysteic acid and methionine sulphone respectively prior to hydrolysis. All the other amino acids determined in unoxidized sample. Oxidation is performed at 0^{o}C with a performic acid/phenol mixture. Excess oxidation reagent is decomposed with sodium disulphite. The oxidised or unoxidised sample is hydrolyzed with hydrochloric acid (HCl = 6mol.l^{-1}) for 23 hours at 110 ^{o}C. The hydrolysate is adjusted to pH 2.20. The amino acids are separated by ion exchange chromatography and determined by reaction with ninhydrin using photometric detection at 570nm

Table1. Scheme and experimental design.

Treatments	Index of utilization type of fat	Replicates	No. of the birds per replicate	Total
C	5% packed fat	4	50	200
T1	2.5% rapeseed oil + 2.5% packed fat	4	50	200
T2	2.5% sunflower oil +2.5% packed fat	4	50	200
T3	2.5% packed fat + 1.25% sunflower oil + 1.25% rapeseed oil	4	50	200

(440nm for proline). And the following principle was preceded.

1. Oxidation:

To the nearest 0.2mg weighed from 0.1 to 1g of the prepared sample into a 100ml bottle was fitted with a screw cap. The weighed sample portions have a nitrogen content of about 10mg. The bottle has placed in an ice-water bath and cooled to 0 ^{o}C, 5ml of oxidation mixture added and mixed, a glass spatula with a bent tip used. The bottle containing the spatula with an air-tight film Sealed, the ice-water bath containing the sealed container placed in a refrigerator at 0 ^{o}C and left for 16 hours. After 16 hours was removed from the refrigerator and decomposed the excess oxidation reagent by the addition of 0.84 g of sodium disulfide.

2. Hydrolysis

A 0.2mg, from 0.1 to 1g of the prepared sample was weighed and added to the 100ml bottle fitted with a screw cap. The weighed sample portion has a nitrogen content of about 10mg. 25ml of hydrolysis mixture was added and mixed with the sample. Closed hydrolysis was used by placing the bottle containing the mixture prepared, in an oven at 110 ^{o}C. After one hour the vessel was closed with the cap and left in the oven for 23 hours. On completion of hydrolysis, the bottle from the oven was removed, and placed in an ice-water bath and left to cool. For evaporation of hydrolyzed sample (5ml) vacuum rotator was used. For pH adjustment, the contents of the bottle quantitatively were transferred to a 100ml round-bottom flask, citrate buffer used (pH 2.2).

3. Adjustment of pH

Depending on the sodium tolerance of the amino acid analyzer, proceeded according to chromatographic system requiring a low sodium concentration:

Using a rotary evaporator reduced the volume to 5-10 ml under vacuum at 40 ^{o}C. The pH Adjusted to 2.20 with sodium hydroxide solution.

4. Chromatography

Before chromatography the extract was fitted to room temperature. The mixture was shaken and filtered a suitable amount through a 0.2 lm membrane filter. The resulting clear solution is subjected to ion exchange chromatography using an amino acid analyzer. The standard or sample is diluted with citrate buffer to give a peak area of the standard of 30-200 % of the sample amino acid peak area. During the chromatography steps the valley: peak height ratios mentioned below were applied, when an equimolar solution (of the amino acids being determined) is analyzed.

5. Calculation of results

The area of the sample and standard peaks is measured for each individual amino acid and the amount, in g amino acid per kg sample, is calculated.

$$\text{g amino acid per kg sample} = \frac{A \times E \times MW \times F}{B \times W \times 1000}$$

A = peak area, hydrolysate or extract

B = peak area, calibration standard solution

MW= molecular weight of the amino acid being determined

E = concentration of standard in lmol.ml^{-1}

W = sample weight (g) (corrected to original weight if dried or defatted)

F = ml total hydrolyzed or ml calculated total dilution volume of extract.

Cystine and cysteine are both determined as cysteic acid in hydrolysates of oxidized sample, but calculated as Cysteine ($C_6H_{12}N_2O_4S_2$, MW 240.30 by using MW 120.15 = 0.5× 240.30). Methionine is determined as methionine sulphone in hydrolysates of oxidized sample, but calculated as methionine by using MW of methionine: 149.21.

The content in feeds was explained in the Tables 2, 3, 4, and 5 for pre-starter, starter, grower and finisher respectively.

Table 2. Amino acid composition of experimental diets at pre-starter period.

Amino acids (g/kg)	C †	T1	T2	T3
Aspartic acid	24.55	24.10	24.69	23.79
Threonine	9.61	9.45	9.55	9.33
Serine	12.13	11.71	11.58	11.40
Glutamic acid	42.46	41.84	42.42	41.13
Proline	15.76	15.68	15.74	15.26
Glycine	10.77	10.58	10.91	10.54
Alanine	11.54	11.35	11.63	11.27
Valine	11.80	11.82	12.47	11.93
Isoleucine	10.42	10.36	10.79	10.47
Leucine	20.15	19.70	20.12	19.54
Tyrosine	8.30	7.97	8.06	7.66
Phenylalanine	11.95	11.60	12.02	11.31
Histidine	6.96	7.04	7.16	6.83
Lysine	15.40	15.21	15.62	15.10
Arginine	18.90	18.53	19.15	18.71
Cystine	4.87	4.83	4.95	4.82
Methionine	8.15	8.42	7.99	8.20
Sum of amino acids (g/kg)	243.72	240.18	244.85	237.28
Nitrogen compounds (%)	26.39	25.71	26.01	25.84
Dry matter (%)	100.00	100.00	100.00	100.00

† Treatments: C: 5% packed fat; T1: 2.5% rapeseed oil + 2.5% packed fat; T2: 2.5% sunflower oil + 2.5% packed fat and T3: 2.5% packed fat + 1.25% sunflower oil + 1.25% rapeseed oil

Table 3. Amino acid composition of experimental diets at starter period.

Amino acids (g/kg)	C †	T1	T2	T3
Aspartic acid	21.93	21.74	22.46	21.77
Threonine	8.65	8.57	8.60	8.45
Serine	11.11	10.77	11.13	10.93
Glutamic acid	38.75	37.63	39.51	39.49
Proline	14.69	14.37	14.38	15.05
Glycine	9.71	9.91	9.78	9.95
Alanine	10.64	10.82	10.55	10.95
Valine	10.32	10.39	10.78	11.00
Isoleucine	8.99	8.95	9.30	9.45
Leucine	18.17	17.76	17.61	18.28
Tyrosine	7.46	7.17	7.20	7.40
Phenylalanine	10.71	10.37	10.70	10.67
Histidine	6.48	6.42	6.60	6.39
Lysine	13.82	14.31	13.99	14.07
Arginine	17.00	16.65	17.19	17.37
Cystine	4.89	4.44	4.79	4.64
Methionine	7.71	8.00	7.32	7.37
Sum of amino acids (g/kg)	221.00	218.27	221.91	223.21
Nitrogen compounds (%)	24.41	23.83	24.22	24.32
Dry matter (%)	100.00	100.00	100.00	100.00

† Treatments: C: 5% packed fat; T1: 2.5% rapeseed oil + 2.5% packed fat; T2: 2.5% sunflower oil + 2.5% packed fat and T3: 2.5% packed fat + 1.25% sunflower oil + 1.25% rapeseed oil

Table 4. Amino acid composition of experimental diets at grower period.

	Treatments †			
Amino acids (g/kg)	C	T1	T2	T3
Aspartic acid	18.92	19.97	19.17	20.04
Threonine	7.23	7.60	7.29	7.85
Serine	9.41	10.14	9.95	10.37
Glutamic acid	36.13	38.57	36.33	37.73
Proline	13.47	14.71	13.77	14.64
Glycine	8.11	8.29	8.07	8.54
Alanine	8.95	8.89	8.58	9.71
Valine	9.52	9.72	8.66	9.20
Isoleucine	8.32	8.75	7.61	7.91
Leucine	15.97	16.54	15.84	16.33
Tyrosine	6.47	6.07	6.27	6.33
Phenylalanine	9.62	9.15	9.30	9.58
Histidine	5.39	5.69	5.65	5.75
Lysine	11.59	11.88	11.39	11.98
Arginine	15.18	15.78	14.15	15.38
Cystine	4.46	4.79	4.55	4.50
Methionine	6.23	6.62	6.25	6.11
Sum of amino acids (g/kg)	194.97	203.17	192.83	201.95
Nitrogen compounds (%)	21.32	22.11	21.41	21.56
Dry matter (%)	100.00	100.00	100.00	100.00

† Treatments: C: 5% packed fat; T1: 2.5% rapeseed oil + 2.5% packed fat; T2: 2.5% sunflower oil + 2.5% packed fat and T3: 2.5% packed fat + 1.25% sunflower oil + 1.25% rapeseed oil

Table 5. Amino acid composition of experimental diets at finisher period.

Amino acids (g/kg)	C †	T1	T2	T3
Aspartic acid	16.66	16.47	16.52	16.58
Threonine	6.97	6.75	6.73	6.72
Serine	9.33	8.95	8.84	9.11
Glutamic acid	36.51	35.75	36.15	36.65
Proline	14.20	13.77	14.33	14.57
Glycine	7.48	7.42	7.44	7.43
Alanine	7.69	7.96	8.10	7.87
Valine	8.46	8.44	8.50	8.49
Isoleucine	7.25	7.12	7.24	7.23
Leucine	14.73	14.32	14.66	14.81
Tyrosine	5.69	5.58	5.76	5.68
Phenylalanine	8.61	8.63	8.91	8.71
Histidine	5.05	4.97	5.11	5.10
Lysine	10.65	10.45	10.67	10.55
Arginine	13.28	12.84	13.20	13.29
Cystine	4.39	4.37	4.36	4.33
Methionine	6.61	6.83	7.08	6.70
Sum of amino acids (g/kg)	183.56	180.62	183.60	183.81
Nitrogen compounds (%)	19.83	19.98	19.73	19.87
Dry matter (%)	100.00	100.00	100.00	100.00

† Treatments: C: 5% packed fat; T1: 2.5% rapeseed oil + 2.5% packed fat; T2: 2.5% sunflower oil + 2.5% packed fat and T3: 2.5% packed fat + 1.25% sunflower oil + 1.25% rapeseed oil

Statistical analysis

For the statistical design and data analyses, complete random design of experiment with four treatments was used. Data were subjected to ANOVA procedures for a completely randomized design and the significance of differences between the means estimated using Duncan's multiple range test. Probability level of $P<0.01$ was considered for significance.. Values in percentage were subjected to transformation of Arc sin v100. All statistical analyses were performed using the software SPSS 17.5 for Windows® (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Effects of diet on amino acid profile of broilers breast muscle

The amino acids profile is important in relation to human health. Influences of diet include different type of fat on the profile of amino acids (Table 6). Not significant differences ($P>0.01$) among groups were found for different type of total amino acids except for threonine ($P<0.01$). Threonine is an essential amino acids for humans, supporting cardiovascular, liver, central nervous, and immune system functions. Furthermore, threonine is needed to create glycine and serine in human's body. From the data obtained, it's found that including packed fat 2.5% + rapeseed 2.5% in the diet increased threonine with higher level in the breast muscle, by comparison to other groups. Serine level in the breast muscle was higher value (35.29g.kg^{-1}) in control group versus T3 which was lowest value (33.11g.kg^{-1}) group.

Cysteine was not affected in the breast muscle by including SF and USF in broilers diets individually, but decreased by the blend of both, this may be due to the antagonism effect of phytochemicals of both mixing. A mathematical differences were observed between C (12.79 g.kg^{-1}) and T3 (12.93g.kg^{-1}) in one side compared to T2 (12.35g.kg^{-1}). Mixing SF and USF with differs level and type in broiler diet tended to increase Cysteine content in the breast muscle compared to group C. In the point of view of human's health, consumption of such meat which modified to contain lower content of Cysteine AA is beneficial, while insignificant differences were observed among groups related to other amino acids.

Cysteinewas not affected in the breast muscle by including saturated fat (SF) and unsaturated fat (USF) in broilers diets individually, but decreased by the blend of both, this may be due to the antagonism

Table 6. Effects of diet on amino acid profile (g.kg^{-1} of the protein) of broilers breast muscle.

Amino acids	C †	T1	T2	T3
Aspartic acid	82.57±3.67	83.71±2.16	81.05±1.77	80.19±1.77
Threonine	38.60±1.27 ^{b}	38.70±0.37 ^{b}	36.80±0.75 ^{a}	37.12±0.35 ^{ab}
Serine	35.29±0.67	34.76±0.39	33.57±1.71	33.11±0.80
Glutamic acid	116.08±7.02	119.44±4.22	114.18±2.53	112.87±5.61
Proline	32.06±2.74	33.95±0.93	31.23±3.05	30.18±2.69
Glycine	37.43±1.55	37.55±0.24	35.63±1.13	36.53±0.99
Alanine	49.79±1.22	49.60±0.32	47.63±1.48	47.83±0.66
Valine	41.07±3.833	42.33±1.06	39.58±3.78	40.33±4.27
Isoleucine	37.82±3.751	39.12±1.52	36.73±3.61	37.38±4.51
Leucine	68.91±1.88	68.31±1.13	65.66±1.10	66.15±1.47
Tyrosine	39.91±2.17	40.30±0.74	38.61±1.19	38.62±1.31
Phenylalanine	34.73±1.07	34.57±0.28	32.96±0.46	33.02±1.00
Histidine	49.07±2.75	47.06±1.61	46.30±2.04	45.86±1.15
Lysine	76.34±4.04	78.16±1.08	74.05±1.72	74.72±2.66
Arginine	55.99±4.37	59.90±2.64	57.61±5.54	57.06±5.29
Cystine	12.79±0.59	12.60±0.14	12.35±0.24	12.93±0.48
Methionine	26.22±2.86	24.22±0.42	22.65±0.55	24.57±1.20

^{a,b} Means with different prescript within row are significantly different ($P<0.01$)

* Values are $\bar{x}$ ± Std. Deviation of 28 chickens

† Treatments: C: 5% packed fat; T1: 2.5% rapeseed oil + 2.5% packed fat; T2: 2.5% sunflower oil + 2.5% packed fat and T3: 2.5% packed fat + 1.25% sunflower oil + 1.25% rapeseed oil

effect of phytochemicals of both mixing. A tends differences were observed between C (12.79 $g.kg^{-1}$) and T3 (12.93$g.kg^{-1}$) in one side compared to T2 (12.35$g.kg^{-1}$) (Table 6). Mixing SF and USF with differs level and type in broiler diet tended to increase Cysteinecontent in the breast muscle compared to group C. In the point of view of human's health, consumption of such meat which modified to contain lower content of CysteineAA is beneficial, while insignificant differences were observed among groups related to other amino acids.

The highest value of aspartic acid was found in group T1 (83.71$g.kg^{-1}$) compared to other groups (Table 6). In general, aspartic acid levels in the breast tended to increase by inclusion of mixing the rapeseed with packed fat in the diet. This is may be due to the higher modification of this amino acid to convert to oxaloacetate, thus increasing energy production in the body of the bird in group T1, on other hand may be the AA was shifted to return the performance of the brain to the normal level when affected by the low level of USF.

The highest content of glutamic acid was found in group T1 compared to other groups. While the content in the breast reduced in other experimental groups compared to control. The lowest amount of glutamic acid was found in group T3, this is may be shifted to the brain instead of concentrating in the muscle under the effect of different type of fat mixture.

Proline tended to be lower in groups T3 and T2 compared to T1 and C. This is could be attributed to the shift of glutamic acid as explained previously, because glutamic acid is the precursor of proline synthesis.

The glysine amino acid contents in the breast muscle of experimental groups were higher than the T1. From the human well being point of view especially for men's, glysine is beneficial to maintaining prostate function and prostate health.

The lowest value of alanine was found in group T2. This is beneficial to human health when consuming meats from birds given diets contents with mixing of sunflower oil with packed fat, in which high alanine intake of alanine correlated with higher blood pressure, energy intake and cholesterol levels.

Valine concentrations in breast muscle of trial group C were higher than other groups this is beneficial for human well growing and muscle metabolism, as energy precursor other than glucose, especially in persons has hyperglycemia.

Isoleucine content in the meat of trial groups was higher in T1 than the control, and other groups which obtained other type of fat in the feed showed lowest mathematical values. In the point of human's health, isoleucine is one of the essential amino acids.

Leucine AA in the breast muscle increased by including packed fat in broiler diets compared to other treatments. lucine function in human body are repair muscles, regulate blood sugar, provide the body with energy and also increases production of growth hormones, and helps burn visceral fat. Leucine is the most effective branched-chain amino acid for preventing muscle loss because it breaks down and is converted to glucose more quickly than isoleucine and valine (Melvin, 2005).

The tyrosine content in breast muscle reduced by inclusion of natural additives compared to control. This is could be attributed to that the tyrosine shifted to regulate appetite and normal functioning of the thyroid, pituitary, and adrenal glands (Roderic and Hans, 2010). Therefore reduced concentrating in muscle.

Phenylalanine content in the breast muscle is increased by inclusion packed fatcompared to other groups. This is may be regulated in birds body to reduce its level in the blood to prevent the brain from the damage by high doses of phenylalanine (Roderic and Hans, 2010). Phenylalanine is an essential amino acid that is needed for normal functioning of the central nervous system of the human.

The histidine content in the breast muscle increased in those groups which consumed diets inclusion with packed fat individual by comparison with mixing with USF. The highest was fund in group C. In the point of human health, histidine is important to normal sexual functioning, because it gets converted into histamine.

The lysine content of breast muscle in experimental groups was higher than in n-control. Lysine is an essential amino acid that is well known for its antiviral properties. It is an essential AA for human and it improves immune system. Moreover, it is needed for hormone production and the growth and maintenance of bones in both children and adults

(Akram *et al*., 2011). The highest value was in group T3.

Mixing packed fat equal proportion with rapeseed increased arginine AA content in the breast muscle of broilers. This is may be due to effects of the phytochemical content of those mixing depressed enzyme activities in the liver to further metabolizing of arginine. Thus, arginine concentrated in the muscles. The highest value was in T1. Arginine stimulates the immune system by increasing the output of T lymphocytes (T- cells) from the thymus gland. Recent studies have focused on the potential of arginine as a treatment for AIDS, cancer; helps detoxify the liver by neutralizing the effects of ammonia and other toxic substances in the body in human body. Laboratory research suggests that arginine may help reduce body fat and speed up weight loss.

Methionine level in the breast muscle of groups consumed the diet packed fat mixed with sunflower oil were slightly lower than the control. This is may be attributed to that the birds used the methionine to the wide metabolism and producing of substance for better immune functions by the effect of types of USFA in sunflower oil.

In general there is an effect of types fat on the amino acids profile in which increased concentration of most amino acids which are essential for humans, this phenomenon may be due to the effect of such development cholesterol level on some hormones functions which involved in the metabolism of protein syntheses such as insulin, growth hormone (GH) and somatomedins, by affecting the affinity of insulin receptors in cell membranes, and secretion and pulsatile of GH. Insulin reduces the concentration of free circulating amino acids and stimulates uricaemia, consequently promoting the entry of amino acids into cells (Larbier and Leclercq, 1992).

The branched amino acids (leucine, isoleucine and valine) levels in the muscle were increased in those groups which the diet content packed fat. This is may be due to the high availability of amino acids in the diet of tested groups by induced by this type of fat and increase formula of cholesterol. There is evidence suggesting that feeding a high protein diet to chicks results in normal levels of most plasma amino acids with the exception of branched-chain amino acids (Featherston, 1969), this is mean that they concentrated in the muscles. In other hand, may be level of cholesterol based affected the insulin activity to the indirection or direction in which concentrating those amino acids in the muscle.

Effects of diet on amino acid profile of broilers thigh muscle

The advantages to broiler performance of feeding increased amino acid (AA) densities have been well documented, but minimal research has been reported on the effects of AA density on meat quality. In the current study, Ross 308 female and male broiler chicks were obtained from a commercial hatchery and fed diets with either deficient, low. Broilers were slaughtered at 42 days of age and evaluated for live performance, carcass traits, breast and thigh composition, and breast and thigh meat quality. As expected, FCR decreased ($P < 0.05$) and yields improved ($P< 0.05$) as the AA density increased, but no differences ($P>0.01$) existed among treatments with regard to final proximate analysis. The deficient AA diet yielded thigh meat with less ($P< 0.05$) moisture, less protein ($P< 0.05$), and more fat ($P< 0.05$) than thigh meat from all other treatments. In addition, the high and excessive AA treatments had higher ($P< 0.05$) concentrations of linoleic and linolenic acids in the thigh meat when compared with the deficient and low AA treatments, and thigh meat from the excessive AA treatment was more susceptible to oxidation ($P<0.05$) than that from the deficient and low AA treatments. Overall, all 4 AA diets yielded high-quality breast and thigh meat, whereas the high AA diet yielded broilers with excellent live performance, carcass traits, and meat quality. Our results agree approximately 90% with results of Lilly *et al*. (2011).

Table 7 indicated that all of AA were insignificant differences ($P<0.01$) and all of their parameter level increase in the muscles compared to contain in diet for pre-starter , starter, grower and finisher period as clear in Tables 2, 3, 4 and 5 respectively.

This is pointed that there were no affect of type fat in diet on significant of AA in thigh muscle. But affect to improve for formula of protein by modification the type of amino acids from diet to each other and improving for building unite of protein by poly peptide and peptide which they combination from smallest unite of protein is amino acids.

Effects of diet on amino acid profile of broilers liver

Some researchers have shown that reduced crude protein-amino acid supplemented diets support good growth and feed consumption of broilers (Aletor *et al*., 2000). Addition of methionine over and above the recommended requirement of broilers improves their performance in terms of body weight gain and food conversion efficiency (Simon *et al*., 1995).

Data from Table 8 pointed there were insignificant differences among all treatment with all of type amino acids expect syctine was significant differences ($P<0.01$) and high value found in group control (15.42g.kg.$^{-1}$ This can be attributing to function of the liver to convert type of AA from carboxyde group and make joiner with group of amide to synthesis of AA. Therefore increase the level of AAin liver compare with the level in diet (Tables 2, 3, 4 and 5) respectively.

CONCLUSION

It is found that amino acids profile changed in the muscle by addition of those mixing different type and level fat to improve the quality of the meat in the point view of human health and well being.

Utilization packed fat individual increase proportion of cholesterol in meat quality. This could be negative affect for human health.

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Table 7. Effects of diet on amino acid profile (g kg^{-1} of the protein) of broilers thigh muscle.

	Treatments			
Amino acids	C †	T1	T2	T3
Aspartic acid	67.21±1.97	65.48±2.35	67.68±2.86	66.54±1.12
Threonine	32.15±1.09	31.15±1.25	32.63±2.11	31.41±1.07
Serine	30.58±0.67	30.00±0.91	30.84±1.40	29.95±1.38
Glutamic acid	102.19±5.89	101.27±5.45	103.08±4.48	95.54±2.90
Proline	31.53±2.74	29.45±1.22	32.92±4.02	40.39±12.05
Glycine	38.01±0.72	35.95±2.13	38.27±3.35	37.30±2.30
Alanine	42.48±1.09	41.22±1.45	43.18±2.61	42.00±1.36
Valine	31.60±2.60	31.24±2.16	32.72±2.77	31.75±2.10
Isoleucine	29.45±2.22	28.98±1.91	30.80±3.35	29.56±1.85
Leucine	56.16±1.90	54.29±1.91	56.75±2.89	55.67±1.34
Tyrosine	28.18±1.32	28.04±0.83	28.82±2.14	27.90±0.53
Phenylalanine	28.35±1.28	27.51±1.08	28.67±2.04	28.04±0.49
Histidine	27.19±1.16	25.81±1.04	26.53±1.95	28.67±5.42
Lysine	63.65±4.23	61.28±2.88	63.02±3.12	61.30±1.77
Arginine	47.45±2.33	47.84±3.56	49.39±2.60	46.74±1.49
Cystine	11.21±0.44	10.85±0.81	11.04±0.33	11.27±0.47
Methionine	20.61±1.49	20.27±1.29	19.77±0.54	20.54±1.08

[a,b] Means with different prescript within row are significantly different ($P<0.01$)

[*] Values are $\bar{x}$ ± Std. Deviation of 28 chickens

† Treatments: C: 5% packed fat; T1: 2.5% rapeseed oil + 2.5% packed fat; T2: 2.5% sunflower oil + 2.5% packed fat and T3: 2.5% packed fat + 1.25% sunflower oil + 1.25% rapeseed oil

LITERATURE CITED

Aftab, U. 2007. Study the extent to which dietary CP could be reduced at the expense of supplemental lysine, methionine, and threonine in broiler feed. Findings of Experiments 1 and 2: Ph.D Thesis submitted to Quid-a-Azam University, Islamabad. Pakistan.

Akram, M.; H. M. Asif, M. Uzair, A. Naveed, M. Asadullah, S. Ali, S. M, Zahoor ul Hassan and U. Asmat. 2011. Amino acids. Journal of Medicinal Plants Research 5 (17): 3997-4000.

Alam, S. and M. Khan. 2000. Poultry farming in Pakistan. Industry and Economy Magazine. 7th August, 2000. www.thepoultrysite.com. (Accessed July 7, 2009).

Aletor, V. A.; I. Hamid, E. Neib and E. Pfeffer . 2000. Low-protein amino acid supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole body composition and efficiency of nutrient utilization. J. Sci. Food Agric. 80: 547-554.

Arbor Acres Index. 2009. Arbor Company. Broiler Nutrition Supplement. p. 5-20.

Featherston, W. R. 1969. Nitrogenous metabolites in the plasma of chicks adapted to high protein diets. Journal of Poultry Science 48: 646-652.

Hesabi, A.; H. Nasiri and M. Birjandi. 2008. Effect of supplemental methionine and lysine on performance and carcass yield characteristics in broiler chicks. Journal of Animal Science 8: 26-30.

Hussein, A. and F. H. Kratzer, 1982. Effect of rancidity on the feeding value of rice bran for chickens. Journal of Poultry Science 61: 2450-2455.

Khan, A. 2009. The domestic food market: Is India ready for food processing? India Development Foundation, Food Processing Five Sector Project, http://www.idfresearch.org/pdf/dommarket.pdf. (Accessed July 7, 2009).

Table 8. Effects of diet on amino acid profile ($g.kg^{-1}$ of the protein) of broilers liver

Amino acids	C †	T1	T2	T3
Aspartic acid	62.29±3.54	61.59±1.96	62.913±1.69	64.58±0.90
Threonine	32.07±2.40	31.00±1.24	31.47±0.84	32.54±0.58
Serine	31.56±2.72	31.25±1.19	32.82±1.08	33.62±0.50
Glutamic acid	77.44±4.23	75.77±2.64	77.08±1.66	77.37±097
Proline	31.10±4.39	32.72±2.57	31.91±1.39	32.78±0.67
Glycine	34.75±1.34	34.5±3.38	34.37±1.18	33.80±0.67
Alanine	42.70±2.75	42.13±2.43	42.27±1.03	42.99±0.88
Valine	39.64±1.69	37.80±3.98	35.57±0.74	35.57±0.74
Isoleucine	29.62±1.47	28.31±3.31	27.47±0.63	27.81±0.25
Leucine	60.89±2.40	59.09±4.10	59.67±1.19	60.68±0.90
Tyrosine	26.14±1.94	25.88±1.92	24.65±1.15	25.65±1.27
Phenylalanine	34.12±1.86	33.13±1.73	33.24±0.68	34.05±0.32
Histidine	19.33±1.37	18.96±0.79	19.06±0.56	19.21±0.52
Lysine	50.77±2.61	49.41±3.06	48.37±1.18	50.40±0.98
Arginine	45.73±2.85	44.24±2.53	44.80±0.49	44.70±0.49
Cystine	15.42±0.58^{b}	14.70±0.42^{ab}	14.46±0.24^{ab}	13.95±0.66^{a}
Methionine	18.01±0.97	17.34±0.51	16.90±0.07	17.73±0.50

^{a,b} Means with different prescript within row are significantly different ($P<0.01$)

* Values are $\bar{x}$ ± Std. Deviation of 28 chickens

† Treatments: C: 5% packed fat; T1: 2.5% rapeseed oil + 2.5% packed fat; T2: 2.5% sunflower oil + 2.5% packed fat and T3: 2.5% packed fat + 1.25% sunflower oil + 1.25% rapeseed oil

Larbier, M, and B. Leclercq. 1992. Nutrition and feeding of poultry. France: Institute National de la Recherche Agronomique,. Nottingham University Press. 305 pp.

Laudadio, V.; L. Passantino, A. Perillo, G. Lopresti, A. Passantino, R. U. Khan and V. Tufarelli. 2012. Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. Poultry Science 91: 265-270.

Lilly, R. A.; M. W. Schilling, J. L. Silva and J. M. Martin. 2011. The effects of dietary amino acid density in broiler feed on carcass characteristics and meat quality. J. Appl. Poult. Res. 20 (1): 56-66.

Melvin, W. 2005, Dietary supplements and sports performance: Amino Acids. Journal of the International Society of Sport Nutrition 2 (2): 63-67.

Rezaei, M.; H. Oghaddam, J. Reza and H. Kermanshahi. 2004. The effects of dietary protein and lysine levels on broiler performance, carcass characteristics and N excretion. International Journal of Poultry Science 3 (2): 148- 152.

Roderick, N. F, and J. F. Hans. 2010. Requirement for amino acids in ontogeny of fish. Aquaculture Research 41: 684-716.

Simon, A.; H. Bergner and D. Buivan. 1995. Methodological investigation on the metabolism oriented determination of methionine requirement of broiler chickens. 3. Degradation of 14C-(CH3)- and 35-Smethionine after feeding of synthetic diets. Archives of Animal Nutrition 47: 229-244.

Wiseman, J.; S. Jaggert, D. J. A. Cole and W. Haresign. 1991. The digestion and utilization of amino acids of heat-treated fish meal by growing/finishing pigs. Animal Production 53: 215-225.