Iranian Journal of Applied Animal Science

The Effect of Formulation Diets Based on Digestible Amino Acids and Lysine Levels on Carcass and Chemical Composition of Broiler

Research Article

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Received on: 26 Sep 2016
Revised on: 14 Jan 2017
Accepted on: 31 Jan 2017
Online Published on: Jun 2017

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Online version is available on: www.ijas.ir

ABSTRACT

This study was conducted to evaluate the carcass yield, abdominal fat deposition and chemical compositions of thigh and breast muscles of male Arian broilers fed three different lysine levels of 1) high lysine (110% NRC), 2) standard level suggested by National Research Council (NRC) and 3) low lysine (90% NRC) based on two expression ways of amino acid contents of feedstuffs, as total amino acids (TAA) or digestible amino acids (DAA). Three hundred one day old male broiler chickens were used in a completely randomized design with 6 treatments of 5 replicates (10 male broilers). All diets were iso-caloric and iso-nitrogenous. The results of this study showed that diet formulation based on DAA significantly influenced breast muscle yield and abdominal fat deposition. High lysine level (110% NRC) significantly improved carcass yield, breast and thigh muscle percentages and weights. The interaction between DAA × lysine levels of the feed affects carcass and breast muscle percentages of the broilers. Feeding the broilers with high level lysine containing diets (110% NRC) resulted in significantly high lysine content of the breast and thigh muscles. The results of this study suggest that diet formulation based on digestible amino acids with additional lysine at the level of 110% of NRC in starter and grower diets optimized body weight gain in Arain male broilers.

KEY WORDS Arian, broiler, carcass, digestible, lysine.

INTRODUCTION

The wide variation in the composition and digestibility of amino acids (AA) present in the poultry feedstuffs is of great concern when using these raw materials. The importance of utilizing the correct amount of balanced dietary protein and AA for poultry is a high priority issue for two reasons: first, the costs of protein and AA the most expensive nutrients in feeds and second, the environmental concerns about liberation of nitrogen compunds from poultry waste. The concentration of protein and AA in broiler diets have a large impact on breast muscle yield, feed/gain ratio, and feeding period of time required to gain the appropriate body weight for each type of market. Depending upon genetic strain and the market objectives for each broiler complex, a broiler integrator probably utilize several different protein and AA dietary programs (Acar *et al.* 1991). In commercial practice, formulating diets to adequate AA minimums is critical to optimize live production and meat yield of broiler chickens. Within the last 10 years, demand for breast fillets and value-added products has contributed to increasing market weights of broiler chickens. Market weight, product mix, live cost and genetic strain are factors that may govern AA supplementation levels. Amino acids are critical for muscle development (Tesseraud *et al.* 1996) and lysine (Lys) content of in breast muscle is relatively higher than those of other AA. Lysine represents approximately 7% of the protein in breast meat (Munks *et al.* 1945).

Dietary Lys inadequacy has been shown to reduce breast meat yield compared with other muscles (Tesseraud *et al.* 1996). Therefore, defining dietary AA needs for optimum growth and meat yield is of utmost importance. Lys, one of the key AAs in protein synthesis and muscle deposition has also been demonstrated to be involved in the synthesis of cytokines, proliferation of lymphocytes and thus in the optimum functioning of immune system in response to infection. An inadequate supply of Lys would reduce antibody response and cell-mediated immunity in chickens (Geraert and Mercier Adisseo, 2010). It is well known that protein and Lys and its interaction is considered as an important factor which affects performance and carcass quality of growing chicks and so, dietary requirement of protein is actually a requirement for the Lys contained in the protein. Essential amino acid recommendations for broilers by the NRC (1994) are largely based on results of the experiments conducted several decades ago (Todd and Roselina, 2014).

Therefore the objective of this study was to evaluate the three different lysine requirement levels, high lysine (HLys, 110% NRC), standard (SLys, NRC) and low lysine (LLys, 90% NRC) and two ways to express amino acids of feedstuffs, either as total amino acid (TAA) or digestible amino acid (DAA) with same protein and energy requirements recommended by NRC (5) effects on the carcass yeilds and chemical composition of thigh and breast muscles of Arian male broiler.

MATERIALS AND METHODS

An experiment with Arian male broilers was conducted from 1 to 6 weeks of age. At day 1, 300 male chicks were placed in 30 floor pens (10 chicks per pen and 0.1 m^2 floor space/chick). Water and feed were also supplied *ad libitum*. The basic chemical composition of the feed materials and breast and thigh muscles were determined according to AOAC (1990). The total amino acid values of the ingredients were assayed by high-pressure liquid chromatography analysis. In order to determine digestibility, the levels of TAA determined in the analysis were multiplied by their respective digestibility coefficients, as determined by Yaghobfar and Zahedifar (2003). A completely randomized experimental design was used, in a factorial scheme (2×3). The first factor was included three different lysine requirement levels, high lysine (110% NRC), standard (NRC) and low lysine (90% NRC) and the second factor was included two levels of total amino acids (TAA) and digestible amino acids (DAA) of feedstuffs. Therefore, the following treatments were applied:

1) diet with high lysine (HLys) requirement level (110% NRC), formulated based on TAA, 2) diet with standard lysine (SLys) requirement level (NRC), formulated based on TAA, 3) diet with low lysine (LLys) requirement level (90% NRC), formulated based on TAA, 4) diet with high lysine (HLys) requirement level (110% NRC), formulated based on DAA, 5) diet with standard lysine (SLys) requirement level (NRC), formulated based on DAA and 6) diet with low lysine (LLys) requirement level (90% NRC), formulated based on DAA.

Diets were formulated according to NRC (1994) recommendations to contain 23 and 19% crude protein (CP) and 3100 and 3200 kcal ME/kg in starter and grower diets, respectively. The diets were formulated as iso-energetic and iso-nitrogenic (Tables 1 and 2). Body weights (BW) was obtained at 42 d of age. Mortality was zero in treatments. At the end of the experimental period (at 42 day of age), in order to evaluate carcass quality, 4 birds from each treatments with body weights as close as to the average body weight of the experimental unit were slaughtered per repetition. These birds were weighed, eviscerated, and weighed again after removal of the head and feet to obtain carcass weight. The breast and thigh muscles, liver and abdominal fat weights were also determined. Yields of the breast and thigh muscles, carcass were determined in relation to body weight, and expressed as percentage of body weight (%). Data were analyzed by factorial analysis of variance (ANOVA) using the general linear models procedure of SAS (2004). Where significance occurred, means were compared with the Duncan multiple range test at 0.05 levels. The data were expressed as means with SEM.

RESULTS AND DISCUSSION

The results of carcass characteristics and chemical composition of thigh and breast muscles are given in Tables 3 and 4. The diet formulated based on DAA did not influence carcass, thigh percentage muscle and liver weight (Table 3). These results are consistent with Rostagno *et al.* (1995), who found no differences in carcass yield with the similar formulation.

The diet formulated on DAA basis significantly ($P<0.05$) influenced breast muscle percentage and abdominal fat deposition (Table 3). The excess AA intake is possibly related to the energy/protein ratio of the diet, and consequently, to carcass composition. In the present study, the diets formulated with the same amino acids (iso-nitrogenic) and same energy contents (iso-energetic) but different AAs containing feedstuffs and lysine levels. Therefore, the broilers given the diet formulated based on DAA, received more AA than those given the diets prepared based on TAA basis.

Table 1 Composition of the experimental diets in starter (0-21 day) period

Amino acids of feed	**Total**			**Digestible**		
Lysine levels	110%	Standard	90%	110%	Standard	90%
	1	2	3	4	5	6
Ingredients						
Corn grain	55.47	56.27	57.32	54.01	55.01	55.39
Soybean meal (48%)	35.56	35.01	33.94	37	36.21	36.01
Soybean oil	3	3	3	2.8	2.8	2.8
Fish meal	2	2	2	2	2	2
Oyster shells	1.88	1.88	1.88	1.88	1.88	1.88
Dicalcium phosphate	1	1	1	1	1	1
Common salt	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin and mineral premix[1]	0. 5	0. 5	0. 5	0. 5	0. 5	0. 5
DL-methionine	0.11	0.09	0.1	0.11	0.1	0.1
L-lysine HCl	0.28	0.05	0.05	0.5	0.3	0.12
Nutrients contents						
Apparent metabolizable energy corrected for nitrogen (AMEn) (Mcal/kg)	3.10	3.10	3.10	3.10	3.10	3.10
Protein (%)	23.00	23.00	23.00	23.00	23.00	23.00
Ether extract (%)	5	5	5	5	5	5
Linoleic acid (%)	2.5	2.5	2.5	2.5	2.5	2.5
Calcium (%)	1.00	1.00	1.00	1.00	1.00	1.00
Avail phosphorus (%)	0.5	0.5	0.5	0.5	0.5	0.5
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16
Lysine (%)	1.25	1.14	1.03	1.25	1.14	1.03
Methionine (%)	0.51	0.51	0.51	0.51	0.51	0.51
TSAA (%)	1.11	1.11	1.11	1.11	1.11	1.11

[1] Provides per kg of diet: vitamin A: 7000 IU; vitamin D_3: 1400 IU; vitamin E: 16.65 mg; vitamin K: 1.5 mg; vitamin B_1: 0.6 mg; vitamin B_2: 2.36 mg; vitamin B_6: 0.6 mg; vitamin B_{12}: 0.013 mg; Biotin: 0.15 mg; Choline: 1.54 g; Pantothenic acid: 9.32 mg; Niacin: 30.12 mg; Folic acid: 1.42 mg; Selenium: 0.65 mg; Iodine: 0.35 mg; Iron: 57.72 mg; Copper: 12.30 mg; Zinc: 141.48 mg and Manganese: 173 mg.
TSAA: total sulfur amino acids.

Table 2 Composition of the experimental diets in grower (22-42 day) period

Amino acids of feed	**Total**			**Digestible**		
Lysine levels	110%	Standard	90%	110%	Standard	90%
	1	2	3	4	5	6
Ingredients						
Corn grain	65.6	65.9	66.78	64.97	65.87	67.6
Soybean meal (48%)	28.72	28.2	27.14	28.93	28.1	27.01
Soybean oil	3	3.24	3.22	3.27	3.4	3
Oyster shells	1.6	1.7	1.8	1.5	1.5	1.4
Dicalcium phosphate	0.2	0.2	0.2	0.23	0.23	0.23
Common salt	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin and mineral premix[1]	0. 5	0. 5	0. 5	0. 5	0. 5	0. 5
DL-methionine	0.1	0.06	0.11	0.2	0.1	0.06
L-lysine HCl	0.08	0	0.05	0.2	0.1	0
Nutients contents						
AMEn (Mcal/kg)	3.2	3.2	3.2	3.2	3.2	3.2
Protein (%)	19	19	19	19	19	19
Ether extract (%)	5	5	5	5	5	5
Linoleic acid (%)	2.5	3	2.5	2.5	3	2.5
Calcium (%)	0.09	0.09	0.09	0.09	0.09	0.09
Avail phosphorus (%)	0.42	0.42	0.42	0.42	0.42	0.42
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16
Lysine (%)	1.1	1	0.9	1.1	1	0.9
Methionine (%)	0.42	0.42	0.42	0.42	0.42	0.42
TSAA (%)	0.88	0.88	0.88	0.88	0.88	0.88

[1] Provides per kg of diet: vitamin A: 7000 IU; vitamin D_3: 1400 IU; vitamin E: 16.65 mg; vitamin K: 1.5 mg; vitamin B_1: 0.6 mg; vitamin B_2: 2.36 mg; vitamin B_6: 0.6 mg; vitamin B_{12}: 0.013 mg; Biotin: 0.15 mg; Choline: 1.54 g; Pantothenic acid: 9.32 mg; Niacin: 30.12 mg; Folic acid: 1.42 mg; Selenium: 0.65 mg; Iodine: 0.35 mg; Iron: 57.72 mg; Copper: 12.30 mg; Zinc: 141.48 mg and Manganese: 173 mg.
TSAA: total sulfur amino acids.

Table 3 Effects of amino acids of feedstuffs and lysine requirement levels on carcass composition at 42 d

Amino acids×lysine levels*	Carcass yeild (%)	Carcass weight (g)	Breast (%)	Breast (g)	Thigh (%)	Thigh (g)	Abdominal fat (g)	Liver (g)
1- TAA × 110% NRC	66.63^{a}	1237^{b}	21.59^{a}	394^{b}	22.62	420^{b}	17.05^{b}	53.85^{ab}
2- TAA × Standard NRC	66.18^{a}	1215^{b}	21.19^{a}	396^{b}	22.79	417^{b}	15.10^{b}	53.63^{ab}
3- TAA × 90% NRC	60.94^{b}	1113^{c}	18.88^{b}	345^{b}	21.88	400^{b}	19.10^{b}	42.55^{b}
4- DAA × 110% NRC	65.18^{a}	1463^{a}	21.92^{a}	472^{a}	23.30	524^{b}	40.33^{a}	60.40^{a}
5- DAA × standard NRC	66.35^{a}	1227^{b}	21.27^{a}	400^{b}	23.71	438^{ab}	12.43^{b}	49.35^{ab}
6- DAA × 90% NRC	63.03^{ab}	1223^{b}	20.78^{ab}	402^{b}	21.63	421^{b}	33.10^{a}	55.75^{ab}
P-value	0.017	0.036	0.087	0.031	0.332	0.039	0.002	0.146
SEM	1.175	52.93	0.381	22.71	0.719	29.35	4.577	4.422
Amino acids of feedstuffs								
Total	64.58	1188^{b}	20.56^{b}	378^{b}	22.43	412	17.08^{b}	50.01
Digestible	64.85	1304^{a}	21.79^{a}	426^{a}	22.88	461	28.62^{a}	55.17
P-value	0.780	0.037	0.042	0.023	0.452	0.056	0.006	0.170
SEM	0.678	78.32	0.293	13.11	0.415	16.94	2.643	2.553
Lysine requiremnet levels								
110% NRC	65.91^{a}	1350^{a}	21.76^{a}	433^{a}	22.96	472	28.69^{a}	57.13
Standard	66.26^{a}	1221^{ab}	21.24^{ab}	398^{ab}	23.25	427	13.76^{b}	51.49
90% NRC	61.98^{b}	1168^{b}	19.83^{b}	374^{b}	21.75	410	26.10^{a}	49.15
P-value	0.003	0.046	0.041	0.045	0.116	0.126	0.010	0.207
SEM	0.831	63.45	0.181	16.06	0.508	20.75	3.237	3.127

* Treatment 1: diet with 110% NRC (1994) lysine requirement level, formulated based on total amino acids (TAA); Treatment 2: diet with standard NRC (1994) lysine requirement level, formulated based on TAA; Treatment 3: diet with 90% NRC (1994) lysine requirement level, formulated based on TAA; Treatment 4: diet with 110% NRC (1994) lysine requirement level, formulated based on digestible amino acids (DAA); Treatment 5: diet with standard NRC (1994) lysine requirement level, formulated based on DAA and Treatment 6: diet with 90% NRC (1994) lysine requirement level, formulated based on DAA.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

If crude protein ratio is increased in a given broiler feed, significant decrease might occur in the energy/protein ratio, which results in carcasses with high fat content (Rosebrough and Steele, 1985).

Protein accretion at post-hatch period in boiler chicks is a result from either increasing protein synthesis or decreasing protein degradation. Diets with low Lys levels can limit breast muscle growth early in the development by reducing synthesis of muscle proteins and RNA content (Tesseraud *et al*. 1996). Significant effects of the dietary Lys levels both on the thigh muscle percentage and liver weight were not observed in this study. The results were in accordance with the previous studies (Kidd *et al*. 1998; Nasr and Kheiri, 2011; Bernal *et al*. 2014) which demonstrated that Lys requirement for growing chicks is higher than that of NRC (1994) recommendation for maximum growth. It is also confirmed that increasing dietary Lys level increases breast meat yield.

Dietary Lys levels significantly influenced the breast muscle percentage (Table 3) and the weights of carcass, breast and thigh muscle (Figure 2). HLys and LLys levels resulted in higher abdominal fat deposition. This result is supported by the finding that the abdominal fat weight significantly (P=0.002) increased both with the increasing or decreasing in Lys levels.

The increase in abdominal fat weight is probably resulted from the imbalanced AA content of the diets. This study showed that increasing Lys level (110% NRC) in diet increased breast muscle yield significantly, as shown in the previous studies (Gorman and Balnave, 1995; Kidd *et al*. 1998; Nasr and Kheiri, 2011; Bernal *et al*. 2014). Feeding broilers with HLys containing diets throughout the feeding period optimizes breast meat yield (Kidd *et al*. 1998; Nasr and Kheiri, 2011; Vieira and Angel, 2012; Bernal *et al*. 2014), although it may not always be economically justified. However, evidence in the literature suggests that feeding diets with high Lys level during the starter period impacts subsequent breast meat yield (Kerr *et al*. 1999). The concentration of dietary Lys can significantly influence the breast meat yield for the following reasons: breast muscle contains a high concentration of Lys (Table 4), which represents a larger portion of carcass meat. Although the breast muscle development is also affected by sex, age, breed and genetics strain, previous studies (Acar *et al*. 1991; Tesseraud *et al*. 1996; Gorman and Balnave, 1995) also demonstrated the increasing effect of additional feed Lys on the breast meat growth.

The weight of breast and thigh muscles of the treatment 4 (HLys-DAA) was significantly (P=0.031 and P=0.039) higher than those of the other treatments (Figure 1).

Table 4 Effects of amino acids of feedstuffs and lysine requirement levels on chemical composition of breast and thigh meat at 42 d

Amino acids×lys levels	Breast meat			Thigh meat		
	Lys (%)	Lipid (%)	CP (%)	Lys (%)	Lipid (%)	CP (%)
1- TAA × 110% NRC	5.83[a]	5.53	57.91	5.05[a]	9.86	60.59
2- TAA × Standard NRC	4.43[b]	3.04	49.26	3.83[b]	9.60	52.22
3- TAA × 90% NRC	3.92[b]	6.49	45.64	3.05[b]	11.39	48.74
4- DAA × 110% NRC	5.89[a]	4.77	59.34	5.40[a]	9.30	64.66
5- DAA × standard NRC	4.29[b]	3.66	52.17	3.92[b]	9.06	53.11
6- DAA × 90% NRC	4.09[b]	3.76	45.10	3.55[a]	11.59	49.82
P-value	0.009	0.295	0.289	0.002	0.517	0.122
SEM	0.424	1.128	5.211	0.362	1.162	4.452
Amino Acids of Feedstuffs						
Total	4.73	5.021	50.94	3.98	10.28	53.85
Digestible	4.76	4.061	52.20	4.29	9.980	55.86
P-value	0.93	0.311	0.769	0.30	0.755	0.587
SEM	0.245	0.651	3.009	0.209	0.671	2.569
Lysine requiremnet levels						
110% NRC	5.86[a]	5.149	58.63	5.23[a]	9.579	62.63[a]
Standard	4.36[b]	3.35	50.72	3.88[ab]	9.33	52.67[b]
90% NRC	4.00[b]	5.13	45.37	3.30[b]	11.49	49.28[b]
P-value	0.001	0.215	0.061	0.000	0.155	0.021
SEM	0.299	0.798	3.685	0.256	0.822	3.148

[*] Treatment 1: diet with 110% NRC (1994) lysine requirement level, formulated based on total amino acids (TAA); Treatment 2: diet with standard NRC (1994) lysine requirement level, formulated based on TAA; Treatment 3: diet with 90% NRC (1994) lysine requirement level, formulated based on TAA; Treatment 4: diet with 110% NRC (1994) lysine requirement level, formulated based on digestible amino acids (DAA); Treatment 5: diet with standard NRC (1994) lysine requirement level, formulated based on DAA and Treatment 6: diet with 90% NRC (1994) lysine requirement level, formulated based on DAA.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
CP: crude protein.
SEM: standard error of the means.

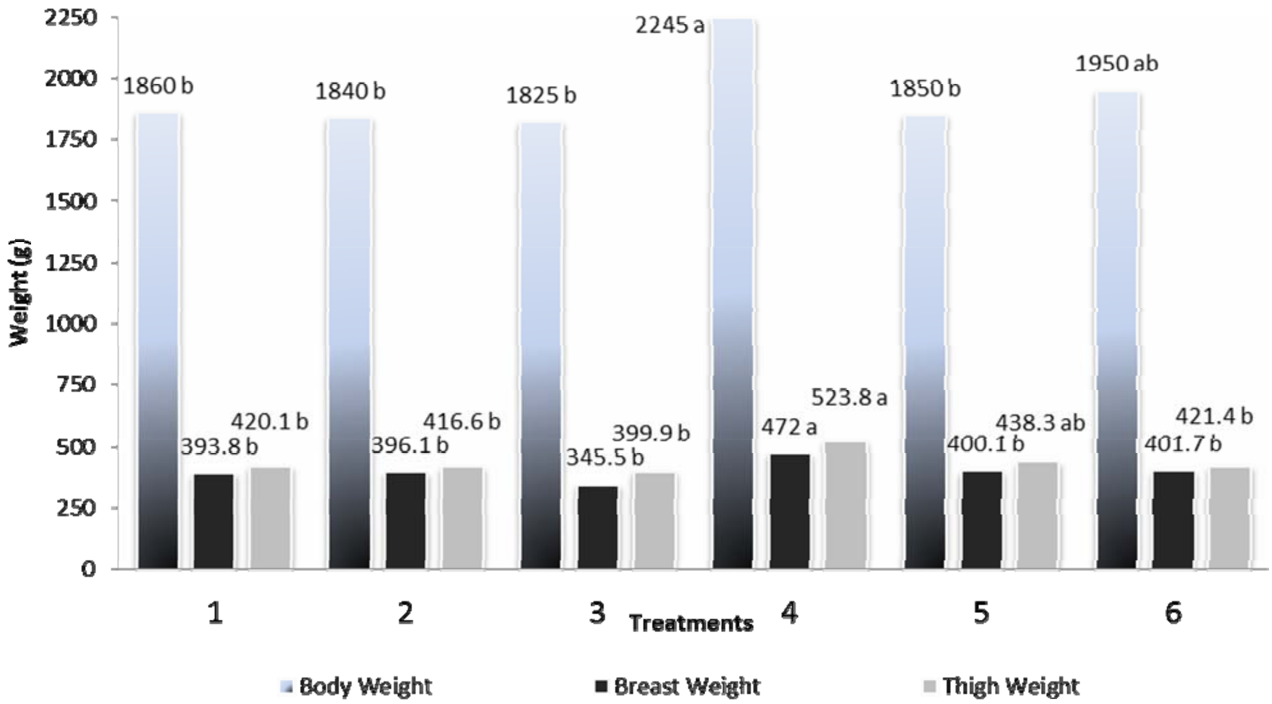

Figure 1 Effects of treatments on mean body, breast and thigh weight (at 42 day)
Treatment 1: diet with 110% NRC (1994) lysine requirement level, formulated based on total amino acids (TAA); Treatment 2: diet with standard NRC (1994) lysine requirement level, formulated based on TAA; Treatment 3: diet with 90% NRC (1994) lysine requirement level, formulated based on TAA; Treatment 4: diet with 110% NRC (1994) lysine requirement level, formulated based on digestible amino acids (DAA); Treatment 5: diet with standard NRC (1994) lysine requirement level, formulated based on DAA and Treatment 6: diet with 90% NRC (1994) lysine requirement level, formulated based on DAA

Previous reserchers (Eits *et al*. 2003; Dozier *et al*. 2007; Bernal *et al*. 2014) also showed that feeding broilers with HLys-AA diets increases breast meat yield. Results of the present study showed that the HLys-DAA had higher efficiency for tissue protein accretion and growth. This result is possibly related to the better availability of AA for synthesis of muscle proteins (Table 3). Diets formulated with HLys levels promoted a better conversion of DAA into carcass and breast and thigh meat yield (Figure 1). However, HLys-DAA groups had excess abdominal fat. This suggests that the excess AA intake caused imbalance and excess fat deposition. The combination of DAA and standard Lys possibly resulted in less AA availability for fat deposition. Leclercq (1998) stated that the Lys level should be at the highest level in the feed for minimizing abdominal fat percentage during maximizing breast meat yield and body weight gain.

Acar *et al*. (1991) found significant interactions between genotype and feed lysine level affecting abdominal fat, breast fillet yield, and breast meat yield. Moreover, dietary protein level has also been found to affect the lysine requirement (expressed as a percentage of the diet) of chicks (Hurwitz *et al*. 1998; Sterling *et al*. 2006).

Differences in the results among the previous researchers in relation to the dietary AA concentrations have been attributed to (Acar *et al*. 1993) differences between broiler strains.

The response to dietary AA/CP density (Smith *et al*. 1998; Sterling *et al*. 2006) and dietary Lys (Acar *et al*. 1991; Pesti *et al*. 1995) differs among the strain sources. A high-yielding strain was shown to contain more breast muscle, total RNA and protein on a weight basis and total DNA content over a low-yielding strain (Corzo *et al*. 2005). Muscle growth is largely related to the number of nuclei or total DNA (Kang *et al*. 1985).

Hence, strains exhibiting rapid muscle growth should have been supplied with high dietary AA needs for muscle accretion. Thus, HLys-DAA had significantly (P<0.05) higher in carcass, breast and thigh weights than those of the other groups, in this study.

The diet formulated based on AA and lysine levels did not affect protein and lipid contents of breast and thigh muscles.

The overall Lys content in carcass of broilers increased as the chicks aged because of relative increase of breast meat percentage (Tables 3 and 4).

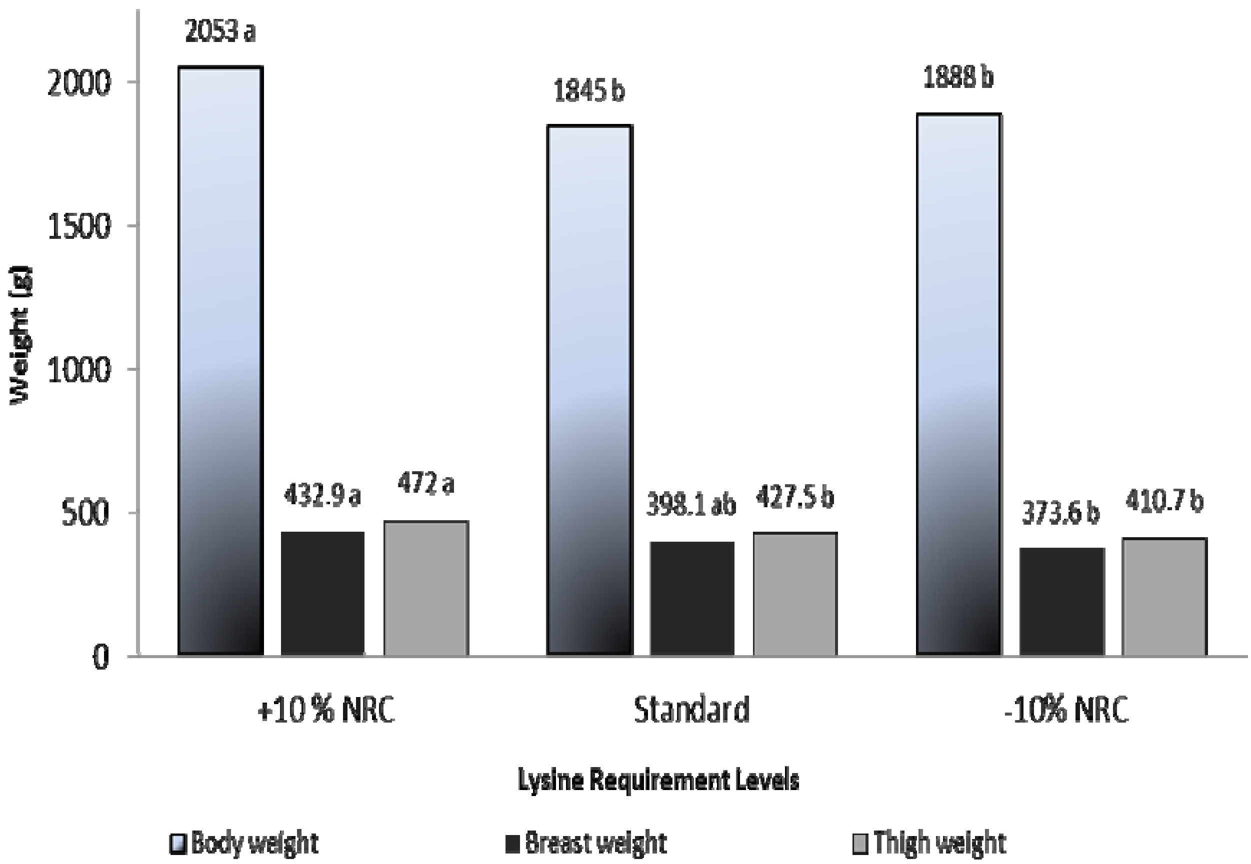

Figure 2 Effects of lysine requirement levels (% of NRC recommendation) on mean body, breast and thigh weight (at 42 day)

The amino acid requirements for different genetic broiler strains will be partially dependent upon the amino acid content of each body components (i.e. breast, thigh and drum) and the extent of changes of the percentages of carcass components.

Nevertheless, the Lys contents of the breast and thigh muscles were 4.742% and 4.133% respectively, which were lower than those of Ross Strain.

CONCLUSION

1- Feeding broilers with HLys diets (110% NRC) increases carcass and breast percentage by 4.4% and 1.81%, the levels was higher than the broilers received LLys diets.

2- HLys-DAA positively affects the carcass and breast muscle percentages of broilers. The interaction between DAA and HLys allows Arian broilers to full express the genetic potential for growth. Formulating broiler diets based on DAA gives a better prediction of dietary protein quality and bird performance than total amino acids.

3- Feeding broilers with high Lys containing diets (110% NRC) significantly increases amount of Lys in breast and thigh meats.

ACKNOWLEDGEMENT

This research was partially supported by Saveh Branch, Islamic Azad University. We thank our colleagues from Saveh Branch, Islamic Azad University who provided insight and expertise that greatly assisted the research.

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