PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 8 (6): 792-796, 2009
ISSN 1680-5194

Effects of Varying Levels of L-Leucine and Metabolizable Energy in Finisher Diet on Carcass Composition and Meat Sensory Characteristics of Broiler Chickens

E. Erwan[1,2], A.R. Alimon[1]*, A.Q. Sazili[3,4], H. Yaakub[3] and R. Karim[5]
[1]Institute of Tropical Agriculture
[2]Department of Animal Science, State Islamic University of Sultan Syarif Kasim, Riau, Indonesia
[3]Department of Animal Science, Faculty of Agriculture,
[4]Halal Products Research Institute, Putra Infoport, Malaysia
[5]Department of Food Technology, Faculty of Food Science and Technology,
Universiti Putra Malaysia, Malaysia

Abstract: A study was conducted to evaluate the effects of leucine supplementation in grower-finisher diets containing varying levels of energy on the sensory characteristics and carcass composition of broiler chickens. In a 2 x 2 factorial arrangement, eighty 21-day old Cobb broiler chicks were divided into 16 groups and fed diets supplemented with 0 or 0.5 % L-leucine and metabolizable energy (ME) concentration at either 3200 kcal/kg or 3000 kcal/kg, for three weeks. Feed intake, growth performance and feed conversion ration were determined on a weekly basis. At the end of the trial, the birds were slaughtered, carcass composition determined and meat samples taken for sensory evaluation. There was no interaction between level of L-leucine and ME on the sensory characteristics and carcass composition at 42 days. Dietary level of L-leucine and ME had no significant effect (P>0.05) on the live-weight, breast meat, lean, bone, fat and skin. Similarly, the flavour, tenderness, aroma, juiciness and overall acceptability scores of breast meat of broiler fed diets supplemented with leucine were also not significantly different. Further research is needed to evaluate the potential impact of excess leucine in diets with reduced levels of crude protein.

Key words: Leucine, broiler, metabolizable energy, sensory, carcass composition

INTRODUCTION

The amount of nutrients in grower-broiler diets plays a significant role in determining the growth rate and meat yield. It is well known that the metabolizable energy (ME) and total amino acids of poultry meat are influenced by feeding treatments, which in turn affect the final meat taste. An improvement in carcass leanness to meet consumer demands for lean meat has also resulted in a reduction in the intra-muscular fat which potentially has a negative effect on the palatability of chickens. A number of approaches have been investigated to improve intramuscular fat by manipulating dietary nutrients including feeding with protein deficient (Castell *et al*., 1994; Blanchard *et al*., 1999) and high leucine diets (Cisneros *et al*., 1996). L-leucine is a ketogenic amino acid, the carbon skeleton of which is converted to acetyl-CoA and acetoacetate in muscle tissue and these intermediates can be used to synthesize fatty acids. Amino acids play a major role in eliciting the characteristics taste of foods. Previous research had identified three compounds, i.e. free glutamic acid (Glu), 5'-inosinic acid (IMP) and potassium ion, as the taste active components in chicken meat extract (Fujimora *et al*., 1995, 1996). Glu and IMP, or also known as *umami* taste, are favorites among consumers as it constitutes a characteristic taste of chicken meat.

In another study, (Cisneros *et al*., 1996) showed that high leucine diet for broilers resulted in an improvement in the muscle color. On the other hand, excessive leucine content in the diets resulted in retarded growth rate and increased feed conversion (FC) for 3-week-old, male broiler (Edmonds and Baker, 1987; Smith and Austic, 1977; Farran and Thomas, 1990; D'Mello and Lewis , 1970). Understanding the effects of limiting amino acids in the diet will allow the nutritionists to minimize or overcome the influence of excess essential amino acids (Kidd *et al*., 2000; Baker *et al*., 2002) and help to reduce costs.

Beside that, energy is also an important nutrient for broiler and other living creatures. Energy is required for body function and an essential constituent of all tissues in the body. High energy broiler starter diets result in extra deposition of fat (Holsheimer and Jensen, 1991) and this is an indication of dietary energy wastage. To ensure the maximum utilization of each and every nutrient of the diet, the right proportion of these nutrients are necessary to optimize the growth of the birds and to minimize the surplus use of vital dietary component.

The challenge for the nutritionist is to formulate economically viable diets which will provide as closely as possible the amino acids and energy requirement of

chickens. To date, no information has been published about the effects of L-leucine supplementation on meat broiler chickens especially at grower-finisher period. Therefore, the aim of this study was to examine the effect of different levels of L-leucine supplement to an energy deficient diet on the live weight and carcass quality of broiler chickens.

MATERIALS AND METHODS

A total of 80 one-day broiler chicks (Cobb) were randomized across 16 floor pens (5 chicks per pen; 0.1m^2 floor space per birds). All birds consumed feed and water *ad libitum* and reared under continuous lighting. Fishmeal, soybean meal and corn were analyzed for crude protein (CP) using the Kjedahl procedure (AOAC, 1990) prior to diet formulation to assure accurate amount of amino acid in the treatment. Four dietary treatments were replicated four times (20 birds per treatment) in a factorial arrangement. Two levels of L-leucine (0 and 0.5%) were added to 3200 and 3000 kcal/kg of ME during the growing periods, respectively (Table 1). Dietary treatments were formulated according to the NRC (1994). From the age of day 1 to 21, all the chicks were fed with a nutritionally complete broiler starter diet. The birds received dietary treatments from the age of 21 to 42 days. One of the formulations i.e. Diet 1 (Table 1) was considered as the positive control and calculated to contain 20% CP and 3200 kcal/kg of ME.

Physical carcass evaluations were carried out to determine the percentage of lean, fat and bone of whole chickens. The birds were dissected for carcass composition determination. The weight of *pectoralis* major and *pectoralis* minor muscles was recorded and included as the lean portion of the carcass composition. These muscles were then used for sensory analysis. Carcasses were taken out from the freezer, thawed for 10 min at room temperature. Then, the carcass without *pectoralis* major and minor muscles was dissected to detach the lean, fat and bone. The factorial arrangement of 4 treatments consisting of two energy levels and two leucine levels with the complete randomized design (CRD) were used and the results obtained were analyzed using the General Linear Models procedure of SAS (SAS Institute, 1997). When differences among means were found, these means were separated using the Duncan's new multiple range test (Steel and Torrie, 1980).

Sensory evaluation: Chicken meat samples were cooked in a boiling water bath for 15-25 min or until the internal temperature reached 80°C. The samples were then cut into small cubes (with dimension of 3 x 1 x 4 cm) using a sharp knife, placed onto a white plate and allowed to rest for 2 minutes prior to tasting. Sensory evaluation was conducted in individual booth at the sensory laboratory of the Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM). Thirty untrained panelists comprising of staffs and students from several departments in UPM were asked to evaluate the degree of liking for the aroma, tenderness, juiciness, flavor and overall acceptability of the samples using a nine-point Hedonic Scale (Score 1 = dislike extremely; Score 9 = like extremely). Panelists were required to rinse their mouth with distilled water before tasting the next sample. The samples were presented on a white plate coded with three-digit random numbers and the order of presentation was randomized. The mean scores for all the sensory attributes were calculated. A high mean score indicates that the particular attribute is acceptable to the panelist. Score 5 is the cut-off point whereby the panelist neither like nor dislike the attribute being tested.

Table 1: Composition (%) and nutrient content of diets with different level of leucine and energy in experiment

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
Corn	61.00	64.17	60.00	64.21
SBM (44%)	24.00	23.80	23.34	24.00
Fishmeal (57%)	6.99	6.7	6.99	5.4
Palm Oil	5.42	2.0	5.70	2.0
Limestone	1.26	1.3	1.27	1.3
Salt	0.28	0.28	0.25	0.28
DCP	0.10	0.8	1	0.8
Mineral Mix	0.25	0.25	0.25	0.25
Vitamin Mix	0.25	0.25	0.25	0.25
L-Lysine	0.20	0.2	0.20	0.2
DL-Methionine	0.15	0.15	0.15	0.15
L-Leucine	0	0	0.5	0.5
Choline chloride	0.10	0.10	0.10	0.10
Calculated values				
ME Kcal/kg	3,204	3,031	3,195	3,053
CP (%)	20.00	20.0	19.92	19.95
Ca (%)	0.99	1.07	1.12	1.03
Available P (%)	0.33	0.33	0.48	0.42
Fiber (%)	3.14	3.19	3.07	3.20
Methionine + Cystine (%)	0.84	0.84	0.83	0.83
Lysine (%)	1.281	1.27	1.26	1.21
Leucine (%[1])	1.707	1.71	2.16	2.19

[1]The NRC (1994) recommends minimum levels of 1.09% Leucine for diets with 3200 ME kcal/kg and 20% crude protein on broiler from 21-42 days of age. [1]Premix provided the following per kg or diet: Vitamin A 50.000 MIU, Vitamin D3 10.000 MIU, Vitamin E 75.000, Vitamin K 20.000 g, Vitamin B1 10.000 g, Vitamin B2 30.000 g, Vitamin B6 20.000 g, Vitamin B12 0.100 g, Calcium D-Panthothenate 60.000 g, Nicotinic Acid 200.000 g, folic acid 5.000 g, Biotin 235.000 mg

RESULTS AND DISCUSSION

The average live weight, carcass composition and sensory mean scores of broiler chickens are shown in Tables 2 and 3. Supplementation of L-leucine had no significant effect on the live weight and carcass composition involving breast meat, lean, bone, fat and skin (P>0.05). Excess L-leucine has been reported to increase the needs for isoleucine and valine and resulted in growth retardation unless these two amino acids were supplemented with L-leucine (Chi, 1984). L-leucine supplementation of a diet adequate in branched

Table 2: Carcass characteristics of broiler chickens fed diets supplemented with L-leucine and at two energy levels (42 days of age)

Treatments			Live	Breast	Lean	Bone	Fat	Skin
	L-Leucine	Energy	Weight (g)	(g)	(g)	(g)	(g)	(g)
L-Leucine (%/kg)								
0.0			2413.5±	524.24±	536.04±	321.02±	44.06±	169.7±
			238.234	58.10	92.49	53.50	17.74	55.66
0.5			2343.1±	547.81±	576.62±	337.90±	43.42±	174.80±
			206.479	46.38	68.36	87.42	21.40	26.41
Metabolizable Energy kcal/kg								
3200			2415.0±	532.29±	562.4±	344.84±	43.16±	157.08±
			194.036	58.32	77.96	75.61	15.32	35.54
3000			2341.6±	539.76±	550.26±	314.08±	44.32±	187.42±
			247.992	49.00	89.35	66.48	23.19	45.11
Level of Significance								
L-Leucine			ns	ns	ns	ns	ns	ns
Metabolizable Energy			ns	ns	ns	ns	ns	ns
L-Leucine x Metabolizable Energy			ns	ns	ns	ns	ns	ns

ns: not significant at $P < 0.05$.

Table 3: Sensory acceptance of chicken meat characteristics as affected by L-leucine supplementation and energy levels (21-42d)

	Odour	Flavour	Tenderness	Juiciness	OA[1]
Main effects: Energy					
3200	6.6379	6.7414	6.8103	6.9310	6.9310
3000	6.7586	6.8966	7.0000	6.7414	6.8793
SEM					
L-Leucine	L-Leucine (%/kg)				
0.0	6.9310	6.6897	7.1379	6.8966	6.9483
0.5	6.4655	6.9483	6.6724	6.7759	6.8621
SEM					
Level of significance					
Energy	ns	ns[2]	ns	ns	ns
L-Leucine	ns	ns	ns	ns	ns
Energy x L-leucine	ns	ns	ns	ns	ns

1- OA: Overall Acceptability. 2- ns : not significant at $P < 0.05$.

Note: Score for Hedonic Scale: (1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like extremely)

amino acids produced the often-reported reduction in growth (Spolter and Harper, 1961; Oestemer *et al*., 1973). However, in this study, addition of 0.50 % leucine to the diets resulted in a total of 2.16 and 2.19 % dietary L-leucine, to the diets containing 3000 and 3200 kcal/kg ME, respectively. The amount of L-leucine in this case was not enough to cause any significant effect on growth. This finding was in agreement with results of Penz *et al*. (1984) who showed that the addition of 1.6% L-leucine (total of 3.23%) in a 22.9% protein diet did not cause a significant decrease in growth performance of chicks. Similarly, in an earlier study, Erwan *et al*. (2008) showed that addition of 0.5% leucine to an iso-nitrogenous diet (20% CP) with either 3200 or 3000 kcal/kg of ME did not show any significant effect on the feed intake, weight gain and FCR of grower broilers. Furthermore, Edmonds and Baker (1987) also found that the excess of 4% L-leucine on diet that contained 23% protein and 3,130 kcal/kg of ME had no significant effect on the growth of broiler chicks. On the other hand, addition of 4.8% L-leucine caused a significant reduction on the growth performance of broiler chicks (Penz *et al*., 1984). Farran *et al*. (2003) reported that addition of 40g leucine/kg did not induce toxicity in starter chicks fed with high protein diet (255 g/kg). Similarly, excessive amount of leucine (70-100 g/kg) in a high protein diet did not affect performance of the rats (Daniel and Waisman, 1968). D'Mello and Lewis (1970) and Ueda *et al*. (1981), indicated a decreasing trend in feed intake with increasing supplementation of dietary leucine. The reason for the toxic effect of excessive L-leucine on chicks fed with low protein diets is not well understood. It is speculated that the consumption of high dietary protein results in an abundance of all amino residues at the absorptive sites of the small intestines. Therefore, L-leucine being the least available amino acids would still be fairly absorbed in adequate amounts. In low protein diets, L-leucine may also accelerate the degradation of other branched chain amino acids (BCCAs), mainly in muscles through the stimulated activity of branched-chain alpha-ketoacid dehydrogenase (Harper *et al*., 1984), which leads to the lowering of valine and iso-leucine in rat tissues. The experiments reported herein (Table 2) indicate that supplementation with leucine at 0.5% did not cause a significant differences in the carcass composition of broiler chickens.

No statistical difference was observed in the body composition or skeletal muscle between any of the chickens fed with any of the experimental diets containing excess leucine (Table 3). However, there was

a small increase in the muscle fat content in diets which contain added L-leucine. The result obtained in this study was in agreement with Donato *et al.* (2006) which indicate that the low-dose L-leucine supplementation increased body fat loss of rats but showed no significant difference in the gastrocnemius muscle mass of rats. This finding showed that L-leucine supplementation caused improvement in live weigh and carcass composition in diet containing low metabolizabe energy compared with the control.

Decreasing dietary energy had no significant effect on the live weigh and carcass composition. Similarly, Zaman *et al.* (2008) noted no differences in breast meat of broiler chickens when dietary levels of metabolizable energy were decreased. The apparent lack of effect of ME density on feed intake in this study was consistent with the revised observations of NRC (1994) which conclude that modern broiler strains did not adjust their feed intake to changes in the dietary ME density. The differences in the effects of energy compound on broiler feed intake observed by other researchers may be due to differences in the range of age of birds used in dietary trials, as well as differences on the formulation techniques used in the experiments.

Mean scores for acceptability of various sensory attributes such as aroma, tenderness, juiciness, flavor, and overall acceptability are shown on Table 3. The mean scores for all attributes evaluated were within 6.63 to 7.14 indicating that in general in terms the acceptability of panelist towards the broiler attributes was between 'like slightly' to 'like moderately'. Addition of 0.5% L-leucine in the diets caused some reduction in the mean scores for all sensory attributes tested except for flavor. However, based on the statistical analysis, no significant differences in the tenderness, juiciness, flavor, aroma, and overall acceptability of the broiler was observed. It is possible that glutamic acids (Glu), which is one of the active taste components may be responsible for the negative effects of L-leucine supplementation on sensory characteristics. With a decrease in dietary L-leucine levels, free glutamic acids, in muscle had tended to increase. However, the results of the present findings that supplementation of 0.5% leucine resulted in increasing of flavor are not in agreement with Fujimora and Kadowaki (2006) who reported that free Glu contents at the recommended level of leucine (NRC) were 34% higher than those supplemented at 130% of the diet in broiler chicks at 10 days of age. From the result of this study, it can be concluded that supplementation of L-leucine may influence the sensory characteristics of poultry meat.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Universiti Putra Malaysia for awarding the Research University Grant Scheme (RUGS) that makes this work possible.

REFERENCES

Association of Official Analytical Chemist, 1980. Official Methods of Analysis. 13th ed. Washington, DC.

Baker, D.H., A.B. Batal, T.M. Parr, N.R. Augspurger and C.M. Parsons, 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, iso leucine, and valine for chicks during the second and third week posthatch. Poult. Sci., 81: 485-494.

Blanchard, P.J., M. Ellis, C.C. Warkup, B. Hardy, J.P. Chadwick and G.A. Deans, 1999. The influence of rate of lean and subcutaneous fat tissue development on pork eating quality. Anim. Sci., 68: 477-485.

Castell, A.G., R.L. Cliplef, L.M. Paste-Flynn and G. Butler, 1994. Performance, carcass, and pork characteristics of castrates and gilts self-fed diets differing in protein content and lysine: energy ratio. Can. J. Anim. Sci., 74: 519-528.

Chi, M.S., 1984. Amino acid balance and its effect on the growth rate of white leghorn female-chickens. Nutr. Rep. Int., 29: 45-53.

Cisneros, F., M. Ellis, D.H. Baker, R.A. Easter and F.K. McKeith, 1996. The influence of shortterm feeding of amino acid-deficient diets and high dietary leucine levels on the intramuscular fat content of pig muscle. Anim. Sci., 63: 517-522.

Daniel, R.G. and H.A. Waisman, 1968. The effects of excess amino acids on growth of the young rat. Growth, 32: 255-265.

Donato, J., G.P. Rogerio, F.C. Vinicius, S.O.P. Ivanir, T. Julio, 2006. Effects of leucine supplementation on the body composition and protein status of rats submitted to food restriction. J. Nutr., 22: 520-527.

D'Mello, J.P.F. and D. Lewis, 1970. Amino acids interaction in chick nutrition. Interrelationships between leucine, isoleucine and valine. Br. Poult. Sci., 11: 313-323.

Edmonds, M.S. and D. Baker, 1987. Comparative effects of individual amino acids excesses when added to a corn-corn soybean meal diet: effects on growth and dietary choice in the chick. J. Anim. Sci., 65: 699-705.

Erwan, E., A.R. Alimon, A.Q. Sazili, H. Yaakub, 2008. Effect of varying levels of leucine and energy on performance and carcass characteristics of broiler chickens. Int. J. Poult. Sci., 7: 696-699.

Farran, M.T. and O.P. Thomas, 1990. Dietary requirement of leucine, iso leucine, and valine in male broiler during the starter period. Poult. Sci., 69: 757-762.

Farran. M.T., E.K. Barbour and V.M. Ashkarian, 2003. Effect of excess leucine in low protein diet on ketosis in 3-week-old male broiler chicks fed different levels of isoleucine and valine. J. Anim. Feed Sci. Technol., 103: 171-176.

Fujimora, S., S. Kawano, H. Takeda, Kadowaki and T. Ishibashi, 1995. Identification of taste-active components in the chickens meat extract by omission test – Involvement of glutamic acid, IMP and potassium ion. Anim. Sci. Technol. (Jpn), 66: 43-51.

Fujimora, S., H. Koga, H. Takeda, N. Tone, M. Kadowaki and T. Ishibashi, 1996. Role of taste-active components, glutamic acids, 5-inosinic acid and potassium ion taste of chicken meat extract. Anim. Sci. Technol. (Jpn), 67: 423-429.

Fujimora, S. and M. Kadowaki, 2006. Improvement of meat taste by dietary components. Bull. Facul. Agri. Niigata Univ., 58: 151-153.

Harper, A.E., R.H. Miller and K.P. Block, 1984. Branched-chain amino acid metabolism. Ann. Rev. Nutr., 4: 409-454.

Holsheimer, J.P. and W.M. Jenson, 1991. Limiting amino acids in low protein maize-soybean meal diets fed to broiler chicks from 3 to 7 weeks of age. Br. Poult. Sci., 32: 151-158.

Kidd, M.T., B.J. Kerr, J.P. Allard, S.K. Rao and J.T. Halley, 2000. Limiting amino acids responses in commercial broilers. J. Appl. Poult. Sci., 9: 223-233.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th edition (Revised). National Academy Press, Washington, D.C.

Oestemer, G.A., L.E. Hanson and R.J. Meade, 1973. Leucine-isoleucine interrelationship in the young pig. J. Anim. Sci., 36: 674-678.

Penz, A.M. JR., A.J. Clifford, Q.R. Rogers and F.H. Kratzer, 1984. Failure of dietary leucine to influence the tryptophan-niacin pathway in chicken. J. Nutr., 33-41.

SAS Institute, 1997. SAS/STAT User's Guide. SAS Institute, Inc, NC.

Smith, T.K. and R.E. Austic, 1977. The branched-chain amino acid antagonism in chicks. J. Nutr., 1180-1191.

Spolter, P.D. and A.E. Harper, 1961. Leucine-isoleucine antagonism in the rat. Am. J. Physiol., 200: 513-518.

Steel, R.G.D. and J.H. Torrie, 1980. Principle and procedures of statistics. 2nd ed. McGraw-Hill Book Co., Inc, New York.

Ueda, H., S. Yabuta, H. Yokota and I. Takashi, 1981. Involvement of feed intake and feed utilization in the growth retardation of chicks given the excessive amounts of leucine, lysine phyenylalanine or methionine. Nutr. Rep. Int., 24: 135-144.

Zaman, Q.U., T. Mushtaq, H. Nawaz, M.A. Mirza, S. Mahmood and T. Ahmad, 2008. Effect of varying dietary energy and protein on broiler performance in hot climate. Anim. Feed Sci. Technol., 146: 302-312.