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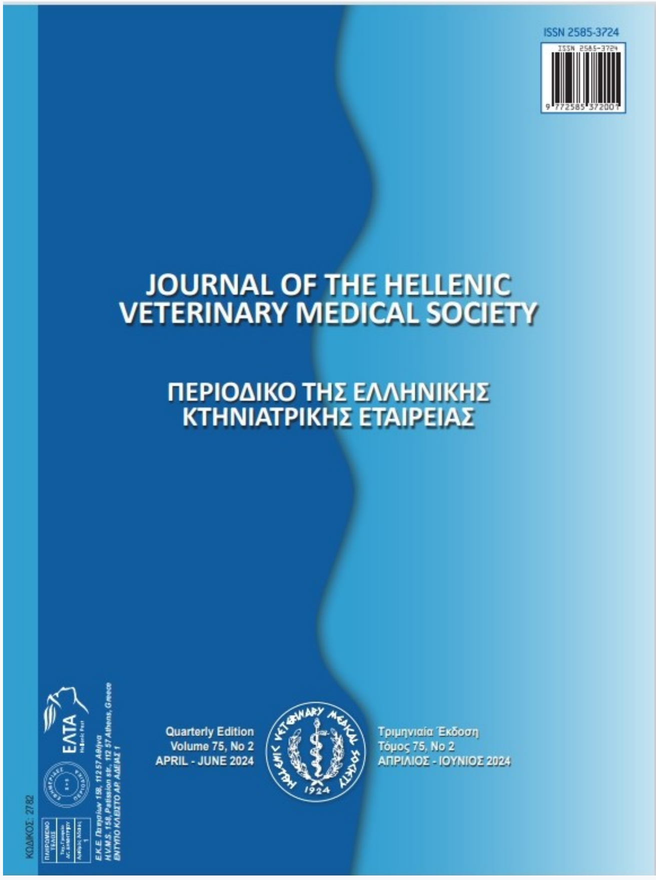

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Influence of increased dietary valine on broiler performance, serum components, nitrogen excretion and jejunum histomorphology

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ABSTRACT: Current experiment was carried out to examine the effects of increasing valine levels in broiler diets based on corn-soybean meal on performance, slaughtering and serum parameters, nitrogen excretion, and jejunum histomorphology. A total of 480 male broiler chicks were randomly allocated to six treatment groups with eight replications, each containing 10 birds. Basal diets were prepared according to the needs of broilers and valine levels were determined as 100% (V100, control), 110% (V110), 120% (V120), 130% (V130), 140% (V140), and 150% (V150). The performance parameters were not affected in the first period, and best results were obtained in V120 group in the second period and 0-42th days. Relative thigh+drumstick weight linearly increased and breast weight decreased. The glucose, total protein, globulin, and creatinine linearly increased, while triglyceride decreased quadratically. Nitrogen excretion decreased linearly with increasing dietary valine. In addition, highest villus height was obtained in V130 and highest villus surface area was obtained in V120, while crypt depth was negatively affected by increasing valine. According to results, highest performance and villus surface area were obtained in V120 group. The high levels administration of valine (140 and 150%) adversely affected relative breast weight and jejunum histomorphology, but nitrogen excreted with feces decreased. Therefore, valine level in broiler diets can be increased by 20% of the recommended level.

***Keywords*:** valine; performance; nitrogen; histomorphology; broiler

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INTRODUCTION

Satisfying the nutritional requirements for optimum performance is the primary goal of poultry feed preparation. Additionally, it is also important to fulfill the nutrient needs at the economic efficiency during performing diet manipulations. Several studies on poultry feeding examine the effects of branched-chain amino acids in terms of maximizing performance. These amino acids are essential for performance parameters (Ren et al., 2015; Batista et al., 2016), protein accumulation and intestine morphology (Allameh and Toghyani, 2019), nitrogen excretion (Alagawany et al., 2014), metabolic homeostasis (Duan et al., 2016), energy stability (Wu, 2009), cell functions, and immunity (Tavernari et al., 2013).

Valine, a hydrophobic amino acid that is one of the branched-chain amino acids (Brosnan and Brosnan, 2006), is used by animals to synthesize various other amino acids and proteins (Ferrando et al., 1995). In broilers fed diets based on corn and soybean meal, valine was reportedly identified as the fourth limiting amino acid (Corzo et al., 2007; Tavernari et al., 2013). According to Lelis et al. (2014) valine is a potentially limiting amino acid after methionine, lysine, tryptophan, and threonine. Besides, it is crucial to understand the optimum level required for the best performance in the addition branched chain amino acids and consequently valine to the diet.

Examining the studies analyzing the influence of valine on various parameters reveals that the main focus is the administration of valine to diets with lower crude protein contents (Ospina-Rojas et al., 2017; Allameh and Toghyani, 2019). Furthermore, although earlier researches on the ideal valine level for broilers was performed, and values for various growth stages were determined, the nutrient requirements of newly generated lines may differ. Therefore, it would be beneficial to re-evaluate the nutritional needs of broilers. The hypothesis of this study is that the addition of increasing levels of valine to broiler diets without crude protein restriction would be advantageous in re-evaluation the optimum valine level. Additionally, in this study, the possibility of reducing the amount of nitrogen excretion by increasing the valine level will be investigated. The purpose of present research is to re-evaluation the optimal needs by examining the effects of gradually increased valine in the diet without crude protein restriction on performance, carcass and some visceral organ weights, serum biochemical constituents, nitrogen excretion, and jejunum histomorphological traits.

Table 1. Experimental diets and calculated nutrient contents

	Treatment Diets																	
Ingredients, %	**V100**			**V110**			**V120**			**V130**			**V140**			**V150**		
	0-10	**11-24**	**25-42**	**0-10**	**11-24**	**25-42**	**0-10**	**11-24**	**25-42**	**0-10**	**11-24**	**25-42**	**0-10**	**11-24**	**25-42**	**0-10**	**11-24**	**25-42**
Corn	49.07	51.83	55.40	49.00	51.86	55.40	49.15	51.86	55.53	49.42	52.05	55.96	49.80	52.03	56.18	50.00	52.03	56.30
Soybean meal	40.90	37.80	33.30	40.88	37.70	33.27	40.60	37.60	33.15	40.30	37.30	32.80	40.00	37.20	32.60	39.68	37.10	32.40
Soybean oil	6.00	7.00	8.30	6.00	7.00	8.30	6.00	7.00	8.20	5.90	7.00	8.00	5.70	7.00	7.90	5.70	7.00	7.88
Limestone	0.73	0.70	0.60	0.71	0.70	0.60	0.73	0.70	0.60	0.73	0.70	0.61	0.73	0.70	0.61	0.73	0.70	0.61
Dicalcium phosphate	2.22	1.95	1.75	2.23	1.95	1.75	2.23	1.95	1.75	2.23	1.95	1.75	2.23	1.96	1.75	2.23	1.96	1.75
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-lysine	0.19	0.10	0.08	0.19	0.10	0.08	0.19	0.11	0.08	0.20	0.12	0.10	0.21	0.13	0.10	0.22	0.13	0.11
DL-methionine	0.36	0.17	0.16	0.36	0.17	0.16	0.36	0.17	0.16	0.37	0.17	0.16	0.37	0.18	0.16	0.37	0.18	0.16
L-threonine	0.15	0.10	0.06	0.15	0.10	0.06	0.15	0.10	0.06	0.16	0.10	0.07	0.16	0.10	0.07	0.16	0.10	0.07
L-valine	0.03	0.00	0.00	0.13	0.07	0.035	0.24	0.16	0.12	0.34	0.26	0.20	0.45	0.35	0.28	0.56	0.45	0.37
Calculated nutrient contents, %																		
Metabolizable energy, kcal/kg	3004	3092	3213	3007	3095	3214	3011	3098	3211	3010	3102	3204	3004	3105	3202	3009	3108	3204
Crude protein	23.00	21.41	19.50	23.09	21.44	19.52	23.00	21.48	19.56	23.08	21.46	19.53	23.07	21.52	19.53	23.06	21.57	19.54
Lysine	1.29	1.15	1.03	1.28	1.14	1.02	1.28	1.15	1.02	1.28	1.15	1.03	1.28	1.15	1.02	1.28	1.15	1.03
Methionine	0.67	0.47	0.44	0.67	0.47	0.44	0.67	0.47	0.44	0.68	0.47	0.44	0.67	0.48	0.44	0.68	0.48	0.43
Methionine + cystine	0.98	0.77	0.72	0.98	0.77	0.72	0.98	0.76	0.72	0.99	0.76	0.71	0.99	0.77	0.71	0.98	0.77	0.71
Calcium	0.97	0.88	0.78	0.96	0.88	0.78	0.97	0.88	0.78	0.97	0.88	0.78	0.96	0.88	0.78	0.96	0.88	0.78
Available phosphorus	0.48	0.43	0.39	0.48	0.43	0.39	0.48	0.43	0.39	0.48	0.43	0.39	0.48	0.43	0.39	0.48	0.43	0.39
Threonine	0.87	0.78	0.68	0.87	0.78	0.68	0.86	0.78	0.68	0.87	0.77	0.69	0.87	0.77	0.68	0.86	0.77	0.68
Valine	0.986	0.907	0.833	1.082	0.973	0.866	1.183	1.058	0.947	1.275	1.149	1.019	1.377	1.234	1.093	1.477	1.329	1.176
Valine/lysine ratio	0.76	0.79	0.81	0.85	0.85	0.85	0.92	0.92	0.93	1.00	1.00	0.99	1.08	1.07	1.07	1.15	1.16	1.14

1Premix provided the following (per kg of diet): manganese 80 mg; iron 60 mg; copper 5 mg; iodine 1 mg; selenium 0.15 mg; vitamin A 8800 IU; vitamin D3 2200 IU; vitamin E 11 mg; nicotinic acid 44 mg; Cal-D-Pan 8.8 mg; vitamin B2 4.4 mg; vitamin B1 2.5 mg; vitamin B12 6.6 mg; folic acid 1 mg; biotin 0.11 mg; choline 220 mg.

V100: Requirement level; **V110:** valine increased by 10% of requirement; **V120:** valine increased by 20% of requirement; **V130:** valine increased by 30% of requirement; **V140:** valine increased by 40% of requirement; **V150:** valine increased by 50% of requirement.

MATERIAL AND METHODS

Birds and Experimental Feeds

The experiment was carried out to randomized arrangement design with six dietary treatments. A total of 480 1-day-old Ross 308 male broiler chicks were randomly distributed among six trial groups. In each experimental group, there were eight subgroups, each with 10 chicks. In all periods (0-10th, 11-24th, and 25-42th days) of the experiment, basal diets (V100) were prepared as isocaloric and isonitrogenic according to the Aviagen (2019) recommendation for broilers (Table 1), and treatment diets were created to contain 100% (V100), 110% (V110), 120% (V120), 130% (V130), 140% (V140), and 150% (V150) valine. Valine levels of experimental diets of all periods as follows. For 0-10th days: 0.986, 1.082, 1.183, 1.275, 1.377, and 1.477%; 11-24th days: 0.907, 0.973, 1.058, 1.149, 1.234, and 1.329%; 25-42th days: 0.833, 0.866, 0.947, 1.019, 1.093, and 1.176%. Treatment diets in mash form and contain other limiting amino acids at required levels. The birds were raised in environmentally controlled house and pens were 150x150 cm. Height-adjustable suspended feeders and drinkers (with diameters 50 and 30 cm, respectively) were used in the experiment. During the trial, ahemeral lighting (23 hours/day) was applied, fresh water and feed were given *ad-libitum.*

Determination of Performance

During the experiment, body weight and feed intake were determined as g/chick by group weighings at the hatching, 10th day, 24th day, and final (42th day) of the trial. Body weight gain was also found from these measurements. Feed conversion ratio was calculated as g feed/g gain with *feed intake / body weight gain* formula.

Determination of Slaughtering Parameters

At the end of the experiment, two broilers at six weeks of age from each subgroup were euthanized by cervical dislocation. Carcass, thigh+drumstick, breast, liver, heart, gizzard, pancreas, and abdominal fat were weighed with a 0.01 g precision scale, and then their relative weights were determined. Relative weights of carcasses and some organs were calculated as percentage of body weight. On the other hand, relative weights of thigh+drumstick and breast were determined as a percentage of the carcass. After data were obtained for slaughtering parameters, jejunum samples were taken for histological analysis.

Determination of Nitrogen Excretion

In order to determine the nitrogen excretion in the feces, the samples were collected immediately after defecation and dried at 70°C. The 0.6 g of each sample placed in 100 mL balloons. Then, samples were kept overnight after 5 mL of 98% sulfuric acid was added and applied the method of wet decomposition by using hydrogen peroxide. The balloons were completed to 100 mL with distilled water and nitrogen values were found according to the Kjeldahl Method (Kjeldahl, 1883). For nitrogen determination, 10 mL sample was placed in kjeldahl tube and treated with 5% sodium hydroxide and 3% boric acid solution. The obtained solution was titrated with 0.043 N sulphuric acid and the %N was found by calculation from the value obtained from the titration. The crude protein (%) was determined by multiplying the found value with 6.25.

Determination of Serum Characteristics

At the end of the experiment, two broilers (96 birds in total) of similar body weight from each subgroup was randomly selected. For blood sampling, 10 mL tubes with serum separator gel were used. Serum were obtained by centrifuging the blood at 4000 rpm for 10 minutes. The serum were stored at -20 °C until analysis, and serum glucose, triglyceride, cholesterol, HDL, total protein, albumin, globulin, and creatinine concentrations were determined by an auto-analyzer (Beckman Coulter LX20) device using commercial kits in a private laboratory.

Determination of Jejunum Histomorphological Traits

The samples taken for jejunum histomorphological measurements were immediately buffered in 10% formalin and they were gently shaken to remove adherent intestinal contents. Samples kept in this solution for 72 hours. After the trimming process, the intact crypt-villus units of each sample were divided into three cross-sectional areas. Preparation and fixation for measurements of villus and crypts were carried out according to the protocol demonstrated by Xu et al. (2003). The process included serial dehydration in graded ethanol solutions, cleaning with xylene and embedding in paraffin. Samples were cut using a microtome, placed on glass slides and stained with hematoxylin and eosin. Measuring with a microscope, villus height was taken from the crypt-villus junction to the tip brush border. In order to obtain accurate results, villus width was measured from the midpoint of the villus between the brush borders of the opposing epithelial cells, as far as possible. The crypt depth was taken at the level of the membranes of the crypt epithelial cells. Villus surface area was calculated with the formula *(2π) × (villus width / 2) × (villus height)* according to Sakamoto et al. (2000).

Statistical Analysis

Data were analysed in the SPSS 18.0 software package (SPSS Inc., Chicago, IL, USA) with a model of one-way ANOVA, using the group mean as an experimental unit. Differences among the group means were determined by Duncan's range tests. Additionally, orthogonal polynomial contrasts were used to evaluate the significance of linear, quadratic, and cubic models to determine the response of the dependent variable to an increasing valine level. A probability value of $P < 0.05$ was considered statistically significant.

RESULTS

Performance

The effects of increased valine levels in corn and soybean meal-based diets on the performance parameters of broilers are demonstrated in Table 2. In the present study, the effect of increasing valine level on 10th day body weight (209.9-220.6 g) and first period body weight gain (164.7-175.4 g), feed intake (206.2-222.9 g), and feed conversion ratio (1.24-1.30) were statistically insignificant ($P>0.05$). However, the effects of treatments on body weight, body weight gain, feed intake, and feed conversion ratio in other periods were found to be considerably significant ($P<0.05$). Treatments had a quadratic effect on body weight in the current study, it statistically increased in the V110 (1161.9 g) and V120 (1154.8 g) groups on day 24 and the V120 (2397.8 g) group on day 42 compared to the control group and other groups. At the end of the trial (42. days), V120 had the highest body weight (2397.8) and V150 had the lowest (2094.7). Again, the V110 and V120 groups in the second period, and the V120 group in the third period and throughout the experiment, had the best numerical values in terms of body weight gain affected quadratically by the treatments. The minimum and maximum means of body weight gain were in the second period: 842.6-946.4 g (V150 vs V110); third period: 1042.1-1243.0 g (V150 vs V120); and 0-42th days: 2049.4-2352.4 g (V150 vs V120). Another parameter quadratically affected by increasing valine content was feed intake. Compared to the control group, there was a statistical increase in all groups except for V150 (1382.5 g) in the second period. In the third period, the only considerably significant outcome compared to the control group was the increase in the V120 (2192.0 g) group. Additionally, the highest feed intake was determined V120 (3890.1 g),

Table 2. Effect of gradually increasing dietary valine on broiler performance

Parameters	Treatment Groups						SEM*	*Probabilities*			
	V100	V110	V120	V130	V140	V150		*P- value*	*Linear*	*Quadratic*	*Cubic*
Body weight, g/bird											
Hatching	45.2	45.4	45.4	45.2	45.1	45.3	0.091	0.898	0.758	0.761	0.245
10th day	220.6	215.5	210.5	212.1	214.6	209.9	1.35	0.211	0.051	0.284	0.228
24th day	1066.4^{cd}	1161.9^{a}	1154.8^{a}	1123.5^{b}	1083.4^{c}	1052.5^{d}	6.92	<0.001	<0.001	<0.001	<0.001
42th day	2142.7^{c}	2343.5^{b}	2397.8^{a}	2294.2^{b}	2170.3^{c}	2094.7^{c}	20.92	<0.001	0.004	<0.001	0.004
Body weight gain, g/ bird											
0-10th days	175.4	170.1	165.1	166.9	169.5	164.7	1.36	0.205	0.055	0.277	0.200
11-24th days	845.9^{d}	946.4^{a}	944.3^{a}	911.5^{b}	868.8^{c}	842.6^{d}	7.00	<0.001	<0.001	<0.001	<0.001
25-42th days	1076.3^{cd}	1181.6^{ab}	1243.0^{a}	1170.7^{abc}	1086.8^{bcd}	1042.1^{d}	16.58	0.001	0.069	<0.001	0.092
0-42th days	2097.5^{c}	2298.1^{ab}	2352.4^{a}	2249.0^{b}	2125.2^{c}	2049.4^{c}	20.90	<0.001	0.004	<0.001	0.004
Feed intake, g/ bird											
0-10th days	222.9	215.5	211.9	206.2	216.7	214.6	1.69	0.104	0.193	0.026	0.614
11-24th days	1361.2^{c}	1490.7^{a}	1486.2^{a}	1471.3^{a}	1427.6^{b}	1382.5^{c}	8.55	<0.001	0.306	<0.001	<0.001
25-42th days	1996.5^{b}	2119.7^{ab}	2192.0^{a}	2082.0^{ab}	2055.6^{b}	2053.5^{b}	17.94	0.031	0.959	0.009	0.036
0-42th days	3580.6^{d}	3825.9^{ab}	3890.1^{a}	3760.0^{bc}	3699.9^{bcd}	3650.6^{cd}	22.30	<0.001	0.656	<0.001	0.004
Feed conversion ratio, g feed/g gain											
0-10th days	1.27	1.27	1.28	1.24	1.28	1.30	0.007	0.105	0.254	0.092	0.242
11-24th days	1.61^{ab}	1.58^{b}	1.58^{b}	1.61^{ab}	1.65^{a}	1.64^{a}	0.008	0.033	0.012	0.109	0.056
25-42th days	1.86^{abc}	1.80^{bc}	1.77^{c}	1.78^{bc}	1.91^{ab}	1.97^{a}	0.020	0.014	0.022	0.003	0.652
0-42th days	1.71^{bc}	1.67^{c}	1.65^{c}	1.67^{c}	1.74^{ab}	1.78^{a}	0.010	<0.001	0.001	<0.001	0.360

*Standard error means.

a,b,c,d:Within a row, values not sharing a common superscript are statistically different; $P \leq 0.05$.

V100: Requirement level; **V110:** valine increased by 10% of requirement; **V120:** valine increased by 20% of requirement; **V130:** valine increased by 30% of requirement; **V140:** valine increased by 40% of requirement; **V150:** valine increased by 50% of requirement.

and there was a noticeable difference between this group and the other treatment groups, with the except for V110 (3825.9 g). The minimum and maximum values for feed intake are as follows: second period: 1361.2-1490.7 g (V100 vs V110); third period: 1996.5-2192.0 g (V100 vs V120); 0-42th days: 3580.6-3890.1 g (V100 vs V120). The feed conversion ratio was linearly affected in the second period and quadratic affected in the third period and throughout the experiment. In the second period, there was a statistical difference between the V110 (1.58) and V120 (1.58) groups and the V140 (1.65) and V150 (1.64) groups, and in the third period, there was a considerable difference between the V120 (1.77) group and the V140 (1.91) and V150 (1.97) groups. Better results were obtained in the V110 (1.67), V120 (1.65), and V130 (1.67) groups compared to the V140 (1.74) and V150 (1.78) groups, despite the fact that there was no significant difference between the control group throughout the experiment. The minimum and maximum means for this parameter are 1.58-1.65 (V110, V120 vs V140) for the second period, 1.77-1.97 (V120 vs V150) for third period and 1.65-1.78 g (V120 vs V150) for 0-42th days.

Slaughtering Characteristics

Table 3 present the effect of increasing dietary valine on some slaughtering traits in broilers. The effects of treatments on relative carcass (73.91-75.16%), liver (1.65-1.70%), heart (0.52-0.56%), gizzard (1.66-1.96%), and pancreas (0.19-0.22%) weights were insignificant (P>0.05). Treatments in the study linearly affected the relative weights of the thigh+drumstick and breast (P<0.05). Compared to the control group (29.75%), the relative thigh+drumstick weight increased significantly in the V130 (30.66%) and V140 (30.61%) groups. In comparison to the V100 (40.15%) and V110 (40.29%) groups, there was a decline in V140 (38.46%) and V150 (38.61%) in relative breast meat. The minimum and maximum means for relative thigh+drumstick and breast meats are 29.75-30.66% (V100 vs V130) and 38.46-40.29% (V140 vs V110), respectively. Abdominal fat was found to be significantly lower in the V130 (0.98%) group compared to the V100 (1.08%), and there was no significant difference between the control group and the other groups. The lowest abdominal fat was found in the V130 group with 0.98% and the highest in the V120 group with 1.12%.

Nitrogen Excretion

Increased valine level linearly affected nitrogen excretion in broilers (Table 4) (P<0.05). The dietary valine level decreased linearly nitrogen excretion from 28.29% (V100) to 25.12% (V150). This decline was noticeable in the V130 (26.27%), V140 (25.54%) and V150 (25.12%) groups compared to the control group (V100).

Table 3. Effect of gradually increasing dietary valine on broiler slaughtering characteristics

Parameters	Treatment Groups						SEM*	Probabilities			
	V100	V110	V120	V130	V140	V150		*P- value*	*Linear*	*Quadratic*	*Cubic*
Carcass[1]	73.98	75.16	74.71	75.16	74.19	73.91	0.23	0.641	0.448	0.299	0.292
Thigh+drumstick[2]	29.75^{c}	29.96^{abc}	29.88^{bc}	30.66^{a}	30.61^{ab}	30.41^{abc}	0.11	0.031	0.004	0.387	0.182
Breast[2]	40.15^{a}	40.29^{a}	39.14^{ab}	39.34^{ab}	38.46^{b}	38.61^{b}	0.18	0.007	<0.001	0.755	0.430
Liver[1]	1.68	1.70	1.65	1.67	1.68	1.65	0.016	0.958	0.563	0.957	0.993
Heart[1]	0.54	0.56	0.52	0.56	0.56	0.55	0.010	0.849	0.733	0.970	0.798
Gizzard[1]	1.96	1.75	1.66	1.84	1.82	1.82	0.034	0.214	0.667	0.088	0.083
Pancreas[1]	0.21	0.22	0.20	0.19	0.19	0.21	0.005	0.496	0.682	0.246	0.136
Abdominal fat[1]	1.08^{ab}	1.02^{bc}	1.12^{a}	0.98^{c}	1.06^{abc}	1.09^{ab}	0.014	0.019	0.934	0.159	0.374

1% of body weight, 2% of carcass. *Standard error means.
a,b,c:Within a row, values not sharing a common superscript are statistically different; P ≤ 0.05.
V100: Requirement level; **V110:** valine increased by 10% of requirement; **V120:** valine increased by 20% of requirement; **V130:** valine increased by 30% of requirement; **V140:** valine increased by 40% of requirement; **V150:** valine increased by 50% of requirement.

Table 4. Effect of gradually increasing dietary valine on nitrogen excretion

Parameter	Treatment Groups						SEM*	*Probabilities*			
	V100	V110	V120	V130	V140	V150		*P- value*	*Linear*	*Quadratic*	*Cubic*
Crude protein, %	28.29^{a}	27.32^{ab}	27.22^{ab}	26.27^{bc}	25.54^{bc}	25.12^{c}	0.291	0.009	0.000	0.965	0.960

*Standard error means.
a,b:Within a row, values not sharing a common superscript are statistically different; P ≤ 0.05.
V100: Requirement level; **V110:** valine increased by 10% of requirement; **V120:** valine increased by 20% of requirement; **V130:** valine increased by 30% of requirement; **V140:** valine increased by 40% of requirement; **V150:** valine increased by 50% of requirement.

Serum Biochemical Constituents

The effect of diet valine level on serum parameters in broilers is given in Table 5. Treatments did not affect cholesterol, HDL, and albumin levels in the study (P>0.05). The minimum and maximum values of these parameters are 93.5-106.0 mg/dL (V110 vs V120), 63.6-73.4 mg/dL (V110 vs V140), and 1.07-1.17 g/dL (V100 vs V110), respectively. Other serum parameters were significantly affected by the treatments (P<0.05). Treatments linearly affected serum glucose and were significantly higher in the V150 (207.8 mg/dL) compared to the V100 (190.5 mg/dL), V110 (188.0 mg/dL), and V120 (182.5 mg/dL) groups. On the other hand, the effect on triglyceride was quadratic and considerably declined in the V110 (28.9 mg/dL) relative to V120 (36.6 mg/dL), V130 (34.6 mg/dL) and V140 (36.1 mg/dL). In addition, increasing dietary valine had a linear effect on total protein and globulin, and were lower in the V100 (2.56 and 1.49 g/dL) group than V120 (2.80 and 1.65 g/dL), V130 (2.79 and 1.68 g/dL), and V150 (2.80 and 1.69 g/dL). Finally, creatinine was linearly affected by valine levels and the highest numerical value was found in V130 with 0.299 mg/dL and the lowest in V100 with 0.266 mg/dL.

Jejunum Histomorphological Traits

Jejunum histomorphological traits in broilers were affected quadratically with increasing valine level (Table 6) (P<0.05). The V130 (728 m) group had the highest villus height, and there was a significant difference between this group and other treatment groups. In addition, villus width considerably increased at V120 (113.7 μm) compared to other groups. A significant decrease was observed in crypt depth in all treatment groups compared to the control group (99.27 μm), except for V110 (98.44 μm). Villus height/crypt depth was higher in V140 (9.10) group compared to other groups. Villus surface area was found to be significantly higher in V120 (0.246 mm^2) ve V130 (0.244 mm^2) groups. Minimum and maximum values of these parameters as follows. Villus height: 672-728 μm (V150 vs V130), villus width: 101.0-113.7 μm (V150 vs V120), crypt depth: 79.77-99.27 μm (V150 vs V100), villus height/ crypt depth: 7.22-9.10 (V110 vs V140), villus surface area: 0.212-0.246 mm^2 (V150 vs V120).

Table 5. Effect of gradually increasing dietary valine on serum biochemical constituents

Parameters	Treatment Groups						SEM*	Probabilities			
	V100	V110	V120	V130	V140	V150		*P- value*	*Linear*	*Quadratic*	*Cubic*
Glucose, mg/dL	190.5^{bc}	188.0^{c}	182.5^{c}	195.8^{abc}	205.9^{ab}	207.8^{a}	2.49	0.011	0.002	0.098	0.215
Triglyceride, mg/dL	32.8^{abc}	28.9^{c}	36.6^{a}	34.6^{b}	36.1^{a}	29.9^{bc}	0.76	0.005	0.693	0.017	0.012
Cholesterol, mg/dL	101.0	93.5	106.0	99.6	105.4	99.8	1.70	0.317	0.509	0.644	0.255
HDL, mg/dL	67.8	63.6	70.4	66.0	73.4	71.1	1.30	0.268	0.121	0.676	0.413
Total protein, g/dL	2.56^{b}	2.73^{ab}	2.80^{a}	2.79^{a}	2.68^{ab}	2.80^{a}	0.244	0.022	0.029	0.067	0.035
Albumin, g/dL	1.07	1.17	1.15	1.11	1.12	1.11	0.010	0.416	0.549	0.253	0.097
Globulin, g/dL	1.49^{c}	1.56^{bc}	1.65^{ab}	1.68^{ab}	1.56^{bc}	1.69^{a}	0.018	0.002	0.002	0.109	0.074
Creatinine, mg/dL	0.266^{b}	0.273^{b}	0.278^{b}	0.299^{a}	0.281^{ab}	0.288^{ab}	0.003	0.027	0.010	0.157	0.664

*Standard error means.
a,b,c:Within a row, values not sharing a common superscript are statistically different; P ≤ 0.05.
V100: Requirement level; **V110:** valine increased by 10% of requirement; **V120:** valine increased by 20% of requirement; **V130:** valine increased by 30% of requirement; **V140:** valine increased by 40% of requirement; **V150:** valine increased by 50% of requirement.

Table 6. Effect of gradually increasing valine on jejunum histomorphology

Parameters	Treatment Groups						SEM*	Probabilities			
	V100	V110	V120	V130	V140	V150		*P- value*	*Linear*	*Quadratic*	*Cubic*
Villus height, μm	704^{b}	682^{cd}	695^{bc}	728^{a}	701^{b}	672^{d}	2.47	<0.001	0.182	0.001	<0.001
Villus width, μm	104.4^{bc}	105.0^{b}	113.7^{a}	106.4^{b}	103.6^{bc}	101.0^{c}	0.51	<0.001	0.007	<0.001	0.174
Crypt depth, μm	99.27^{a}	98.44^{ab}	94.16^{c}	88.61^{d}	79.77^{e}	94.89^{bc}	0.56	<0.001	<0.001	<0.001	<0.001
Villus height/ Crypt Depth	7.45^{cd}	7.22^{d}	7.67^{c}	8.63^{b}	9.10^{a}	7.55^{cd}	0.057	<0.001	<0.001	<0.001	<0.001
Villus surface Area, mm^2	0.231^{b}	0.225^{b}	0.246^{a}	0.244^{a}	0.227^{b}	0.212^{c}	0.001	<0.001	0.001	<0.001	0.022

*Standard error means.
a,b,c,d,e:Within a row, values not sharing a common superscript are statistically different; P ≤0.05.
V100: Requirement level; **V110:** valine increased by 10% of requirement; **V120:** valine increased by 20% of requirement; **V130:** valine increased by 30% of requirement; **V140:** valine increased by 40% of requirement; **V150:** valine increased by 50% of requirement.

DISCUSSION

Performance

According to the data in Table 2, the high performance obtained by increasing the diet valine level by 20% (V120) reduced in the V140 and V150 groups, although it was similar to the control group. The V120 group had the highest values for body weight (2397.8 g), body weight gain (2352.4 g), feed intake (3890.1 g), and feed conversion ratio (1.65) in throughout the trial. Literature data demonstrate that the results obtained are inconsistent because the strain, diet crude protein source and level, gender, and age employed in these researches are frequently different from one another. In one of these, Ospina-Rojas et al. (2017) reported that increasing level addition of valine to the diet (from 0.52% to 1.12%) linearly improved feed intake, body weight gain, and feed efficiency in Cobb 500 broilers. According to Corzo et al. (2008), higher valine content (from 0.73 to 1.15) resulted in enhanced body weight gain, feed intake, and feed efficiency. In addition, Corzo et al. (2007) stated that with gradually increasing levels of valine (0.59, 0.64, 0.69, 0.74, 0.79, 0.89%) body weight gain, feed intake, and feed efficiency improved linearly. Furthermore, Corzo et al. (2004) tried four different levels of valine (0.60, 0.72, 0.80, 0.83%) in Ross 308 broiler diets at 42-56 days and body weight, body weight gain, feed intake, and feed efficiency quadratically increased. Schedle et al. (2019) stated that increasing valine-lysine ratio (68, 73, 78, 83, 88%) improved performance in Ross 308 broilers at 8-21, 22-36 days and the whole rearing period and the optimum ratio was 80%. Moreover, although there are studies noting that dietary valine did not have a significant effect on broiler performance (Corzo et al., 2009; Maynard et al., 2021:2022; Attia et al., 2022), Berres et al. (2011) and Tavernari et al. (2013) documented that performance quadratically changed as the valine:lysine ratio increased from 0.69 to 0.85 for growing birds (8-21 and 30-43 days). These researches indicated that the need for valine had increased over time. This is mainly due to genetic selection, which increases the metabolic needs of nutrients in poultry, primarily amino acids, and strengthens the need for continuous updating of the nutritional requirements of broilers.

Slaughtering Characteristics

In the present study, relative thigh+drumstick, breast, and abdominal fat weights were significantly affected by the treatments, and high-level valine (140 and 150%) had a negative effect on the relative breast weight. The best values of these parameters were 30.66% (V130), 40.29% (V110) and 0.98% (V130), respectively. The effect of dietary valine administration on slaughtering characteristics was apparent, but different groups of birds performed best in terms of these traits. The early post-incubation period is crucial for the mechanisms controlling the overall capacity of body protein deposition (Vieira and Angel, 2012). Muscle fiber growth occurs is mainly due to the accumulation of myofibrillar proteins such as actin and myosin, which are proteins composed of about 18% branched-chain amino acids (Baracos and Mackenzie, 2006). Branched-chain amino acids make up 35-40% of essential amino acids in the body (Ferrando et al., 1995), so the inclusion of these amino acids can affect the diameter of muscle fibers. Adequate dietary valine is critical to supporting optimal growth, feed efficiency, and carcass characteristics (Corzo et al., 2007). Additionally, the effect of valine on reducing abdominal fat can be attributed to its reduction in fatty acid synthesis (Ospina-Rojas et al., 2017). Just as in the performance parameters, there is a contradiction among the literature data in terms of carcass characteristics. Ospina-Rojas et al. (2017) clarified that different levels of valine (from 0.52 to 1.12%) increased thigh+drumstick and reduced abdominal fat. Corzo et al. (2011) indicated that the addition of valine from 0.26 g/kg to 1.30 g/kg linearly increased carcass and breast meat, and decreased abdominal fat as quadratic. In addition, Corzo et al. (2008) expressed that carcass, breast, and thigh+drumstick improved with increasing valine level (from 0.73 to 1.15%), and in another study it was reported that rising the valine level from 0.59% to 0.89% increased breast meat (Corzo et al., 2007). These studies are partially similar to the current research. On the other hand, there are a considerable number of studies reporting that carcass characteristics are unaffected by the valine level (Corzo et al., 2004; Berres et al., 2011; Tavernari et al., 2013; Maynard et al., 2021:2022; Attia et al., 2022). However, as stated in the previous sections, the results on this issue are in conflict. Indeed, Dozier et al. (2011) expressed that the addition of valine was needed for broilers, but Agostini et al. (2019) ve Kop Bozbay et al. (2021) reported that valine supplementation was not critical in promoting higher carcass and breast weights or yield in broilers.

Nitrogen Excretion

Reducing dietary crude protein has become a trend, as excess crude protein in the diet poses significant problems for gut health and the environment. Although the crude protein level was not reduced in the current study, a linear decrease in nitrogen excretion was observed with the addition of valine, and decreased from 28.29% (V100) to 25.12% (V150). The decrease in the level of crude protein in the feces is important in terms of improving the use of diet protein as well as reducing the amount of nitrogen released into the environment. However, it was observed that the decrease in the fecal nitrogen did not reflect positively on the performance further the level of 120% (V120). This can be explained by the use of dietary nitrogen in processes such as feathering that need a high nitrogen concentration. The report of Maynard et al. (2021) who stated that feathering significantly improved with an increase in dietary digestible valine level from 0.57% to 0.78%, supports this opinion. According to these results, it can be said that diet nitrogen may have been used for feathering, but since feathering was not examined in the current study, this issue needs to be investigated in future studies. In the literature, a similar study was found with the present study in terms of materials and methods used in the experiment. In this study, Corzo et al. (2004) reported that four different levels of valine (0.60, 0.72, 0.80, 0.83%) performed in broiler diets did not affect nitrogen excretion. These outcomes disagree with the present research. However, although not conducted on broilers, Alagawany et al. (2014) stated that the addition of 0.1% valine to quail diets with reduced crude protein diminished fecal nitrogen compared to diets containing optimum crude protein.

Serum Biochemical Constituents

In the present research, cholesterol, HDL, and albumin were not significantly affected by the treatments, whereas there was a noticeable effect on glucose, triglyceride, total protein, globulin, and creatinine as quadratic or linear. Numerically, the highest serum glucose concentration was 207.8 mg/dL in the V150 group, and this result was found to be significant compared to the V100 (190.5 mg/dL), V110 (188.0 mg/dL), and V120 (182.5 mg/dL) groups. The glucostatic theory of appetite management is based on the connection between blood sugar and appetite. The increase in feed intake (0-42th days) in the current study also supports this opinion. Birds are characteristically hyperglycemic (Krzysik-Walker et al., 2008) and insulin resistant (Dupont et al., 2004), making them an interesting model for understanding the role of branched-chain amino acids in the regulation of hepatic fat metabolism. Bai et al. (2015) announced that low levels of branched-chain amino acids inhibit fatty acid synthesis and disrupt the lipid composition of the cell. In fact, the fluctuating effect seen on triglyceride in the current study can be explained by this. Total protein, albumin and globulin concentrations are important indicators of the protein status of the blood. In this study, total protein and globulin also improved with increasing valine level. As with the other parameters examined, there are differences between the results found in the literature and this study. In some of these studies (Zhang et al., 2007; Higuchi et al., 2011), it was indicated that branched-chain amino acids affect glucose metabolism. On the other hand, Corzo et al. (2009) in a study investigated the critical limits of a in broiler diets, stated that the inclusion of valine 0.15% more than the requirement did not affect serum glucose and uric acid, but increased total protein. In another, Ospina-Rojas et al. (2017) expressed that the addition of increased levels of valine (from 0.52 to 1.12%) to the diet did not affect serum glucose, triglyceride, total protein, and albumin. Apart from these, Alagawany et al. (2011) and Zeweil et al. (2011), reported that with the increase of dietary protein in laying hens, total plasma protein and globulin increased. In the same context, Mosaad and Iben (2009) stated that as dietary nitrogen intake increases, plasma total protein increases. Additionally, with the administration of 0.1% valine to the low crude protein diet, blood total protein and globulin concentrations decreased, but creatinine concentration was not affected (Alagawany et al., 2014).

Jejunum Histomorphological Traits

All poultry needs longer villi to create more surface area for better absorption of nutrients. Villus epithelial cells are falling into regularly due to interaction with food and intestinal microbes (Singh and Kim, 2021). These cells are regenerated from actively dividing crypts and crypt depth increases during villus atrophy, so the villus/crypt ratio is a good indicator of gut health in young birds (Wang et al., 2020; Singh et al., 2021). Despite the recognition of the important role of branched-chain amino acids in modulating gut health, no specific mechanism for their interaction was described in the available literature (Kim et al., 2022). In the present study, villus height, villus width, crypt depth, villus height/crypt depth, and villus surface area were quadratically affected by gradually increasing valine levels. It can be said that the digestion rate of branched chain amino acids in broilers is slow due to their hydrophobicity (Liu et al., 2013).

The fluctuating response to valine inclusions in the current study may be related to amino acid digestibility coefficients. The number of studies examining the effects of adding valine to the diet on small intestine morphology is limited in the literature. In one of these studies, Allameh and Toghyani (2019) determined that the supplementation of valine to the diet containing low crude protein increased the villus height in the jejunum and ileum by 29% and 17%, respectively, and positively affected protein accumulation. In addition, Jian et al. (2021) clarified that villus height and villus height/crypt depth increased with rising dietary valine (0.59-0.79%) in chickens, but crypt depth was not affected. In this study, the highest value in terms of villus surface area, which is the place of absorption of nutrients, was obtained in the V120 group, and this status was also reflected in the performance of the birds.

CONCLUSIONS

In conclusion, the performance parameters were significantly affected by the dietary valine level and the best results were obtained by increasing the valine level by 20%. However, this positive effect was not observed with increasing by 40% and 50%. Moreover, with the increase of valine level by 40% or 50%, the relative breast weight was negatively affected along with performance. Additionally, slaughtering characteristics, serum parameters, and jejunum histomorphology were also positively affected by the 20% increase of dietary valine level, but the effect was fluctuating. However, nitrogen excretion decreased with increasing valine level. Therefore, it can be said that increasing valine level in broiler diets by 20% performance and absorption area in the small intestine can enhance, but these fluctuations in other parameters demonstrated that the issue needs to be investigated in future studies.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest to disclose.

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