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RESEARCH ARTICLE

Impact of Different Dietary Lysine Regimens on Blood Biochemical Profile and Immune Response in Indigenous Aseel Varieties

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ABSTRACT

This study was planned to evaluate the effect of different dietary lysine regimens on blood biochemistry and immune response in Lakha, Mianwali, Mushki and Peshawari varieties of indigenous Aseel chicken. One-day-old chicks (n=240), 60 from each of the variety were randomly selected, sub-divided equally into three groups (A, B and C) and offered three dietary lysine regimens (L1, L2 and L3). L1 (1.3% lysine) was offered to group A from 0-6th week and L2 (1.4-1.2% lysine), wherein 1.4% lysine from 0-3rd week and 1.2% lysine from 4-6th week was offered to group B. In L3 (1.5-1.3-1.1% lysine), 1.5, 1.3 and 1.1% lysine from 0-2nd, 3-4th and 5-6th week, respectively, was offered to group C. These birds were kept under RCBD having factorial arrangement of 3 (lysine regimens) × 4 (varieties) × 20 (replicates) with one bird in each replicate. After six weeks, 72 birds including 18 from each variety were selected to collect the blood samples for analysis of blood biochemicals and immune response. The collected data were analyzed by ANOVA and treatment means were compared by DMR test. The findings of this study showed that birds fed on L3 lysine regimen had significantly higher (P≤0.05) serum glucose, total protein, globulin, high density lipoprotein, triglycerides and antibody titer against Newcastle disease virus (NDV) and Infectious bronchitis virus (IBV). While, Lakha and Peshawari varieties presented an overall improved picture of their blood biochemical profile and immune response against NDV and IBV.

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INTRODUCTION

Under the impact of growing population of animals, there always exist increased chances of diseases which manifest such a wide range of symptoms that mere physical analysis insufficient for diagnosis. However, the altered blood components might indicate the physiological status in a true sense (Szabo *et al*., 2005). For example, when integrity of the cell membrane is lost under the impact of hypoxia or trauma, the enzymes leak out into extracellular fluid which may be an indicative of the degree of tissue lesion. Similarly, the quality of dietary protein can be determined through serum total protein (Alikwe *et al*., 2010; Bhatti *et al*., 2015), glucose and triglycerides that provide the information about the availability of energy required to maintain the physiology of the body. Hematological and biochemical analyses are very important to diagnose the immune status and genetic distinctiveness (Kral and Suchy, 2000). Moreover, blood biochemical profiling will also be very helpful to provide baseline information about the required factors or genes i.e., genes for disease resistance to be improved and used in management as well as breeding programs for the conservation of concerned breeds (Sonaiya, 2007). Blood biochemical profiling can help monitor bird's health and physiological status and is also interlinked with many variables like sex, age, breed, feeding routine, management and anxiety level (Lloyd and Gibson, 2006). Such analyses have been discussed in a number of studies with respect to domestic animals, but are very rare with respect to broiler chicken and even for Aseel chicken. Domestic chicken presents the most practical model of avian species for immune response towards bacterial and viral infections which provide a plenty of knowledge to understand the bird's performance (Haunshi *et al*., 2011). Aseel is well known native chicken breed of Indo-Pak

region. The supremacy of Aseel on other native breeds is its largest body size, strong physique, stamina, hardiness, resistance against diseases, low mortality and conserved gene pool (Usman *et al*., 2014). The exotic and commercial strains were mainly focused to import, flourish and are still being given more consideration, while, the native gold mines of genome are going to be extinct even before their recognition (Jatoi *et al*., 2014). The conservation and the genetic improvement of the local breeds under local conditions are inevitable without the availability of adequate information and data regarding performance and genetic distinctiveness. As there is inadequacy of proper reference ranges with respect to Aseel, however, the data accessible is based upon restricted numbers, parameters and frequently out-of-date analytical methods regarding the blood biochemistry of Aseel chicken. The present study was planned to investigate the effect of different dietary lysine regimens on blood biochemical profile in different varieties of indigenous Aseel chicken.

MATERIALS AND METHODS

The present experiment was conducted at Indigenous Chicken Genetic Resource Center (ICGRC), UVAS, Lahore, Pakistan, for a period of six weeks duration and standard instructions for the care and welfare of the experimental birds were followed. Two hundred and forty, day-old chicks including 60 from each of the four varieties i.e., Lakha, Mianwali, Mushki and Peshawari, were arbitrarily selected and divided into three sub-groups A, B and C, with 20 birds in each. These birds offered three lysine regimens i.e., L1 from 0-6th week in one phase to group A, and L2, wherein 1.4% lysine was offered from 0-3rd week and 1.2% lysine from 4-6th week in two phases to group B. Further, L3 lysine regimen having 1.5, 1.3, 1.1% lysine was offered from 0-2nd, 3-4th and 5-6th week, respectively, in three phases to group C. The composition of lysine regimens is mentioned in Table 1. These experimental birds were kept in an independent open sided poultry house equipped with 3-tiered battery cages having removable dropping trays, trough feeders and nipple drinking system. The birds were placed under RCBD with a factorial arrangement of 3 (dietary lysine regimens) × 4 (Aseel chicken varieties) × 20 (replicates) with one bird in each replicate. At the end of sixth week, 72 birds including 18 birds from each of the four Aseel varieties with 6 birds from each treatment group were arbitrarily picked and 3mL of blood sample/bird was collected through brachial vein in disposable syringes of 5mL. Serum from each blood sample was separated out and preserved at -20^{o}C till biochemical analyses of glucose (GLU), Alkaline phosphate (ALP), total proteins (TP) and fractions [albumin (ALB) and globulin (GLO)], total lipids and fractions [cholesterol (CH), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL)], liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine phosphokinase (CPK), creatinine kinase CK MB (myocardial/brain) and creatinine (CR), urea (UR), uric acid (UA) for renal function tests, using commercially available kits and semi-automated chemistry analyzer (Model 5010, Robert Riele GmbH & amp; Co KG. D-13467 Berlin, Germany).

For sampling of titer against ND and IB, all the birds were vaccinated intra-ocularly against ND and IB one week prior to the sampling (La Sota and H 120 viral strains were used as antigens to titrate antibodies against NDV and IBV, respectively). Hemagglutination inhibition (HI) technique was implemented to determine the antibody response for NDV (Beard, 1989), while, Enzyme-linked Immunosorbent antibody assay (ELISA) was employed for IBV (Munir *et al*., 2012) using commercially available kits. The antigens of NDV and IBV were obtained from University Diagnostic Laboratory, UVAS, Lahore, Pakistan. The expression of HI titers was made as log2 of the reciprocal of highest dilution of serum which resulted into HA inhibition. The collected data after being observed for level of standardization and normality were exposed to two-way ANOVA and General Linear Model (GLM) of SAS (SAS Institute Inc., 2002–03) software under randomized complete block design. Dietary lysine regimens and Aseel varieties and their interactions were taken as main variables and results were expressed as means and their standard errors. The treatment means were compared by Duncan's Multiple Range (DMR) test (SAS Institute Inc., 2002–03) and differences among treatment means were considered as significant at P≤0.05.

RESULTS

Lysine supplementation in 3-phased (L3) and 2-phased (L2) dietary regimens characterized a noteworthy (P≤0.05) response concerning the blood biochemical profile of Aseel. Table 2, 3 and 4 represent mean values for different serum biochemical parameters, whereas, Table 5 and 6 display the enzymatic activities of liver enzymes and immune response against NDV, IBV, respectively, as affected by lysine regimens, Aseel varieties and their interactions.

GLU: Significantly (P≤0.05) higher GLU (214.08±9.15 mg/dL) was found in L3 than other lysine regimens and among varieties, Peshawari depicted higher GLU (210.11±14.87 mg/dL) than other varieties. However, in interactions among lysine regimens and Aseel varieties, Peshawari showed higher GLU (284.33±8.71 mg/dL) with L3 (Table 2).

Table 1: Composition of experimental diets for Aseel birds

Ingredients	Dietary lysine levels (%)				
	1.1	1.2	1.3	1.4	1.5
Corn	59.08	59.08	59.08	59.08	59.08
Sunflower Meal (24%)	18.9	18.9	18.9	18.9	18.9
Soya bean Meal (44%)	7.04	7.04	7.04	7.04	7.04
Rapeseed Meal	3	3	3	3	3
Fish Meal (52%)	3	3	3	3	3
Poultry by-product Meal	3	3	3	3	3
Molasses	3	3	3	3	3
Limestone	1.14	1.14	1.14	1.14	1.14
Lysine Sulphate	0.7	0.9	1.1	1.3	1.5
Mono Calcium Phosphate	0.45	0.45	0.45	0.45	0.45
Vitamin-Mineral Premix	0.2	0.2	0.2	0.2	0.2
Sodium Chloride	0.18	0.18	0.18	0.18	0.18
Alimet (Novus)	0.17	0.17	0.17	0.17	0.17
Betaine HCl	0.05	0.05	0.05	0.05	0.05
Threonine	0.04	0.04	0.04	0.04	0.04

Table 2: Effect of lysine regimens, Aseel varieties and their interactions on glucose, total proteins and fractions in indigenous Aseel varieties at 6th week of age

Parameters / Variables		Glucose (mg/dL)	Total Proteins (g/dL)	Globulin (g/dL)	Albumin (g/dL)
Lysine Regimens					
1.3% (L1)		174.33±3.97^{b}	3.50±0.10^{b}	2.09±0.10^{b}	1.41±0.03^{a}
1.4-1.2% (L2)		171.21±5.69^{b}	3.96±0.09^{b}	2.65±0.10^{a}	1.31±0.02^{b}
1.5-1.3- 1.1% (L3)		214.08±9.15^{a}	4.13±0.16^{a}	2.93±0.15^{a}	1.19±0.03^{c}
Verities					
Lakha		172.83±3.82^{b}	3.72±0.16	2.53±0.15	1.19±0.03^{b}
Mianwali		171.83±4.13^{b}	3.94±0.15	2.56±0.17	1.38±0.04^{a}
Mushki		191.39±4.10^{ab}	3.94±0.13	2.61±0.14	1.33±0.03^{a}
Peshawari		210.11±14.87^{a}	3.83±0.19	2.52±0.20	1.31±0.03^{a}
Lysine Regimens × Varieties					
1.3% (L1)	Lakha	164.83±1.35^{de}	3.33±0.21^{b}	2.10±0.19^{cd}	1.23±0.04^{cd}
	Mianwali	149.17±0.65^{e}	3.67±0.21^{ab}	2.10±0.23^{cd}	1.57±0.04^{a}
	Mushki	187.17±1.35^{cd}	3.67±0.21^{ab}	2.23±0.22^{bcd}	1.43±0.02^{b}
	Peshawari	196.17±3.54^{bc}	3.33±0.21^{b}	1.92±0.24^{d}	1.42±0.06^{b}
1.4-1.2% (L2)	Lakha	176.33±11.09^{cd}	4.00±0.00^{ab}	2.73±0.03^{abcd}	1.27±0.03^{cd}
	Mianwali	182.50±4.12^{cd}	4.00±0.26^{ab}	2.68±0.27^{abcd}	1.32±0.03^{bcd}
	Mushki	176.17±5.38^{cd}	4.00±0.00^{ab}	2.65±0.02^{abcd}	1.35±0.02^{bc}
	Peshawari	149.83±17.34^{e}	3.83±0.31^{ab}	2.52±0.30^{abcd}	1.32±0.04^{bcd}
1.5-1.3-1.1% (L3)	Lakha	177.33±2.16^{cd}	3.83±0.40^{ab}	2.75±0.36^{abcd}	1.08±0.06^{e}
	Mianwali	183.83±1.40^{cd}	4.17±0.31^{ab}	2.90±0.31^{abc}	1.27±0.03^{cd}
	Mushki	210.83±3.94^{b}	4.17±0.31^{ab}	2.95±0.31^{ab}	1.22±0.05^{cd}
	Peshawari	284.33±8.71^{a}	4.33±0.33^{a}	3.13±0.31^{a}	1.20±0.04^{d}

Values have been mentioned as Mean±SEM and various superscripted alphabets show significant (P≤0.05) differences among them (order of significance is as: a>b>c>d…).

Table 3: Effect of lysine regimens, Aseel varieties and their interactions on various blood biochemicals (mg/dL) in indigenous Aseel varieties

Parameters / Variables		Creatinine	Urea	Uric Acid
Lysine Regimens				
1.3 %(L1)		0.40±0.01^{a}	4.77±0.06^{a}	3.56±0.04^{a}
1.4-1.2% (L2)		0.36±0.01^{ab}	4.60±0.05^{b}	3.34±0.03^{b}
1.5-1.3-1.1% (L3)		0.35±0.02^{b}	4.56±0.05^{b}	3.32±0.03^{b}
Varieties				
Lakha		0.35±0.02	4.48±0.04^{b}	3.39±0.05
Mianwali		0.40±0.01	3.35±0.05^{b}	3.35±0.05
Mushki		0.37±0.01	4.80±0.05^{a}	3.46±0.05
Peshawari		0.36±0.02	4.89±0.04^{a}	3.42±0.04
Lysine Regimens × Varieties				
1.3% (L1)	Lakha	0.37±0.02^{ab}	4.65±0.06^{c}	3.52±0.12^{abc}
	Mianwali	0.42±0.02^{a}	4.47±0.07^{d}	3.51±0.09^{abc}
	Mushki	0.40±0.03^{ab}	4.92±0.07^{ab}	3.64±0.07^{a}
	Peshawari	0.40±0.04^{ab}	5.07±0.04^{a}	3.57±0.03^{ab}
1.4-1.2% (L2)	Lakha	0.32±0.03^{bc}	4.36±0.05^{d}	3.33±0.06^{cde}
	Mianwali	0.40±0.03^{ab}	4.45±0.05^{d}	3.28±0.04^{de}
	Mushki	0.35±0.02^{abc}	4.84±0.07^{b}	3.39±0.07^{bcde}
	Peshawari	0.38±0.03^{ab}	4.76±0.04^{bc}	3.36±0.07^{bcde}
1.5-1.3-1.1%(L3)	Lakha	0.37±0.03^{abc}	4.43±0.03^{d}	3.34±0.07^{cde}
	Mianwali	0.38±0.03^{ab}	4.33±0.04^{d}	3.26±0.07^{e}
	Mushki	0.35±0.02^{abc}	4.63±0.04^{c}	3.35±0.05^{bcde}
	Peshawari	0.28±0.03^{c}	4.86±0.08^{b}	3.32±0.05^{cde}

Values have been mentioned as Mean±SEM and various superscripted alphabets show significant (P≤0.05) differences among them (order of significance is as: a>b>c>d…).

TP, ALB and GLO: TP (4.13±0.16 g/dL) and GLO (2.93±0.15 g/dL) were found highly significant (P≤0.05) in L3 than L2 and L1. Whereas, ALB (1.41±0.03 g/dL) was highest in L1 followed by L2 and L3. Among Aseel varieties, significant (P≤0.05) variations were specified in ALB, wherein all three varieties, except Lakha, presented higher serum ALB level. As far as, interactions among lysine regimens and varieties are concerned, Peshawari variety showed higher TP and GLO with L3, while, ALB was higher in Mianwali with L1 lysine regimen than other varieties and regimens (Table 2).

CR, UR and UA: Significantly higher (P≤0.05) CR (0.40±0.01 mg/dL), UR (4.77±0.06 g/dL) and UA (3.56± 0.04 mg/dL) levels were observed in L1 than L2 and L3 lysine regimens, whereas, birds of Mushki and Peshawari revealed significantly higher (P≤0.05) UR level than other two varieties. However, significant differences were perceived in interactions between lysine regimens and varieties and higher CR, UR, UA were specified with L1 by birds of Mianwali, Peshawari and Mushki varieties, respectively (Table 3).

CH, TG, HDL and LDL: Significant influence of lysine regimens was observed in CH, TG and HDL as lowest CH (16.33±0.68), highest TG (91.54±5.58) and HDL (34.13±2.54) mg/d/L were displayed by L3. Moreover, significant (P≤0.05) variations of all biochemical parameters were displayed among Aseel varieties as well, wherein, birds of Lakha variety showed highest levels of TG and HDL. Furthermore, Mushki displayed higher levels of serum cholesterol, and LDL levels than other varieties. Lakha variety displayed higher TG and HDL levels in L3 among interactions between lysine regimens and Aseel varieties, whereas, Mianwali depicted higher CH in L1 than other regimens and varieties (Table 4).

ALT/SGPT, AST/SGOT, ALP, CPK and CK MB: All serum enzymes except CPK showed significant (P≤0.05) variations with respect to lysine regimens, wherein levels of ALT/SGPT, AST/SGOT were higher in both L1 and L2 than L3, however, levels of both ALP and CK MB were higher in L1 than both L2 and L3 lysine regimens. Among Aseel varieties, birds of Mushki variety showed a significantly higher level of ALP, CPK and CK MB than other varieties. Higher levels of ALT by Peshawari, while, AST by both Lakha and Mianwali varieties were displayed in interaction with L1 regimen. However, significantly higher ALP, CPK and CK MB levels were shown by Mushki variety in interaction with L1 (Table 5).

Antibody response to NDV and IBV: L3 reflected significantly (P≤0.05) elevated antibody titer against NDV and IBV than L2 and L1. Among Aseel varieties, Lakha and Peshawari both showed higher antibody titer against NDV and IBV than Mianwali and Mushki varieties. Among interactions between dietary lysine regimens and

Aseel varieties, higher antibody titer against ND and IB vaccine was depicted by Lakha variety with L3 (Table 6).

DISCUSSION

GLU: Birds fed on L3 presented higher (P≤0.05) serum GLU level than L2 and L1 which indicated that L3 can provide more instant energy as higher GLU within the normal range depicts the available energy for metabolic activities. Among Aseel varieties, Peshawari variety showed more GLU level than Mushki, Mianwali and Lakha varieties which may be due to the dwarf body posture (Ibrahim *et al*., 2012). Moreover, the genetic impact of strains and varieties can also affect the level of GLU and other blood biochemicals (Dutta *et al*., 2013).

TP, ALB and GLO: TP and GLO were higher in L3 followed by L2 and L1, while ALB was highest (P≤0.05) in L1 than L2 and L3. Significant variations were observed among varieties in ALB level, wherein, Mianwali, Mushki and Peshawari varieties showed higher level of ALB than Lakha, while, they all were non-significantly different among themselves. Significantly higher TP and fractions (ALB) were found when lysine was supplemented according to NRC standards in the diet of broilers (Faluyi *et al*., 2015). It was also reported that serum TP content increased when lysine was increased in the diet of broiler breeders (Sahir *et al*., 2006). A study on red-legged partridge females has shown that plasma TP greatly depended upon dietary protein and its decreased value was observed when birds were offered poor dietary protein (Rodriguez *et al*., 2005). The present findings of serum TP and fractions indicated that lysine supplementation in phases is more better to provide energy for enhanced growth and metabolism, while L3 provided the lysine rightly as per growth requirement of bird.

Table 4: Effect of lysine regimens, Aseel varieties and their interactions on lipids and fractions (mg/dL) in indigenous Aseel varieties

Parameters / Variables		Cholesterol	Triglycerides	HDL	LDL
Lysine (%)					
1.3% (L1)		22.42±0.83^{a}	64.21±3.50^{c}	22.63±2.73^{c}	13.79±1.26
1.4-1.2% (L2)		19.42±0.63^{b}	79.08±4.20^{b}	29.13±2.49^{b}	13.79±1.09
1.5-1.3-1.1% (L3)		16.33±0.68^{c}	91.54±5.58^{a}	34.13±2.54^{a}	13.25±0.72
Verities					
Lakha		19.78±0.38^{a}	107.17±5.49^{a}	43.39±1.68^{a}	12.22±0.67^{b}
Mianwali		17.44±1.43^{b}	85.83±1.81^{b}	35.78±1.27^{b}	10.44±0.68^{b}
Mushki		21.17±0.36^{a}	55.83±2.12^{c}	14.33±1.32^{d}	18.61±1.55^{a}
Peshawari		19.17±1.22^{ab}	64.28±2.82^{d}	21.00±2.00^{c}	13.17±0.66^{b}
Lysine (%) × Varieties					
1.3% (L1)	Lakha	21.00±0.37^{abc}	81.50±3.03^{de}	35.83±2.56^{bc}	11.67±0.76^{b}
	Mianwali	24.00±2.61^{a}	79.00±1.53^{de}	34.33±1.76^{bc}	9.67±0.42^{b}
	Mushki	22.33±0.67^{ab}	47.33±1.76^{g}	9.50±1.12^{g}	21.50±3.12^{a}
	Peshawari	22.33±2.08^{ab}	49.00±1.93^{g}	10.83±1.25^{g}	12.33±1.43^{b}
1.4-1.2% (L1)	Lakha	20.00±0.37^{bc}	107.17±2.10^{b}	45.17±1.51^{a}	10.67±1.33^{b}
	Mianwali	14.83±0.60^{d}	86.50±1.67^{cd}	33.67±0.92^{cd}	11.50±0.92^{b}
	Mushki	21.17±0.48^{abc}	54.33±1.52^{g}	14.33±2.12^{fg}	20.00±2.73^{a}
	Peshawari	21.67±0.76^{abc}	68.33±0.56^{f}	23.33±0.56^{e}	13.00±0.97^{b}
1.5-1.3-1.1% (L1)	Lakha	18.33±0.71^{c}	132.83±5.46^{a}	49.17±1.08^{a}	14.33±0.88^{b}
	Mianwali	13.50±0.76^{d}	92.00±3.37^{c}	39.33±2.96^{b}	10.17±1.83^{b}
	Mushki	20.00±0.37^{bc}	65.83±2.32^{f}	19.17±1.64^{ef}	14.33±1.31^{b}
	Peshawari	13.50±0.76^{d}	75.50±1.41^{e}	28.83±2.26^{d}	14.17±1.05^{b}

Values have been mentioned as Mean±SEM and various superscripted alphabets show significant (*P≤0.05*) differences among them (order of significance is as: a>b>c>d…).

Table 5: Effect of lysine regimens, Aseel varieties and their interactions on serum enzymes (U/L) in indigenous Aseel varieties

Parameters / Variables		ALT	AST	ALP	CPK	CK MB
Lysine Regimens						
1.3% (L1)		6.52±0.05^{a}	214.92±3.13^{a}	2053.38±91.10^{a}	1621.50±68.13	1619.21±123.13^{a}
1.4-1.2% (L1)		6.43±0.05^{a}	207.71±2.62^{a}	1777.58±45.65^{b}	1650.50±39.90	1168.00±32.36^{b}
1.5-1.3-1.1% (L1)		6.25±0.04^{b}	190.83±2.66^{b}	1767.58±48.63^{b}	1631.21±53.02	1238.42±67.50^{b}
Verities						
Lakha		6.38±0.08	205.72±5.42	1854.06±55.45^{ab}	1532.22±55.45^{b}	998.22±42.55^{b}
Mianwali		6.47±0.04	205.56±4.38	1724.72±61.04^{b}	1634.83±33.80^{b}	1535.39±97.69^{a}
Mushki		6.36±0.05	204.78±2.71	1977.28±88.99^{a}	1865.94±68.61^{a}	1673.22±113.74^{a}
Peshawari		6.39±0.06	201.89±3.20	1908.67±67^{ab}	1504.61±51.63^{b}	1160.67±69.79^{b}
Lysine Regimens × Varieties						
1.3% (L1)	Lakha	6.50±0.13^{abcd}	222.00±2.92^{a}	2087.17±221.08^{ab}	1389.67±120.93^{c}	837.17±74.12^{f}
	Mianwali	6.58±0.08^{abc}	220.33±9.79^{a}	1847.67±165.92^{abc}	1663.00±68.09^{abc}	2027.67±47.19^{a}
	Mushki	6.36±0.12^{cd}	208.83±4.85^{abc}	2130.67±190.89^{a}	1914.83±144.41^{a}	2206.83±104.40^{a}
	Peshawari	6.64±0.07^{a}	208.50±4.52^{abc}	2148.00±167.85^{a}	1518.50±121.89^{c}	1405.17±162.56^{bc}
1.4-1.2% (L1)	Lakha	6.63±0.10^{ab}	218.00±4.59^{ab}	1678.17±48.71b^{c}	1570.83±39.80^{bc}	995.17±20.75^{def}
	Mianwali	6.48±0.08^{abcd}	203.17±2.61^{bcd}	1632.67±48.30^{c}	1589.50±13.89^{bc}	1298.67±57.19^{bc}
	Mushki	6.37±0.05^{bcd}	203.17±5.33^{bcd}	2049.33±101.41^{abc}	1849.50±110.59^{ab}	1247.00±57.97^{cd}
	Peshawari	6.25±0.07^{d}	206.50±6.34^{abcd}	1750.17±47.49^{abc}	1592.17±68.30^{bc}	1131.17±36.02^{cde}
1.5-1.3- 1.1% (L1)	Lakha	6.02±0.06^{e}	177.17±4.90^{e}	1796.83±60.30^{abc}	1636.17±92.87^{abc}	1162.33±44.34^{cde}
	Mianwali	6.35±0.03^{cd}	193.17±4.21^{cd}	1693.83±56.85^{bc}	1652.00±78.72^{abc}	1279.83±138.22^{c}
	Mushki	6.35±0.07^{cd}	202.33±4.20^{bcd}	1751.83±134.68^{abc}	1833.50±119.12^{ab}	1565.83±148.71^{b}
	Peshawari	6.27±0.07^{d}	190.67±2.33^{de}	1827.83±128.33^{abc}	1403.17±63.51^{c}	945.67±23.98^{ef}

Values have been mentioned as Mean±SEM and various superscripted alphabets show significant (*P≤0.05*) differences among them (order of significance is as: a>b>c>d…); ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline Phosphatase; CPK: Creatinine Phospho Kinase; CK MB: Creatinine Kinase (dimer).

Table 6: Newcastle disease (ND) and infectious bronchitis (IB) titer means, for the main and interactive effects of different dietary lysine regimens and Aseel chicken varieties

Parameters / Variables		ND (HI titer, log2)[2]	IB (ELISA titer)[3]
Lysine (%)			
1.3 (L1)		10.44±0.49^{b}	3212.19±90.12^{b}
1.4-1.2 (L2)		11.74±0.57^{b}	3238.00±72.85^{b}
1.5-1.3-1.1 (L3)		13.28±0.49^{a}	3484.89±66.26^{a}
Varieties			
Lakha		12.76±0.80^{a}	3499.70±55.39^{a}
Mianwali		11.32±0.60^{ab}	3295.30±83.44^{ab}
Mushki		10.64±0.54^{b}	3128.11±102.75^{b}
Peshawari		12.56±0.54^{a}	3323.68±105.00^{ab}
Lysine Levels (%) × Varieties			
1.3	Lakha	10.74±1.43^{cd}	3486.56±69.21^{ab}
	Mianwali	10.48±0.51^{cd}	3327.45±151.33^{abc}
	Mushki	8.70±0.79^{d}	2917.93±176.64^{c}
	Peshawari	11.85±0.71^{bc}	3116.83±231.72^{bc}
1.4-1.2	Lakha	11.54±1.04^{bcd}	3322.93±106.19^{abc}
	Mianwali	12.33±1.74^{bc}	3193.59±142.19^{bc}
	Mushki	11.55±0.99^{bcd}	3129.17±203.36^{bc}
	Peshawari	11.52±0.89^{bcd}	3306.31±140.01^{abc}
1.5-1.3- 1.1	Lakha	16.00±0.45^{a}	3689.60±41.59^{a}
	Mianwali	11.16±0.30^{cd}	3364.85±156.86^{abc}
	Mushki	11.67±0.48^{bcd}	3337.22±132.46^{abc}
	Peshawari	14.31±0.89^{ab}	3547.91±143.11^{ab}

Values have been mentioned as Mean±SEM and various superscripted alphabets show significant (*P≤0.05*) differences among them (order of significance is as: a>b>c>d); [2]HI antibody titer articulated as log2 geometric means (HI titers ± standard errors); [3]ELISA antibody titer to IBV expressed as ELISA titers ± standard errors.

CR, UR and UA: Birds fed on L3 and L2 had significantly lower values of serum CR, UR and UA than L1, whereas, among varieties, birds of both Peshawari and Mushki showed significantly lower UR level than other varieties. As serum CR is an intramuscularly found waste product of creatinine phosphate, its accumulation causes increase in muscle mass and related physiological and behavioral changes in birds (Ladokun *et al*., 2008). The lower values of CR, UR and UA in L3 may be due to the supply of lysine rightly as per growth requirement of birds. Moreover, serum CR, UR and UA values depend upon age, species, quality and quantity of dietary protein (Simaraks *et al*., 2004). Genetic impact of varieties and strains is also a consistent argument to justify the variations in the serum values of these biochemicals (Dutta *et al*., 2013).

CH, TG, HDL and LDL: Serum CH was drastically lowest in L3 followed by L2 and L1 while, birds of Mianwali variety showed its lower level than other varieties. As decrease in CH (hypocholesterolemia) is associated with dietary components of feed, species, sex and age (Toghyani *et al*., 2010) and dietary lysine in proper proportions acts as antioxidant agent to lower the CH (Al-Beitawi and El-Ghousei, 2008). The protein supplementation in feed also acted as hypocho-lesterolemic factor to reduce serum CH by its increased degradation and lower synthesis (Rita *et al*., 2014). L3 regimen and Lakha variety displayed remarkably highest level of serum HDL followed by L2, L1 and Mianwali, Peshawari, Mushki varieties, respectively. Serum LDL was lower (numerically but not statistically) in L3 than L2 and L1 and was statistically higher in Mushki than other varieties. TG was significantly highest in L3 and Lakha followed by L2 and L1 lysine regimen and Mianwali, Mushki and Peshawari varieties, respectively. As TG are main organic components which act as important primary energy source for metabolic activities of body. The present findings of serum TG may be correlated to the nutritional effect (lysine supplementation) rather than genetic factor (Ladokun *et al*., 2008; Rehman *et al*., 2016). Etim *et al*. (2014) also defined the nutrition as main non-genetic factor amongst environment, anxiety, behaviour and physiological condition of birds that affect TG value. It was also found in a study on growing pigs that when dietary lysine was increased, blood TG was also increased (Zeng *et al*., 2013).

ALT, AST, ALP, CPK and CK MB: Birds fed on L3 showed significantly lower values of ALT and AST than L2 and L1 which in turn were not significantly different by themselves, while serum ALT and AST values indicated non-significant variations among varieties. Both of these enzymes are important indicators of acute liver diseases and are linked with pathophysiological index of liver malfunctioning. Thus, highly increased values of these enzymes into blood stream demonstrate the liver dysfunction. The values of ALT and AST enzymes, in the present study, are within the normal range and reveal the normal activity of liver and hence the normal health status of Aseel birds. Serum ALP and CK MB values were also significantly affected by lysine regimens and were lower in L3 than L2 and L1, while CPK varied non-significantly with respect to lysine regimens. Among varieties, significant variations were portrayed by Mushki in ALP, CPK and CK MB values than other varieties. The values of ALT, AST, ALP and CPK are lower in comparison with values of those found in a study on broiler chicks fed with single cell protein mixed in basal diet at the rate of 10% of dry mass (Yakoub *et al*., 2011). The present results of serum enzymes indicate the normal function of liver and kidney and L3 proved to be the best to provide the lysine as per growth requirements of birds sustaining the good health and body activity.

Antibody response to NDV and IBV: There always exists probability of disease challenges in poultry procedures. The bird's immune and humoral system remains involved adjusting itself against the disease condition and environmental stresses, so, nutritional components may be enhanced at that time. Now-a-days, much attention is given to improve the quality of poultry nutrition by supplementing the feed with different synthetically available micronutrients to improve the bird's immunity against the infections and epidemic diseases.

The birds fed on L3 showed higher antibody response towards NDV and IBV than those fed on L2 and L3, furthermore, Peshawari as well as Lakha performed better in context of immune response. According to present study, L3 might have provided the lysine content as idyllic requirement of Aseel birds than L2 and L1 lysine regimens. Mehardad (2012) also reported the increased immune response of broilers chicken under the impact of increased lysine supplementation in feed higher than NRC recommendations. The improved immune response by adding lysine in poultry feed was also observed in another study (Eduardo *et al*., 2009). It was also proved that when lysine deficient diets are offered to broilers, their humoral (evaluated in the form of ND titer against ND vaccination)

as well as cell-mediated immune response was decreased (Chen *et al*., 2003).

Conclusions: The results of the present study revealed that L3 lysine regimen has a positive influence on various blood biochemicals of native Aseel chicken, particularly on serum GLU, TP and fractions (GLO), TCH, HDL, TG, enzymes (ALT, AST) and antibody titer against NDV and IBV. While, Lakha and Peshawari varieties presented an overall improved picture of their blood biochemical profile and immune response towards ND and IB vaccine.

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Authors contribution: TB executed the whole experiment, collected and statistically analyzed the data along with AR and prepared the manuscript while, NR and AM critically analyzed and revised the manuscript in its final form.

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