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Effect of Dietary Lysine Regimens on Growth Performance and Meat Composition in Aseel Chicken

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ABSTRACT

A study was designed with the objective, to evaluate the effect of dietary lysine (Lys) regimens on growth performance and meat composition of Aseel chicken. In total, 540 day old chicks, 180 from each variety, were randomly assigned to 9 experimental groups in a 3 (Varieties: Mianwali (MW), Peshawari (PW), and Lakha (LK)) × 3 (Lys regimens: L1, L2 and L3:1.35, 1.30 and 1.25%) factorial arrangement under randomized complete block design (RCBD) with sex as block. Each experimental group was replicated 6 times with 10 birds in each with average weight of 29 gram. Feed intake (FI), weight gain (WG) and feed:gain ratio (F:G) parameters of growth performance and Dry Matter (DM), Ash, Crude Protein (CP) and Ether Extract (EE) parameters of meat composition were evaluated. The results indicated better (p=0.0006) WG and (p=0.0006) F:G was observed in MW verities. Among different Lys regimens, higher and medium level in the diet improved WG (p<.0001), F:G (p<.0001) and reduced (p=0.0001) FI. Similarly increased (p<.0001; 0.0150) ash content in thigh and breast due to increased level of lysine in the early life period. Dry matter was found to be higher (p=0.0036) only in medium Lys regimen, whereas meat CP was observed to be higher (p=0.0064) in control diet. It was concluded that, 1.30% digestible Lys level regimen can be used to improve the early growth rate of Aseel chicken. Similarly, Mianwali variety due to its better early growth can be used as a meat type chicken.

INTRODUCTION

Aseel is the oldest Asian game fowl having superior meat quality. The pugnacity, upright posture, firm muscular thighs with strong legs (Usman *et al.* 2014) and heavy body weight (Jatoi *et al.* 2014) are well-known characteristics of the Aseel. With large body size Aseel has body weight (BW) range of three to five kg; probably the reason why Aseel was used in the breeding plan of Cornish breed grand parent of modern broiler (Usman *et al.* 2014). On the basis of its body weight, sturdiness, diseases resistance, adaptability, survival in harsh climatic conditions and excellent meat producing qualities the Aseel can be used as a meat-type bird (Khan *et al.* 2016). It is speculated that an improved Aseel chicken may help in the revival of family poultry and can bring the small poultry farmers back into business, helping in poverty alleviation. The main hurdle in the use of Aseel as a meat type bird, however, is it's poor early growth rate and F:G. Different techniques including genetic selection, managemental related and nutritional manipulations can be applied to improve the early poor growth of Aseel chicken (Rehman *et al.* 2017).

The concept of providing individual amino acids for proper growth is not new. Specific amount of individual amino acids is necessary for proper growth performance (Baker & Han, 1994; Ayasan & Okan, 2014).

Lysine, the basic building block for protein synthesis, is involved directly or indirectly in the regulation of the protein synthesis (Dozier *et al.* 2007). Lysine is critical and accounts for 7% of the protein in breast meat (Eits *et al.* 2003; Dozier *et al.* 2007). Dietary Lys is important for maintenance and skeletal muscle accretion (Dozier *et al.* 2007; Ayasan & Okan, 2010). Supplementation of Lys during early growth stages (Ayasan & Okan, 2006; Campestrini *et al.* 2010) is beneficial because it involves the regulation of the protein synthesis and increases muscle growth (Eits *et al.* 2003), improve weight gain and feed efficiency (Quentin *et al.* 2003; Si *et al.* 2004). Chickens grow faster at a relatively high dietary Lys concentration in the diet compared with diet deficient in Lys (Li *et al.* 2013). Deficiency of Lys in the diet results in decreased protein synthesis and accretion (Liao *et al.* 2015). Keeping in view all above discussion, it was hypothesized that balanced diet supplemented with Lys may ameliorate the early poor growth rate of indigenous Aseel chicken. The present study was, therefore, planned to evaluate the effect of different Lys regimens on growth performance and meat composition in different varieties of indigenous Aseel chicken.

MATERIALS AND METHODS

Ethical note

The experimental procedures were in accordance with the guidelines and code of practice of the University of Veterinary and Animal Sciences Lahore, Lahore. Ethical approval was granted before the conduct of the study via protocol number UVAS-DAS-4901.

Housing, Experimental Birds and Diets

The study was conducted at the Indigenous Chicken Genetic Resource Center, Ravi Campus, University of Veterinary and Animal Sciences Lahore. In total, 540 day old chicks, 180 from each variety, were randomly assigned to 9 experimental groups in a 3 (Varieties: Mianwali (MW), Peshawari (PW), and Lakha (LK)) × 3 (Lys regimens: L1, L2 and L3:1.35, 1.30 and 1.25%) factorial arrangement under randomized complete block design (RCBD) with sex as block. Each experimental group was replicated 6 (three for male and female each) times with 10 birds in each, the experiment was conducted in multi deck cage houses with stocking density of 0.2-0.4 ft^2 /bird. House preparation for the experiment was done by cleaning and washing of the equipments, rinsing with potassium per magnate ($KMnO_4$) and sun drying. Fumigation (35ml formalin + 17.5g $KMnO_4$ = 1X concentration) of the house was completed before one week of the chicks arrival. Management conditions like temperature, relative humidity and lighting schedule were maintained according to intensive broiler rearing. Water and feed were provided *ad-libitum* through trough feeders and nipple drinking system.

Nine different diets with variable Lys levels formulated on the bases of ideal amino acid concept were used. The diets were formulated in such a way that the average allowance of Lys (1.20%) in all treatments were the same. All diets were iso-nitrogenous and iso-caloric (Table 1). Before the start of the trial, experimental diets were analyzed for dry matter (DM), crude protein (CP), crude fiber, ether extract (EE), ash, calcium and phosphorus by following AOAC (2005) while, amino acid analysis was executed using Biochrome 30+ Series amino acid analyzer resulting in ±2% from the calculated values and metabolizable energy was calculated through regression equation as described by NRC (1994). Linear formulation method was used for the diet's formulation. Experimental diets were offered for the duration of 6 wk.

Parameters

Cumulative feed intake was determined by combining the weekly feed intake (FI). Initial and final body weights (FBW) were recorded to calculate the WG, feed:gain ratio (F:G) and Lys efficiency ratio (LER). In total, 162 birds, three from each replicate with average weight were picked, kept off feed for six hours before slaughtering. Thereafter, birds were slaughtered to collect meat samples from the thigh and breast portion of the carcass. Proximate analysis (DM, Ash, CP and EE) of the meat samples was performed using standard methods, DM was determined through hot air oven at 80 C° for 48 hours (Haunshi *et al.* 2012), CP by Kjeldahl method (AOAC 1999). Ether extract was determined by the Soxhlet apparatus method (AOAC 1999) and ash was determined by complete burning of the sample using furnace with heating temperature of 600°C (AOAC 1999).

Statistical Analysis

Before analysis, data were checked for uniformity and homogeneity of variance and verified for the normality. After that, the data were analyzed through ANOVA technique in factorial arrangement under RCBD by using GLM procedure of SAS assuming the following mathematical model:

$$Yijk = \mu + \beta i + Vj + Lk + (VL)jk + \varepsilon ijk$$

Table 1 – Ingredients and nutrients composition of diets*

Ingredients (%)	1 to 14 day			15 to 28 day			29 to 42 day		
	L1	L2	L3	L1	L2	L3	L1	L2	L3
Corn	61.97	62.44	62.66	65.78	64.03	65.78	71.9	61.98	61.45
Soybean Meal	23.05	19.94	21.97	20.20	23.72	20.20	13.7	25.82	26.59
Fish meal	8.00	11.13	9.04	6.09	5.46	6.09	6.34	0.69	0.66
Corn Gluten 60%	3.00	3.00	3.00	3.00	2.00	3.00	3.00	3.00	3.00
$CaCO_3$	0.97	0.97	0.99	0.95	0.80	0.95	0.96	0.80	0.80
DCP	0.20	0.20	-	-	0.10	-	-	1.00	1.00
Common Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
$NaHCO_3$	0.20	0.34	0.20	0.24	0.26	0.24	0.24	0.34	0.34
Vegetable Oil	0.20	-	-	1.26	1.94	1.26	2.3	4.29	4.47
Vit-Min Premix	0.50	0.50	0.50	0.50	0.50	0.50	0.5	0.50	0.50
Lysine Sulphate	0.61	0.46	0.40	0.67	0.37	0.67	0.47	0.58	0.45
DL-Methionine	0.35	0.29	0.26	0.34	0.26	0.34	0.21	0.32	0.27
L-Threonine	0.25	0.21	0.18	0.23	0.14	0.23	0.13	0.17	0.13
L-Valine	0.09	0.03	0.38	0.11	0.01	0.11	0.03	0.08	0.03
L-Isoleucine	0.11	0.04	0.01	0.10	0.00	0.10	0.05	0.05	0.01
L-Tryptophan	-	-	-	0.01	-	0.01	-	-	-
L-Arginine	0.19	0.16	0.10	0.23	0.11	0.23	0.14	0.11	0.03
Nutrients[1]									
CP (%)	23	23	23	21	21	21	20	20	20
Metabolizable Energy (Kcal/Kg)	3000	3000	3000	3100	3100	3100	3200	3200	3200
Ca	1.00	1.00	1.00	0.80	0.80	0.80	0.80	0.80	0.80
Ava. P	0.40	0.40	0.40	0.30	0.30	0.30	0.30	0.30	0.30
Dig. Lysine	1.35	1.30	1.25	1.20	1.15	1.25	1.05	1.15	1.10
Dig. TSAA	1.02	0.98	0.94	0.95	0.88	0.95	0.77	0.89	0.85
Dig. Threonine	0.95	0.91	0.88	0.86	0.79	0.86	0.68	0.78	0.75
Dig. Tryptophan	0.22	0.21	0.20	0.20	0.19	0.20	0.16	0.18	0.18
Dig. Arginine	1.42	1.37	1.32	1.33	1.22	1.33	1.07	1.23	1.18
Dig. Valine	1.02	0.98	0.94	0.95	0.88	0.95	0.77	0.89	0.85
Dig. Isoleucine	0.89	0.86	0.83	0.84	0.77	0.84	0.68	0.78	0.75
Dig. Phenylalanine	0.85	0.82	0.79	0.79	0.72	0.79	0.63	0.72	0.69

*L1=High lysine regimen, L2=Medium lysine regimen and L3=Low lysine regimen 1 Analyzed amino acids is in the range of ±2% of the calculated values

Where, Y_{ijk} is Dependent variables, μ is overall population mean, βi are the blocks that is sex, V_j is effect of i[th] treatment (i=3; Varieties), L_k is effect of j[th] treatment (j=3; Lysine Regimens), $(VL)_{ij}$ is interaction effect and ε_{ijk} is residual error. Aseel variety and Lys regimens were taken as main effects. The interaction of these were also tested. Treatment means were compared through Tukeys HSD test at 5% probability level. Each replicate was considered as an experimental unit.

RESULTS AND DISCUSSIONS

Feed Intake, Weight Gain and Feed:Gain Ratio

Different Lys regimens separately (p=0.0001) and in interaction with different Aseel varieties (p=0.0496) showed variations in feed intake, whereas Aseel varieties independently (p=0.1005) could not show their influence on feed intake. Reduced FI was observed in higher and medium Lys regimens. Reduced FI in higher and medium Lys regimen birds may be attributed to the fact that more amino acid dense diets in less quantity might have fulfilled nutrients requirement of the birds and made them satiated. Similar results were reported in other studies also, where lower FI due to high amino acid dense diets was observed in broilers (Sklan & Plavnik, 2002; Corzo *et al.* 2005). Likewise, supplementation of branched chain amino acids (valine, leucine and isoleucine) resulted in decreased FI in animals (Trottier & Easter, 1995).

All treatments including Aseel varieties (p=0.0002), Lys regimens (p<.0001) and their interaction (p=0.0034) indicated marked difference in weight gain. High and medium level of Lys regimens demonstrated higher weight gain compared to low level regimen. Among different varieties, (p=0.0006) birds of MW variety showed enhanced weight gain followed by those of PW and LK. Feed:gain ratio showed marked variations (p<.0001) with respect to Lys regimens, Aseel varieties (*p*=0.0006), and their interaction (*p*=0.0026). Higher and medium Lys regimens resulted in improved F:G than low level of Lys. Birds of MW variety exhibited better F:G than those of LK and PW. Lysine efficiency showed similar trend to that of F:G in dietary treatments (*p*<.0001) and varieties (*p*=0.0007). Interaction of dietary treatments and varieties showed differences (*p*=0.0496, 0.0034, 0.0026 and 0.009) in FI, WG, F:G and LER, where higher FI was found in birds of all varieties fed low Lys diets. Weight gain was higher in medium Lys diet fed birds of MW variety, F:G and LER were better in all varieties fed high and medium Lys diet. Phase wise WG and F:G showed linear response (figure 1).

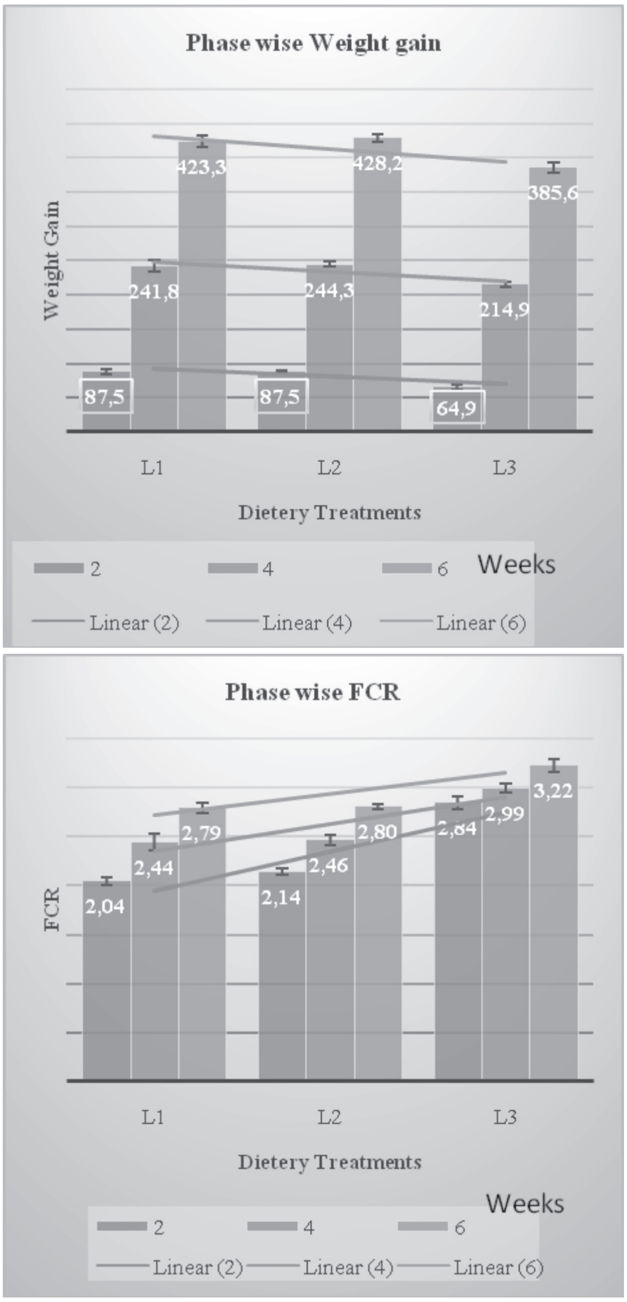

Figure 1 – Phase wise weight gain and feed conversion ratio in varying lysine diets

Higher and medium level Lys regimens in the diets of Aseel chicken improved WG and F:G. It is quite possible that the supplementation of higher and medium level of Lys as compared to lower level might have promoted muscle accretion in early two weeks of growth, resulting in increased final weight gain. It is reported that supplementation of Lys during early growth stages (Campestrini *et al.* 2010) is beneficial. It involves the regulation of the protein synthesis and increases muscle accretion (Dozier *et al.* 2007; Eits *et al.* 2003). Hence, chickens grow faster at a relatively high dietary Lys concentration in the diet compared with diet having less Lys (Li *et al.* 2013). Moreover, increased weight gain in higher and medium Lys diets may also be attributed to its distinct characteristic of increasing villous height in jejunum and ileum with increasing Lys levels (Vaezi *et al.* 2011; Wang *et al.* 2009) and increasing crypt depth in duodenum and jejunum (Franco *et al.* 2006) ultimately accelerating early growth through improved nutrient digestion and uptake.

Similar to these findings, increment in Lys over and above NRC (1994) recommendation was reported to improve WG and F:G (Si *et al.* 2004). It was reported that meat type birds performed better with increasing amino acids densities (Zhai *et al.* 2013, 2014). Panda *et al.* (2011) fed broiler 1.3% Lys with a specific ratio to other amino acid and, observed better results, indicating that concentrated diets can improve F:G in broiler (Quentin *et al.* 2003). Moreover, improved F:G in high and medium Lys groups may be attributed to the higher WG and lower FI in the same groups. On the other hand, decreased WG diet may be explained by the fact that inadequate Lys supply depresses the immune system of the birds (Geraert & Mercier2010) showing adverse effects on livability and growth performance (Liao *et al.* 2015). Similarly, it was reported that depressed growth in Lys deficient diets may be due to decreased protein synthesis and accretion (Tesseraud *et al.* 1996).

The observed difference in growth performance between varieties may be the result of genetic diversity between the breeds and varieties (Leeson *et al.* 1997). Similarly, differences in WG and F:G were observed in different varieties of Aseel by Jatoi *et al.* (2014).

Mortality percentage also differed significantly among different (*p*=0.028) dietary treatments, where higher mortality was found in groups fed low Lys diets (Table 2). Higher and medium Lys regimens in the diets showed reduced mortality. Higher mortality in low Lys diet may be attributed to the fact that inadequate Lys

Table 2 – Growth performance, lysine efficiency ratio and mortality at six weeks of age

Effects		Parameters[§]				
		FI	WG	F:G	LER	Mortality
Dietary Treatments[¶]						
L1		1175.8±3.7^{c}	423.3±8.2^{a}	2.79±0.05^{b}	27.97±0.58^{a}	0.56± 0.56^{b}
L2		1198.0±5.4^{b}	428.2±4.9^{a}	2.80±0.03^{b}	27.80±0.30^{a}	1.12± 0.76^{b}
L3		1232.9±4.5^{a}	385.6±7.2^{b}	3.22±0.06^{a}	24.15±0.46^{b}	3.89± 1.18^{a}
Varieties[‡]						
LK		1199.3±7.2	401.5±9.3^{c}	3.02±0.08^{a}	25.99±0.74^{b}	1.12± 0.76
MW		1198.5±7.5	424.1±8.1^{a}	2.84±0.05^{b}	27.51±0.56^{a}	1.67± 0.91
PW		1208.9±7.1	411.5±6.3^{b}	2.95±0.05^{a}	26.43±0.49^{b}	2.78± 1.09
Dietary Treatments × Varieties						
L1	LK	1174.0±5.62^{b}	394.7±15.3^{ab}	3.0±0.12^{d}	28.02±1.12^{a}	1.12
	MW	1176.7±5.43^{b}	396.0±18.7^{ab}	3.0±0.14^{d}	28.04±1.28^{a}	0.56
	PW	1177.0±8.67^{b}	393.4±9.34^{ab}	3.0±0.08^{d}	27.86±0.69^{a}	2.78
L2	LK	1191.6±6.09^{b}	393.0±4.04^{ab}	3.03±0.03^{d}	27.49±0.25^{a}	1.12
	MW	1184.4±9.86^{b}	406.2±12.5^{a}	2.93±0.07^{d}	28.56±0.71^{a}	2.78
	PW	1218.0±6.21^{a}	400.0±7.12^{ab}	3.05±0.05^{d}	27.36±0.42^{a}	3.89
L3	LK	1232.4±9.45^{a}	332.2±10.5^{d}	3.73±0.11^{a}	22.47±0.72^{d}	2.78
	MW	1234.4±6.62^{a}	384.2±10.0^{b}	3.22±0.07^{c}	25.39±0.57^{b}	2.78
	PW	1232.0±8.14^{a}	355.8±6.58^{c}	3.47±0.05^{b}	24.06±0.34^{c}	3.89
p-value						
DT[*]		<.0001	<.0001	<.0001	<.0001	0.028
VA[†]		0.1005	0.0002	0.0006	0.0007	0.4238
DT×VA		0.0496	0.0034	0.0026	0.009	0.9727

[*]DT, Dietary Treatments [†]VA= Varieties [‡]LK=Lakha, MW=Mainwali, PW=Pashwari [¶]L1=High lysine regimen, L2=Medium lysine regimen and L3=Low lysine regimen [§]FI=feed intake, BWG=body weight gain, F:G= feed:gain ratio, LER= lysine efficiency ratio

Value with different superscript within column differ significantly ($p>0.05$)

supply may depresses immune system of the birds (Geraert & Mercier 2010), as deficiency of dietary Lys leads to increased susceptibility of birds to diseases (Datta *et al.* 2001; Li *et al.* 2007). The reason behind is because the fact that Lys integral component for protein synthesis, any deficiency of Lys can limits the immune related protein synthesis includes antibodies and cytokines. Chen *et al.* (2003) reported that Lys deficiency negatively affects antibody response f and cell mediated immunity, also showing adverse effects on growth performance (Liao *et al.* 2015). Similar to the current study, Mushtaq *et al.* (2015) fed higher Lys level to the birds and observed reduced mortality, concluding that higher Lys levels in the diets improves livability.

Meat Composition

Birds fed with medium level Lys regimens showed higher (p=0.0036) percentage of DM content in thigh whereas DM content in breast remained unchanged (p=0.1728). Varieties independently indicated similar pattern in DM contents in both thigh and breast (p=0.6635; 0.0786). Birds fed with higher Lys demonstrated increased (p<.0001; 0.0150) ash contents in both thigh and breast. Among different varieties, birds of PW variety indicated higher (p=0.0002) ash content in thigh whereas ash content of breast was found to higher (p<.0001) in LK variety of Aseel. Birds fed with lower Lys indicated higher (p=0.0064) CP content in thigh whereas CP remained unchanged (p=0.7139) in breast. Moreover, Aseel varieties remained unresponsive (p=0.7447; 0.3339) regarding CP in both thigh and breast. All treatments separately and in interaction did not show any pronounced (p=0.6340; 0.3272; 0.9806 and 0.1159) effect on EE in both thigh and breast meat samples (Table 3). Findings of the current study confirm that different dietary Lys regimens affect meat composition (DM, Ash, CP and EE) of indigenous Aseel chicken that might be the result of incremental levels of amino acids modifying the muscle growth by increasing myofiber size (Tesseraud *et al.* 1996). Lower Lys level during early growth stages in the present study did not show adverse effect on proximate meat composition. However, opposite to present study it was observed that amino acid deficiency can lead to protein decrease and crude fat increase in meat (Corzo *et al.* 2005; Lilly *et al.* 2011).

Among different varieties, ash content in thigh was found to be greater in birds of PW variety, whereas

Table 3 – Effect of dietary amino acid regimens on meat composition traits of Aseel varieties

Variables*	Thigh				Breast			
	DM	Ash	CP	EE	DM	Ash	CP	EE
Dietary Treatments								
L1	73.21±0.34[ab]	1.40±0.04[a]	22.32±0.22[ab]	0.63±0.02	71.62±0.21	1.42±0.04[a]	24.26±0.15	0.51±0.02
L2	73.80±0.17[a]	1.23±0.03[b]	21.97±0.17[b]	0.62±0.04	71.60±0.25	1.36±0.04[ab]	24.49±0.19	0.55±0.04
L3	72.72±0.19[b]	1.23±0.04[b]	22.57±0.17[a]	0.66±0.04	71.22±0.26	1.30±0.04[b]	23.66±1.26	0.52±0.03
Varieties								
LK	73.15±0.30	1.26±0.04[b]	22.33±0.20	0.63±0.03	71.79±0.25	1.47±0.03[a]	24.15±0.19	0.49±0.02
MW	73.19±0.29	1.23±0.03[b]	22.32±0.22	0.64±0.04	71.38±0.23	1.30±0.03[b]	23.35±1.24	0.52±0.04
PW	73.40±0.21	1.37±0.04[a]	22.21±0.16	0.63±0.04	71.27±0.23	1.31±0.05[b]	24.90±0.18	0.56±0.03
p-value								
DT	0.0036	<.0001	0.0064	0.6340	0.1728	0.0150	0.7139	0.3272
VA	0.6635	0.0002	0.7447	0.9806	0.0786	<.0001	0.3339	0.1159
VA x DT	0.3528	<.0001	0.0005	0.4449	0.0025	<.0001	0.6189	0.1190

*VA= Varieties, DT, Dietary Treatments, LK=Lakha, MW=Mainwali, PW=Pashwari, L1=High lysine regimen, L2=Medium lysine regimen and L3=Low lysine regimen, DM= Dry Matter C*p*=Crude Protein EE= Ether Extract

Value with different superscript within column differ significantly ($p>0.05$)

in breast it was found to be higher in LK variety of Aseel. However, DM, CP and EE contents in both thigh and breast remained unchanged among all varieties. Greater ash content in PW and LK might be due to their genetic effect. Similarly, differences in ash content among different genotypes of poultry have already been reported as Wattanachant *et al.* (2004) observed that Thai Indigenous chicken meat contained lower percentages of ash contents than those of commercial broiler, indicating that ash content varies with genotype (Wattanachant 2008; Sogunle *et al.* 2010) and variety (Okarini *et al.* 2013). Samooel *et al.* (2015) observed significantly higher ash content in meat proximate composition of yellow-brown plumage color birds.

In the current study, no variety effect on DM, CP and EE was observed. In contrast, difference in DM (Tougan *et al.* 2013), CP (Wattanachant 2008) and EE (Samooel 2015) was reported among different genotypes as well as phenotypes of poultry, indicating these parameters of meat composition may vary from breed to breed (Mohan *et al.* 2008; Okarini *et al.* 2013), strain to strain (Sogunle *et al.* 2010) and genotypes to genotypes (Liu *et al.* 2012). Higher ash contents in the present study was reported in indigenous chicken meat (2%) (Ogunmola *et al.* 2013), while lower ash contents (0.95±0.5%) by (Tougan *et al.* 2013; Chepkemoi *et al.* 2017). Whereas protein content are higher than those presented in the literature for indigenous chicken meat 18.15% by (Chepkemoi *et al.* 2017), 20.5±0.5 (Tougan *et al.* 2013). Moreover these variations in nutritional contents can be attributed to variation in the breed, moreover to feed, sex, production system, processing, age at slaughter, and the part of the cut (Haunshi *et al.* 2010).

From the findings it can be concluded that 1.30% digestible Lys level regimen with ideal amino acid ratio during early weeks of growth can be used to improve early growth of native Aseel chicken. Furthermore, Mianwali variety due to its better early growth performance can be used in future breeding plans for the development of a meat type breed.

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