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Effects of Levels of L-Leucine Supplementation with Sub-optimal Protein in the Diet of Grower-finisher Broiler Chickens on Carcass Composition and Sensory Characteristics*

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ABSTRACT : An experiment involving 180 straight run one-day-Cobb broilers was conducted to evaluate the effect of supplementation of L-leucine with different levels of crude protein (CP) on carcass composition and sensory characteristics of broiler grower-finisher chickens. Six experimental diets comprising two levels of crude protein (CP) i.e., 20 and 20% with three levels of L-leucine i.e. 0, 0.5 and 0.67%, were offered to birds from 21-42 d of age. The birds were randomly divided into 36 experimental pens, 5 chickens in each pen, and there were 6 replicates under each diet. L-leucine supplementation did not affect the bone and lean, whereas fat was decreased ($p<0.05$) when L-leucine was added at 0.5%. Similarly, there were no significant differences ($p>0.05$) in the lean, fat and bone among chickens fed two levels of CP. No significant differences between dietary treatments were observed on any sensory characteristics affected by dietary L-leucine and CP. From this study, it is obvious that supplementation of up to 0.5% L-leucine reduced fat. However, other characteristics were not affected by supplementation of L-leucine. Similarly, reduction of body composition and sensory characteristics were not apparent on a diet low in CP. (**Key Words :** L-leucine, Crude Protein, Broiler, Carcass Composition and Sensory Characteristics)

INTRODUCTION

It is now well documented that dietary composition can have a major effect on performance and body composition of chickens (Busye et al., 1992; 1997; Nieto et al., 1997; Collin et al., 2003). As a broiler chicken grows, its carcass composition changes. The most evident change is the increase in carcass fat. Some researchers reported that lowering dietary CP did not affect growth performance (Morran and Stilborn, 1996), however carcass composition becomes inferior in broilers fed diets in which CP has been reduced by more than 3%, even with adequate nutrient requirements (Sterling et al., 2005; Waldroup et al., 2005). Generally, amino acids (AA) and CP in the diet influence the carcass composition of broilers, and reducing dietary CP effects a decrease in carcass fat content (Si et al., 2004). On the other hand, increasing dietary leucine decreases carcass fat content (Donato et al., 2006).

In addition, studies on the relationship between feed and taste-active components of grower-finisher broilers are still limited. It was reported that diets have no effect on taste-active components (Farmer, 1990; Erwan et al., 2009). However, in other reports, a relationship was found between feed and meat qualities including texture (Kristensen et al., 2002), aroma (Bou et al., 2001) and color (Cisneros et al., 1996; Smith et al., 2002). In previous research, three compounds were identified, namely free glutamic acid (Glu), 5'-inosinic acid (IMP), and potassium ion, as taste-active components in chicken meat extract (Fujimora et al., 1995, 1996). Glu and IMP, known as *umami* taste, are favored by consumers and constitute a characteristic taste of chicken meat. Several studies showed that restricted feeding and low metabolizable energy (ME) levels decreased Glu

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content and meat taste deteriorated (Fujimora et al., 1997; 2001). There is no information available, at present, on the effect of leucine supplementation and different levels of CP on the carcass composition and sensory characteristics of grower-finisher broilers. The objective of this study was to assess the effect of L-leucine supplementation on carcass composition and sensory characteristics in grower-finisher broilers fed different levels of CP.

MATERIALS AND METHODS

Experimental materials and methods

An experiment was conducted with 180 broilers (Cobb) from 21 to 42 day of age. Initially all birds were placed in four stainless steel cages measuring 2 feet×2 feet×1 ft high, fitted with feeders and waterers. The chicks were fed a standard commercial starter ration until 21 days of age. Feed and water were given *ad libitum* and they were subjected to continuous lighting. At day 21, the chicks were individually weighed and randomly allotted to 6 dietary treatments, with each treatment replicated five times and with 5 birds in each replicate.

The six dietary treatments comprised 3 levels of Leucine supplementation (0, 0.5 and 0.67%) and two levels of dietary CP (20 and 18%) as shown at Table 1. The basal diet was formulated to meet NRC requirements (1994) by addition of L-leucine. All diets were formulated using corn, soybean meal and DL-methionine to meet or exceed NRC (1994) nutrient recommendations. Fishmeal, soybean meal and corn were analyzed for CP prior to diet formulation using the Kjedahl procedure (AOAC, 1990). The calculated ME was 3,200 kcal per kg for all diets. Diet 1 was considered a positive control and calculated to contain 20% CP and 3,200 kcal/kg of ME. Birds were given the experimental diets from 21 to 42 days of age.

Carcass composition measurement

Physical carcass composition was 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, which were dissected for sensory analysis, was also recorded and included as lean in carcass composition. Carcasses were taken from the freezer, thawed for 10 minutes at room temperature, and then carcass without *pectoralis* major and minor muscle were dissected

Table 1. Composition (%) and nutrient content of experimental diets with different levels of leucine and protein

Ingrediants	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Corn	61	65.31	61	65.51	61	65.54
SBM (44%)	24	25	24	25	24	25
Fishmeal (57%)	6.99	2.1	6.49	1.6	6.32	1.4
Palm oil	5.42	5	5.42	4.8	5.42	4.8
Limestone	1.26	1.26	1.26	1.26	1.26	1.26
Salt	0.28	0.28	0.28	0.28	0.28	0.28
DCP	0.1	0.1	0.1	0.1	0.1	0.1
Mineral mix	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin mix[1]	0.25	0.25	0.25	0.25	0.25	0.25
L-lysine	0.2	0.2	0.2	0.2	0.2	0.2
DL-methionine	0.15	0.15	0.15	0.15	0.15	0.15
L-leucine	0	0	0.5	0.5	0.67	0.67
Choline cloride	0.1	0.1	0.1	0.1	0.1	0.1
Calculated analysis						
ME kcal/kg	3,204	3,207	3,208	3,202	3,207	3,204
CP (%)	20.00	18.00	20.01	18.05	20.02	18.04
Ca (%)	0.92	0.68	0.89	0.66	0.89	0.65
Available P (%)	0.33	0.20	0.32	0.19	0.31	0.18
Fiber (%)	3.14	3.27	3.13	3.27	3.13	3.27
Methionine+cystine (%)	0.84	0.76	0.83	0.75	0.83	0.75
Lysine (%)	1.281	1.09	1.25	1.07	1.25	1.06
Leucine (%)[2]	1.71	1.58	2.18	2.06	2.34	2.22

[1] Vitamin mix provided the following per kg of diet: vitamin A, 50.000 MIU; vitamin D_3, 10.000 MIU; vitamin E, 75.000; vitamin K, 20.000 g; vitamin B_1, 10.000 g; vitamin B_2, 30.000 g; vitamin B_6, 20.000 g; vitamin B_{12}, 0.100 g; calcium; D-panthothenate, 60.000 g; nicotinic acid, 200.000 g; folic acid, 5.000 g; and biotin, 235.000 mg.

[2] The NRC (1994) recommends minimum levels of 1.09% leucine for diets with 3,200 kcal/kg of metabolizable energy and 20% of crude protein for broiler chickens from 21 to 42 days of age.

Table 2. Final carcass composition of broilers as affected by L-leucine supplementation and protein levels (42 days of age)[1] (mean±standard deviation)

Treatments	Lean (g)	Bone (g)	Fat (g)
L-leucine (%/kg)			
0.0	737.250±155.47	533.12±77.89	69.608[a]±22.02
0.5	747.716±132.21	509.73±49.60	58.216[b]±16.88
0.67	698.433±110.13	683.27±985.35	72.058[a]±23.31
Protein (%)			
20	758.65±121.73	521.10±78.74	65.578±22.52
18	696.950±139.61	629.64±802.21	67.678±20.70
Interaction (Leucine×protein)			
0.0×20	788.583±113.70	546.72±92.33	81.133±22.33
0.0×18	685.917±178.50	519.52±61.30	58.083±14.99
0.5×20	769.250±154.55	501.47±56.31	55.350±19.57
0.5×18	726.183±107.94	518.00±47.72	83.867±22.57
0.67×20	718.117±86.27	515.12±89.79	60.250±17.95
0.67×18	678.750±130.67	851.42±1,400.56	61.083±13.95
Probability			
L-leucine	NS	NS	*
Protein	NS	NS	NS
Interaction	NS	NS	*

[1] n = 6 pens of 12 birds per treatment. [2] NS = Non significant.

to detach lean, fat and bone.

Sensory evaluation

Sensory evaluation was carried out by 27 panelists/ session consisting of students and staff of various departments at UPM. For practical reasons, it was difficult to engage more people during the period in question when field work occupied most panels. However, the consumer's cultural attachment to the sensory test was considered an advantage for judging the samples. The sensory test was conducted in isolated booths. Whole breast meat was boiled in a water bath for 15-25 minutes at 80°C (internal temperature). After cooking was completed the samples were cut into small cubes (3×1×4) cm and served using a plate and allowed to stand for 2 min prior to individual presentation to consumers. Each plate was labeled using 3-digit random code numbers before it was served to consumers. Samples were randomly allocated at room temperature and distilled water was also provided to cleanse palates of consumers between tastings. Hedonic ratings using 9-point scales were adopted. Attributes evaluated including aroma, tenderness, juiciness, flavor and overall acceptance. The higher rating indicated a good quality attribute, that is, (1), dislike very much and (9), like very much.

Statistical analyses

The data obtained from the experiment was analyzed to study the effect of dietary treatments using analysis of variance (3×2 factorial design) and the means were compared for significance by Duncan's new multiple range test (Steel and Torrie, 1980) at $p<0.05$.

RESULTS AND DISCUSSION

Carcass compositions

Table 3 shows the carcass composition as affected by L-leucine supplementation and different levels of CP. There was no difference in lean and bone weight after supplementation with L-leucine up to 0.67%. However, supplementation of 0.5% L-leucine significantly ($p<0.05$) decreased the relative weight of fat. In addition, carcass composition was not significantly affected by CP levels. In the present study, broilers supplemented with 0.5% L-leucine presented approximately 16.4% less carcass fat than the control group. This observation confirms the effect of leucine supplementation on body fat described by Donato et al. (2006), which indicated that a low-dose of L-leucine supplementation of rats resulted in almost 25.0% less carcass fat than the control diet. The results regarding the body composition of the animals are not surprising because some human studies have also provided evidence indicating that an increase in branched chain amino acid (BCAA) intake affects weight-loss (Mourier et al., 1997; Layman et al., 2003a, b, c).

Furthermore, studies comparing diets with different

Table 3. Chicken meat sensory characteristics as affected by L-leucine supplementation and protein levels (21-42 d) (mean±standard deviation)

Treatments	Aroma	Flavour	Tenderness	Juiciness	OA
L-leucine (%/kg)					
0.0	6.389±1.68	6.444±1.75	6.241±1.70	6.037±1.90	6.644±1.56
0.5	6.333±1.60	6.407±1.80	6.260±1.54	6.167±1.63	6.389±1.63
0.67	6.52±1.61	6.260±1.88	6.815±1.61	6.389±1.57	6.426±1.80
Protein (%)					
20	6.395±1.60	6.407±1.81	6.543±1.57	6.210±1.60	6.494±1.64
18	6.321±1.64	6.333±1.80	6.333±1.70	6.185±1.82	6.346±1.68
Interaction (Leucine×protein)					
0.0×20	6.074±1.82	6.185±1.98	6.148±1.94	5.926±1.84	6.222±1.63
0.0×18	6.703±1.49	6.704±1.46	6.333±1.47	6.148±1.99	6.667±1.49
0.5×20	6.260±1.70	6. 370±1.93	6.593±1.12	6.333±1.41	6.370±1.76
0.5×18	6.407±1.53	6.444±1.70	5.926±.84	6±1.84	6.407±1.53
0.67×20	6.852±1.20	6.667±1.52	6.889±1.53	6.370±15	6.889±1.53
0.67×18	5.852±1.83	5.582±2.12	6.741±1.72	6.407±1.65	5.963±1.95
Probability					
L-leucine	NS[1]	NS	NS	NS	NS
Protein	NS	NS	NS	NS	NS
Interaction	NS	NS	NS	NS	NS

NS = Non significant.

protein levels resulted in favorable body composition, especially in a greater fat decrease for high-protein diets, with part of these alterations being attributed to an increase of leucine (Layman et al., 2003; Plantenga et al., 2004). These observations are in accordance with Summers and Leeson (1984) who showed that increasing the dietary protein level resulted in an increase in yield of edible meat and it would be reasonable to assume that increasing the dietary protein level would cause a reduction in the fat content of the edible portion. Several mechanisms have been proposed to described the effect of high protein intake in weight-loss diets on body composition (Halton and Hu, 2004). Some nutritionists have suggested that part of the beneficial effect of these diets might be attributed to the higher ingestion of BCAAs, especially leucine (Layman et al., 2004; Zemel et al., 2004).

The present result was also in agreement with Elvery **(**1983**)** who found that different dietary levels during the finisher phase **(**24-49 d) had no significant effect on body-weight but there was a tendency for body-weight to increase with each increment of dietary protein. Neither the protein level of the starter nor the finisher diet had a significant effect on fat deposition at market age, but there was a general trend for fat deposition to decrease as dietary protein increased. Thus, it appears that increasing the dietary protein level can be used as a means to reduce carcass fat levels without a concomitant decrease in body-weight.

Sensory evaluation

Mean scores for various sensory attributes including aroma, tenderness, juiciness, flavor, and overall acceptability are shown on Table 3. The sensory analysis indicated no significant differences in tenderness, juiciness, flavor, aroma, and overall acceptability.

The aroma score ranged from 5.85 to 6.85, flavor score ranged from 5.58 to 6.70, tenderness score ranged from 6.15 to 6.89, juiciness score ranged from 5.93 to 6.41 and overall acceptability score ranged from 5.96 to 6.89. In general, the mean scores ranged from 5.85 to 6.89 which indicated that the acceptability of all attributes studied were between ‘like slightly’ to ‘like moderately’.

The sensory evaluation indicated no significant differences in tenderness, juiciness, flavor, aroma, and overall acceptability. However, there was a numerical decrease in aroma, flavor and overall acceptability with increasing levels of leucine. Other studies have also found a decrease of free glutamic acid (Glu) as a taste-active component in chickens when fed a high level of leucine during the starter period (Fujimora and Kadowaki, 2006).

On the other hand, a higher level of CP caused an increased sensory score when compared to low level of CP (18%), though not significantly different ($p>0.05$), and might be attributable to an increase of Glu caused by higher protein. This finding is an agreement with a previous study (Fujimora and Kadowaki, 1996) which found that by giving 30.7% CP overall preference, density and *umami* taste were increased compared to control groups.

CONCLUSION

It is concluded that relative weight of fat of grower-finisher chicken can be reduced by supplementation of up to 0.5% L-leucine. However, increasing dietary leucine tends to reduce the sensory characteristics. In addition, reducing CP by up 2% has no significant effect on carcass composition and sensory characteristics.

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