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Full Length Article

Effect of Valine to Lysine Ratios on Growth Performance, Carcass Characteristics and Immune Response in Broiler Chickens

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Abstract

The present study aimed to investigate the effect of dietary valine supplementation on growth, carcass characteristics, and immune response in broilers. One day old eight hundred and forty broiler chicks were raised on iso-nitrogenous and iso-calorie diets (starter (1-10 days) CP 22%, ME 2975 kcal/kg), grower (11-21 days) CP 20%, ME 3025 kcal/kg) and finisher (22-35 days) CP 19%, ME 3100 kcal/kg) respectively. These birds were randomly consigned to one negative control group (V:L68, 68% valine to lysine ratio), one control group (V:L73, 73% valine to lysine ratio) and four treatments; V:L78 (78% valine to lysine ratio), V:L83 (83% valine to lysine ratio), V:L88 (88% valine to lysine ratio), V:L93 (93% valine to lysine ratio) with seven replication per treatment and 20 birds per replicate. During both the starter (0-3 weeks) and finisher phase (4-5 weeks), parameters such as body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and FCR remained unaffected ($P > 0.05$) by treatments in ration diet. However, during the overall period (0-35 days) average daily feed intake (ADFI) was higher ($P < 0.05$) of birds fed V:L88 (88% valine to lysine ratio) diets compared to other treatments (V:L68, V:L73, V:L78, V:L83, V:L93). Dressing percentage and relative thymus weight were higher ($P < 0.05$) in birds offered V:L88 diets than in the respective treatments. Both on days 17 and 35 Antibody titer averse to Newcastle disease (ND) virus was better ($P < 0.05$) in birds receiving V:L93 (93% valine to lysine ratio) diets than the rest of the treatments. Birds offered V:L93 diets had higher ($P < 0.05$) Ca and P concentrations in bones than in other treatments. Overall, enhancing valine to lysine ratio than the recommendation of Cobb-Vantress did not improve growth performance, or carcass characteristics, however, it improved bone mineralization and immune status in broilers.

Keywords: Valine; Supplementation; Growth; Carcass characteristics; Immunity

Introduction

Feed is a major cost item in modern poultry production that comprises almost 70% of the total cost of production. Amongst feedstuff, protein sources are paramount cost items. Diet costs could be reduced either by purchasing ingredients at least cost prices or ameliorating bird's feed efficiency. On the other hand diet costs could also be reduced by leaving off protein sources while inducting synthetic essential amino acids (Kaplan and Yildiz 2017). Synthetic derivatives of indispensable amino acids are utilized in broiler production to lesser diet cost meanwhile providing good amino acid balance. Supplementation of L-Valine in corn and -Soybean Meal (SBM) diets may lower production fetch without fluctuating the broiler's performance (Duarte *et al*. 2014). Essential amino acids play a predominant vital role in protein synthesis and breast meat portion (Zeitz *et al*. 2019).

Valine belongs to the category of branched-chain amino acid that is primarily metabolized in the region of skeletal muscle. Amongst its prime functions, it is involved in protein synthesis as well as protein balance in the body (Rogero and Tirapegui 2008). Dietary lysine is more important for breast meat than for growth (Sterling *et al*. 2006). Feather and leg abnormalities were typically affected by a deficiency of valine in broilers (Farran and Thomas 1992). Growth performance, feed conversion and carcass yield were affected adversely if adequate levels of valine were not used in broiler rations (Corzo *et al*. 2008). Body weight gain (BWG) and FCR were decreased when inadequate dietary valine level was used (Corzo *et al*. 2004; Duarte *et al*. 2014). Valine deficiency affects antibody titers in broilers (Bhargava *et al*. 1971). Valine deficiency causes failure of lymphoid organ development. The weights of the thymus and bursa were significantly reduced when broiler birds were offered a valine-lacking ration (Konashi *et al*. 2000).

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Several studies (Baker *et al.* 2002; Corzo *et al.* 2007; Corzo *et al.* 2008; Corzo *et al.* 2009; Rostagno *et al.* 2011) concluded that in broilers receiving diets based upon corn and SBM, valine becomes potentially 4th limiting amino acid. This limitation is more prominent in the grower and finisher phases when grains are a major part of the broiler diet (Corzo *et al.* 2004). The minimum crude protein content of the diet is variable and depends upon 4th limiting amino acids (Kidd and Hackenhaar 2006). Dietary protein could be reduced reasonably without any negative influence on broiler performance (Berres *et al.* 2011) when supplemented with valine (Duarte *et al.* 2014). Valine requirements for optimum BWG and FCR are 0.90% and 0.82% during starter (0-21 days) and finisher (21-42 days) of broiler age respectively (NRC 1994). The ideal valine-to-lysine ratio for starter and grower phase is 82% (NRC 1994).

Cobb's requirements are slightly different than those recommended by National Research Council (NRC), United States. For optimum BWG and FCR digestible valine requirements are 0.89% for the starter, 0.85% for the grower, and 0.76% for the finisher respectively (Vantress 2018). The FCR was better ($P < 0.05$) when digestible valine inclusion levels of 1.028% for the starter phase, 0.905% for the grower, and 0.853% for the finisher phase were used in the formulation (Nascimento *et al.* 2016). Feed intake (FI), BWG, and FCR were better ($P < 0.05$) when a valine inclusion level of 0.816 (starter), 0.848 (grower) and 0.903% (finisher) corresponds to valine to lysine (digestible) ratio of 76.00%, 79.00% and 84.12% were used. For the overall best performance, a 0.903% valine level was suggested (Duarte *et al.* 2014). Factors such as environmental temperature, health status, production state, genotype, and quality of protein could change amino acid need which in turn may change the need for dietary valine (Tavernari *et al.* 2013; Agostini *et al.* 2019). It was hypothesized that valine supplementation could improve production performance in broilers. Therefore, a research trial was planned to investigate the effect of dietary valine supplementation on production parameters, carcass characteristics, and immunity in broilers.

Materials and Methods

The current experiment was conducted at the R&D Farm of Five Star Feed Mills Gujranwala, Punjab, Pakistan. The approval for the present study was obtained from the Animal Ethics and Review Protocol committee of the University of Agriculture, Faisalabad, using letter no. IADS/3204.

Experimental birds, diet and housing

A total of 840-day-old broiler chicks were assigned to 6 different treatments randomly. Each treatment had 7 replicates and each replicate comprised 20 birds. Six diets were formulated containing negative control, positive control, and four other treatment diets. All the formulated diets were categorized into three phases *i.e.*, starter (01-10), grower (11-21) and finisher (22-35 days). According to (Vantress 2018) recommendation the control group was fed with digestible valine to lysine ratio of 73% (0.89:1.22, 0.81:1.12, 0.74:1.02) for starter, grower, and finisher while negative control was fed with valine to lysine ratio of 68% (0.82:1.22, 0.76:1.12, 0.69:1.02) and four other treatments were also be fed with valine to lysine ratio of 78% (0.95:1.22, 0.87:1.12, 0.79:1.02) 83% (1.01:1.22, 0.95:1.12, 0.84:1.02) 88% (1.07:1.22, 0.98:1.12, 0.89:1.02) 93% (1.13:1.22, 1.02:1.12, 0.94:1.02) for starter, grower and finisher phase as shown in Table 1, 2 and 3, respectively.

Table 1: Ingredient composition of experimental diets for 01-10 days

Ingredients	V:L68	V:L73	V:L78	V:L83	V:L88	V:L93
Corn grain	57.31	57.28	57.26	57.24	57.22	56.89
Soybean meal 45%	26.73	26.46	26.2	25.88	25.62	25.47
Canola meal 37%	7.00	7.22	7.45	7.74	7.97	8.00
Guar meal 39%	3	3	3	3	3	3
Poultry fat	1.9	1.9	1.9	1.9	1.9	1.9
Limestone	1.17	1.17	1.17	1.17	1.17	1.17
DCP	1.10	1.10	1.10	1.10	1.10	1.10
Lysine sulphate	0.44	0.44	0.45	0.45	0.46	0.47
Dl-Methionine	0.31	0.31	0.31	0.31	0.31	0.31
L-Threonine	0.17	0.17	0.17	0.17	0.17	0.17
L-Isoleucine	0.06	0.06	0.06	0.06	0.06	0.07
L-Valine	0.03	0.08	0.12	0.17	0.21	0.27
Vit-mineral premix	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.06	0.06	0.06	0.06	0.06	0.06
Phytase ®	0.005	0.005	0.005	0.005	0.005	0.005
Znic Bactricin (AGP)	0.015	0.015	0.015	0.015	0.015	0.015
Sodium Chloride	0.31	0.31	0.31	0.31	0.31	0.31
Sodium Bicarbonate	0.29	0.29	0.29	0.29	0.29	0.29
Total	100	100	100	100	100	100
Nutrient composition of experimental diets						
ME (Kcal/kg)	2975	2975	2975	2975	2975	2975
CP %	22	22	22	22	22	22
EE %	4.67	4.68	4.68	4.68	4.68	4.72
CF %	4.37	4.38	4.39	4.4	4.41	4.44
Ca %	0.96	0.96	0.96	0.96	0.96	0.96
Av. P %	0.44	0.44	0.44	0.44	0.44	0.44
Na %	0.23	0.23	0.23	0.23	0.23	0.23
Cl %	0.23	0.23	0.23	0.23	0.23	0.23
Dig lysine	1.22	1.22	1.22	1.22	1.22	1.22
Dig met + cys	0.91	0.91	0.91	0.91	0.91	0.91
Dig threonine	0.83	0.83	0.83	0.83	0.83	0.83
Dig tryptophan	0.23	0.23	0.23	0.23	0.23	0.23
Dig arginine	1.58	1.57	1.57	1.57	1.57	1.56
Dig valine	0.85	0.89	0.93	0.98	1.02	1.07
Dig isoleucine	0.77	0.77	0.77	0.77	0.77	0.77
Dig leucine	1.58	1.57	1.57	1.57	1.57	1.56
Linoleic acid	1.75	1.75	1.75	1.75	1.75	1.75

Birds offered feed ad libitum during the 35 days of the research trial. Birds were kept in a house with the dimension of replication pens of 4.0’× 4.0’× 3.0’. All pens were fitted with manual tube feeders and automatic nipple drinkers. The two-inch-deep layer of wood shaving was used as a bedding material. The research trial was conducted under strict hygienic and standard management conditions.

Data collection

Growth performance: Body weight (g): Body weight was measured weekly starting with day 1st, then on days 7, 14, 21 and 35 with the assistance of digital weighing balance.

Table 2: Ingredient composition of experimental diets for 11-21 days

Ingredients	V:L68	V:L73	V:L78	V:L83	V:L88	V:L93
Corn grain	58.68	58.74	58.81	58.90	58.96	59.03
Soybean meal 45%	20.80	20.71	20.63	20.52	20.43	20.34
Canola meal 37%	7.5	7.5	7.5	7.5	7.5	7.5
Guar meal 39%	3.0	3.0	3.0	3.0	3.0	3.0
Rice polish	4.0	4.0	4.0	4.0	4.0	4.0
Poultry fat	2.14	2.10	2.07	2.03	2.00	1.97
Limestone	1.0	1.0	1.0	1.0	1.0	1.0
DCP	0.99	0.99	0.99	0.99	0.99	0.99
Lysine sulphate	0.50	0.50	0.51	0.52	0.52	0.53
Dl-Methionine	0.3	0.3	0.3	0.3	0.3	0.3
L-Threonine	0.13	0.14	0.14	0.14	0.14	0.14
L-Isoleucine	0.09	0.09	0.09	0.09	0.09	0.09
L-Valine	0.07	0.11	0.15	0.20	0.25	0.29
Vit-mineral premix	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.06	0.06	0.06	0.06	0.06	0.06
Phytase ®	0.01	0.01	0.01	0.01	0.01	0.01
Znic Bactricin (AGP)	0.02	0.02	0.02	0.02	0.02	0.02
Sodium Chloride	0.31	0.31	0.31	0.31	0.31	0.31
Sodium Bicarbonate	0.28	0.28	0.28	0.28	0.28	0.28
Total	100	100	100	100	100	100
Nutrient composition of experimental diets						
ME (Kcal/kg)	3025	3025	3025	3025	3025	3025
CP %	20	20	20	20	20	20
EE %	5.51	5.48	5.45	5.42	5.39	5.36
CF %	4.55	4.55	4.55	4.55	4.54	4.53
Ca %	0.86	0.86	0.86	0.86	0.86	0.86
Av. P %	0.42	0.42	0.42	0.42	0.4	0.42
Na%	0.23	0.23	0.23	0.23	0.23	0.23
Cl%	0.23	0.23	0.23	0.23	0.23	0.23
Dig lysine	1.12	1.12	1.12	1.12	1.12	1.12
Dig met + cys	0.85	0.85	0.85	0.85	0.85	0.85
Dig threonine	0.73	0.73	0.73	0.73	0.73	0.73
Dig tryptophan	0.2	0.2	0.2	0.2	0.2	0.2
Dig arginine	1.09	1.09	1.09	1.09	1.09	1.09
Dig valine	0.81	0.85	0.89	0.94	0.98	1.02
Dig isoleucine	0.72	0.72	0.72	0.72	0.72	0.72
Dig leucine	1.44	1.43	1.43	1.43	1.43	1.42
Linoleic acid	1.9	1.9	1.9	1.89	1.89	1.88

Table 3: Ingredient composition of experimental diets for 22-35 days

Ingredients	V:L68	V:L73	V:L78	V:L83	V:L88	V:L93
Corn grain	56.67	56.74	56.81	56.88	56.94	57.01
Soybean meal 45%	18.09	18.00	17.91	17.82	17.74	17.65
Canola meal 37 %	8.0	8.0	8.0	8.0	8.0	8.0
Guar meal 39%	3.0	3.0	3.0	3.0	3.0	3.0
Rice polish	8.0	8.0	8.0	8.0	8.0	8.0
Poultry fat	2.96	2.92	2.90	2.86	2.82	2.80
Limestone	0.89	0.89	0.89	0.89	0.89	0.89
DCP	0.75	0.75	0.75	0.75	0.75	0.75
Lysine sulphate	0.42	0.42	0.42	0.42	0.42	0.42
Dl-Methionine	0.27	0.27	0.27	0.27	0.27	0.27
L-Threonine	0.09	0.10	0.10	0.10	0.10	0.10
L-Isoleucine	0.08	0.08	0.08	0.08	0.08	0.08
L-Valine	0.00	0.05	0.09	0.13	0.18	0.22
Vit-mineral premix	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.06	0.06	0.06	0.06	0.06	0.06
Phytase ®	0.005	0.005	0.005	0.005	0.005	0.005
Znic Bactricin (AGP)	0.015	0.015	0.015	0.015	0.015	0.015
Sodium Chloride	0.31	0.31	0.31	0.31	0.31	0.31
Sodium Bicarbonate	0.29	0.28	0.28	0.28	0.28	0.28
Total	100	100	100	100	100	100
Nutrient composition of experimental diets						
ME (Kcal/kg)	3075	3075	3075	3075	3075	3075
CP %	19	19	19	19	19	19
EE %	6.83	6.80	6.78	6.75	6.72	6.69
CF %	4.87	4.89	4.86	4.86	4.86	4.85
Ca %	0.86	0.86	0.86	0.86	0.86	0.86
Av. P %	0.42	0.42	0.42	0.42	0.42	0.42
Na %	0.23	0.23	0.23	0.23	0.23	0.23
Cl %	0.23	0.23	0.23	0.23	0.23	0.23
Dig lysine	1.02	1.02	1.02	1.02	1.02	1.02
Dig met + cys	0.80	0.80	0.80	0.80	0.80	0.80
Dig threonine	0.66	0.66	0.66	0.66	0.66	0.66
Dig tryptophan	0.19	0.19	0.19	0.19	0.19	0.19
Dig arginine	1.05	1.05	1.05	1.05	1.05	1.05
Dig valine	0.72	0.76	0.8	0.84	0.88	0.92
Dig isoleucine	0.67	0.67	0.67	0.67	0.67	0.67
Dig leucine	1.36	1.36	1.36	1.36	1.36	1.35
Linoleic acid	2.15	2.14	2.14	2.13	2.13	2.12

Body weight gain (g): The BWG of broilers was calculated at the termination of every week by subtracting the final BW from the antecedent body weight.

Feed intake (g): Feed intake was calculated weekly and 5 times during the whole experiment by extracting the feed refusal from the feed offered.

Feed conversion ratio: Although FCR was derived weekly yet final FCR was calculated once after the termination of the trial by dividing feed intake (g) by weight gain (g).

Mortality (%): The mortality record was maintained daily. Dead birds were weighed and used to adjust the BWG and FCR.

Slaughter data: At the termination of the research trial, 2 birds from each pen were erratically selected. Birds were individually weighed and analyzed to collect data on meat attributes. Feathers were removed, and evisceration was followed to attain carcass weight as well as breast and thigh meat weight. The weight of giblet organs will be chronicled for every treatment replicate to compute relative giblet organ weight. Data acquired were utilized to compute: Live weight, breast meat weight, thigh meat yield, internal organ weight, thymus weight, bursa of fabricius and abdominal fat.

Antibody titers

Blood samples (2 birds/replicate) were taken on days 17 and 35 to determine antibody titers against the Newcastle disease virus with a standard Hemagglutination Inhibition test (Allan and Gough 1974).

Ca and P determination

From the right pelvic limb, the femur and the tibiotarsus were attained. The bones of chicken birds having 15 days of age were gutted from lenient tissue. Fat was partially removed from bones by ether during twenty-four hours. Bones were ashed at 550°C. Then from dried ash, Ca and P were determined using different techniques that include, ashing, extraction, and titration. These techniques were performed according to the protocol followed in (AOAC 2000).

Table 4: Effect of dietary valine to lysine ratios on growth performance in broiler chickens during starter phase (0-3 weeks), finisher phase (4-5 weeks) and overall period (0-5 weeks)

Parameters	Treatments						SEM	*P*-value
	V:L68	V:L73	V:L78	V:L83	V:L88	V:L93		
Initial body weight (g)	44.4	44.8	45.1	45.2	44.8	44.9	0.1	0.5
0-3 Weeks (final body weight at the end of 3rd week)								
Body weight (g)	847.3	878.4	874.8	881.2	878.6	882.5	4.9	0.3
ADG (g)	38.3	39.7	39.5	39.8	39.7	39.9	0.2	0.3
ADFI (g)	50.8	51.4	51.7	51.5	51.5	51.9	0.3	0.9
FCR	1.33	1.29	1.31	1.29	1.29	1.30	0.01	0.9
Mortality (%)	3.34	2.04	1.36	2.04	1.36	1.36	0.51	0.9
4-5 Weeks (final body weight at the end of 5th week)								
Body weight (g)	2200.6	2223.7	2179.7	2204.4	2235	2190.5	9.5	0.85
ADG (g)	96.9	94.3	93.3	96.0	95.4	94.7	0.7	0.81
ADFI (g)	149.2	149.9	151.7	149.9	149.6	149.8	0.8	0.97
FCR	1.5	1.6	1.6	1.5	1.6	1.6	0.01	0.67
Mortality (%)	1.32	1.36	0.75	0.00	1.39	0.00	0.28	0.47
0-5 Weeks (final body weight at the end of 35 day)								
Body weight (g)	2200.6	2223.7	2179.7	2204.4	2235	2190.5	9.5	0.58
ADG (g)	61.7	62.3	60.9	61.7	62.6	61.3	0.3	0.55
ADFI (g)	88.1^{ab}	91.2^{a}	89.6^{ab}	91.5^{a}	93.3^{a}	91.7^{a}	0.5	0.02
FCR	1.4	1.4	1.4	1.4	1.5	1.5	0.0	0.31
Mortality (%)	1.3	1.7	0.8	0.00	1.4	0.0	0.3	0.47

V:L68 = 68% valine to lysine ratio, V:L73 = 73% valine to lysine ratio V:L78 = 78% valine to lysine ratio, V:L83 = 83% valine to lysine ratio, V:L88 = 88% valine to lysine ratio, V:L93 = 93% valine to lysine ratio

ADG = Average daily gain, ADFI = Average daily feed intake, FCR = Feed conversion ratio

$P > 0.05$ (non-significant), $P < 0.05$ (significant)

Table 5: Effect of dietary valine to lysine ratios on the carcass parameters in broiler chickens slaughtered at the end of day 35

Variables (%)	Treatments						SEM	*P*-value
	V:L68	V:L73	V:L78	V:L83	V:L88	V:L93		
Dressing percentage	50.1^{e}	55.5^{cd}	57.6^{c}	62.9^{ab}	66.1^{a}	63.8^{ab}	0.033	0.04
Breast meat	63.8	63.8	63.9	63.6	63.3	63.0	0.23	0.87
Thigh meat	36.1	36.4	34.8	35.4	36.3	36.4	0.29	0.55
Gizzard	1.4	1.4	1.4	1.6	1.5	1.5	0.02	0.13
Heart	0.5	0.6	0.6	0.6	0.6	0.6	0.01	0.53
Liver	3.2	3.3	3.5	3.2	3.1	3.2	0.05	0.33
Spleen	0.19	0.21	0.18	0.17	0.18	0.21	0.008	0.72
Thymus	0.08^{b}	0.10^{a}	0.12^{a}	0.13^{a}	0.16^{a}	0.14^{a}	0.005	0.00
Bursa of fabricius	0.20	0.23	0.20	0.25	0.24	0.25	0.006	0.08
Abdominal fat	2.90	3.2	3.2	3.2	2.9	2.9	0.08	0.55

V:L68 = 68% valine to lysine ratio, V:L73 = 73% valine to lysine ratio V:L78 = 78% valine to lysine ratio, V:L83 = 83% valine to lysine ratio, V:L88 = 88% valine to lysine ratio, V:L93 = 93% valine to lysine ratio

$P > 0.05$ (non-significant), $P < 0.05$ (significant)

Statistical analysis

Minitab Statistical Software (2010) analyzed the collected data *via* the ANOVA technique under a Completely Randomized Design. Tukey's test equated differences among treatment means (Steel *et al*. 1997).

Results

Growth performance

Dietary inclusion of valine among all treatments did not differ ($P > 0.05$) BW, ADG, ADFI and FCR during the starter phase (0-3 weeks). Similarly, during the finisher phase (4-5 weeks) BW, ADG, ADFI and FCR remained unaffected ($P > 0.05$) due to dietary treatments. However, during the overall experimental period (0-5 weeks) ADFI was better ($P < 0.05$) among birds offered V:L88 diets (88% valine to lysine ratio) than other treatments. Nevertheless, BW, ADG and FCR remained unaffected ($P > 0.05$) due to treatments in the ration diet. Mortality was not affected by dietary treatments during the starter, finisher and overall period (Table 4).

Organ Index

Birds fed V:L88 diets had the highest ($P < 0.05$) dressing percentage than other dietary treatments. Similarly, the highest ($P < 0.05$) relative weight of thymus was noted among birds fed V:L88 diets than the rest of the dietary treatments. However, the relative weight of breast meat, thigh meat, giblet organs, bursa, and abdominal fat did not differ ($P > 0.05$) among all

Table 6: Effect of valine to lysine ratios on immune response in broiler chickens at 17 and 35 days of age

Parameters (%)	Treatments						SEM	*P*-value
	V:L68	V:L73	V:L78	V:L83	V:L88	V:L93		
HI titer (log_2 day 17)	4.16[e]	4.68[de]	5.04[cd]	5.42[bc]	5.83[ab]	6.22[a]	0.118	0.000
HI titer (log_2 day35)	6.62[e]	6.99[de]	7.34[cd]	7.70[bc]	8.11[ab]	8.43[a]	0.106	0.000

V:L68 = 68% valine to lysine ratio, V:L73 = 73% valine to lysine ratio V:L78 = 78% valine to lysine ratio, V:L83 = 83% valine to lysine ratio, V:L88 = 88% valine to lysine ratio, V:L93 = 93% valine to lysine ratio
P > 0.05 (non-significant), *P < 0.05* (significant)

Table 7: Effect of valine to lysine ratios on bone mineralization in broiler chickens at the end of day 15

Parameters (%)	Treatments						SEM	*P*-value
	V:L68	V:L73	V:L78	V:L83	V:L88	V:L93		
Ca (mg/g of dried bone)	54.49[e]	58.00[de]	61.30[d]	66.85[c]	76.18[b]	81.55[a]	1.55	0.00
P (mg/g of dried bone)	7.86[b]	7.77[b]	7.93[b]	8.56[ab]	8.58[ab]	8.96[a]	0.10	0.001

V:L68 = 68% valine to lysine ratio, V:L73 = 73% valine to lysine ratio V:L78 = 78% valine to lysine ratio, V:L83 = 83% valine to lysine ratio, V:L88 = 88% valine to lysine ratio, V:L93 = 93% valine to lysine ratio
P > 0.05 (non-significant), *P < 0.05* (significant)

dietary treatments (Table 5).

Immune response

Antibody titers against NDV (Newcastle disease virus) were influenced (*P < 0.05*) by enhancing the valine-to-lysine ratio in the broiler ration. On day 17, the highest (*P < 0.05*) antibody titer was noticed among broiler birds that were offered V:L93 diets while the lowest (*P < 0.05*) antibody titer was observed among birds that were fed V:L68 diets respectively. Similarly, on day 35, the highest (*P < 0.05*) antibody titer was seen among birds that were offered V:L93 diets while the lowest (*P < 0.05*) antibody titer was noted among birds given V:L68 diets respectively (Table 6).

Bone mineralization

The highest (*P < 0.05*) calcium concentration was detected in birds given V:L93 diets than other dietary treatments. However, the lowest (*P < 0.05*) Ca concentration was noticed among birds that were offered V:L68 diets respectively. The highest (*P < 0.05*) phosphorus concentration was detected among the bird group offered V:L93 diets than respective treatments. However, the lowest P concentration in dried bone was observed among birds fed V:L73 diets (Table 7).

Discussion

Protein plays a vital role in broiler nutrition. Dietary treatments showed no impact (*P > 0.05*) during the starter phase on BW, ADG, ADFI and FCR. Mortality was unaffected during the overall starter period due to the treatments offered. Our results are comparable to those of Kaplan and Yildiz (2017), who explained that BWG, feed consumption, and FCR were unaffected (*P > 0.05*) by using various levels of valine supplementation in the ration diet. During the finisher phase, BW, ADG, ADFI, and FCR were not affected (*P > 0.05*) by treatments offered in the diet. Our outcomes are in tune with Caetano *et al*. (2015) suggested that growth performance remained unaffected (*P > 0.05*) due to different valine-to-lysine ratios in the broiler diet.

The reason might be that according to the National Research Council (1994), the valine requirement in broiler rations could be 0.82%. The supplementation of valine above that level is unnecessary because it does not have any direct impact either on bird performance or the immune system. In the present research, during the overall experimental period (0-35 days) ADFI was higher (*P < 0.05*) among birds offered V:L88 diets then followed by V:L93, V:L83, V:L73, V:L78 and V:L68, respectively. The possible reason might be that a high intake of valine increases the efficiency of valine utilization for protein synthesis in the body which leads to greater protein accumulation. That is why valine and other branched-chain amino acids can change muscle protein degradation.

Our results are consistent with Duarte *et al*. (2014) concluded that FI, BWG, and FCR were better (*P < 0.05*) when valine levels of 0.816 (starter), 0.848 (grower) and 0.903% (finisher) correspond to digestible ratios of (valine to lysine) 76.00%, 79.00%, and 84.12% were used from 22-42 day of age. They also reported that for the overall best performance of the broiler, a 0.903% level of valine was suggested. However, during the overall experimental period, BW, ADG, FCR, and mortality did not differ (*P > 0.05*) by treatments offered in broiler ration. Our findings are in close agreement with Kaplan and Yildiz (2017) who determined that BWG, feed consumption and FCR have remained unaffected (*P > 0.05*) when various levels of valine supplementation were used in a broiler diet. The difference in current research and previous studies might be due to chick strain, feed composition, and more importantly synergism and antagonism between branched-chain amino acids.

In the present study, a higher (*P < 0.05*) dressing percentage was seen among birds that were given V:L88 diets than other treatments. These findings are consistent with Daware *et al*. (2018) concluded that carcass characteristics were higher (*P < 0.05*) up to 27.06% when a valine level of 0.04% and valine to lysine ratio of 80% for broiler pre-starter, 85% for starter and 88% for broiler finisher were used. Similarly, Nascimento *et al*. (2016) reported that the dressing percentage was higher when valine was supplemented in a diet devoid of valine.

In the current study, thymus relative weight was higher (*P < 0.05*) among birds offered V:L88 diets than respective treatments. These findings are in line with Kaplan and Yildiz (2017) summarized that valine supplementation had an improved effect on thymus weight when a 1.00% valine level was used. However, in the current study relative weight of breast meat, thigh meat, gizzard, heart, liver, spleen, thymus, bursa and abdominal fat remained unaffected due to dietary treatments. The recent observations are consistent with Tavernari *et al*. (2013) suggested that carcass characteristics and bird organ weight were not influenced by different levels of dietary valine. Akin outcomes were suggested by Berres *et al*. (2011) evaluated that carcass yield and breast meat yield were not improved by using various levels of valine in the ration diet.

In the current research, antibody titer averse to NDV was better (*P < 0.05*) by enhancing the valine supplementation level. The possible reason behind this might be that the synthesis of the glutamine α-amino group is provided by a branched-chain family of amino acids which includes leucine, isoleucine, and valine. The skeletal muscles, which are an integral component of the immune system, are predominantly responsible for the Synthesis of glutamine (Newsholme and Calder 1997). These results are in tune with Daware *et al*. (2018) reported that dietary L-valine showed a greater antibody response against Newcastle disease virus (NDV) @ 0.12% as compared with the control diet without L-valine. Similar findings were reported by Foroudi and Rezamand (2014) showed that hemagglutination inhibition titer was better (*P < 0.05*) by using valine supplementation in broiler ration and valine to lysine ratio of 86% was optimum for immunity from 0-15 days of broiler. They also concluded that the valine requirement for immunity was slightly greater than the National Research Council (1994) recommendation to better support the immune system.

In the present study, as valine supplementation level was increased antibody titer was increased. These results are in exact tune with Bhargava *et al*. (1971) suggested that immunity was better (*P < 0.05*) due to increasing levels of valine in the diet. They also concluded that with the increase of valine level in diet antibody titer was increased. Current results and previous research are in contrast with Kaplan and Yildiz (2017) reported that at 1.1% dietary valine level highest antibody titer was obtained. They summarized that although the highest vaccination antibody titer was gained, it remained unaffected (*P > 0.05*) due to dietary treatments. According to Thornton *et al*. (2006), immunity remained unaffected (*P > 0.05*) due to valine supplementation in broiler ration. They concluded that marginal valine deficiency did not adversely affect the immune system of broiler chickens. Therefore, valine needs for immunity may be lesser than that of growth.

Li *et al*. (2007) concluded that birds fed with amino acids or protein deficient diet had decreased their immune function and consequently increased the chances of birds to various infectious diseases. In the current study, Ca and P concentration in bone was improved (*P < 0.05*) with different varying levels of valine supplementation in broiler chicken diet. Our findings are consistent with Foroudi and Rezamand's (2014) conclusion that calcium concentration in bone was higher (*P < 0.05*) due to valine supplementation in broiler ration.

Coto *et al*. (2009) studied one of the most important nutritional aspects and there is a bonding relation between calcium absorption and excretion and amino acid levels in the diet. Farran and Thomas (1992) concluded that there is an incidence of leg abnormality among birds fed valine deficient diet compared with valine supplemented diet. They also investigated that among three comparative studies, the content of bone ash and calcium was lowest among birds fed valine-deficient diets. The reason behind this might be that a deficiency of valine causes to increased rate of calcium excretion in urine that tends to induce leg abnormality in younger chickens.

Conclusion

The findings of the current investigation lead to the conclusion that enhancing the valine-to-lysine ratio more than Cobb-Vantress's (2018) recommendation did not improve growth performance or carcass characteristics; however, it improved bone mineralization and immune status. The valine-to-lysine ratio of 93% was optimum for bone mineralization and immune response in broilers.

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Author Contributions

WA and SAB Conceptualization and designed the experiment, WA, MAZ and MS data collected and interpreted results, WA and ZZ prepared the manuscript, WA, SAB and MS critically reviewed the draft and approved the final manuscript.

Conflict of Interest

No potential conflict of interest was reported by the authors.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

The approval for this study was taken from the Animal Ethics and Review Protocol committee of the University of Agriculture, Faisalabad *via* letter no. IADS/3204.

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