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Research Paper

The effects of dietary supplementation levels of valine on performance and immune System of broiler chickens

Murat KAPLAN[1]* • Gültekin YILDIZ[2]

[1]Virology Department, İzmir/Bornova Veterinary Control Institute, Erzene Mah. Ankara Cad. No:172/155 35040, Bornova, İzmir, Turkey.
[2]Animal Feeding and Nutritional Diseases Department, Veterinary Faculty of Ankara University, İrfan Baştuğ Cad. 06110, Dışkapı, Ankara, Turkey.

*Corresponding author. E-mail: kaplanmurat10@gmail.com. Tel: +90 544 232 84 82.

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Abstract. The aim of this work was to evaluate the effects of adding different levels of valine in broiler diets on broiler performance, weights of immune system organs, that is, thymus, bursa of Fabricius and spleen. Also, measure the antibody titers of birds due to feeding diets containing different levels of valin for different feeding periods. Five dietary treatments groups (Control, D_1, D_2, D_3 and D_4) each containing 49 birds were arranged. The valine levels of the control group were 0.9% for 0 to 21 days and 0.82% for 21 to 42 days as recommended by NRC (1994). D_1 and D_2 groups were 0.9% for 0 to 21 days as recommended by NRC (1994) and 0.92 and 1.02% valine upper value of 0.1 and 0.2% recommended by NRC (1994) for 21 to 42 days. D_3 and D_4 groups were 1 and 1.1% valine upper value of 0.1 to 0.2% recommended by NRC (1994) for 0 to 21 days, 0.82% for 21 to 42 days as recommended by NRC (1994). Different dietary valine levels in different feeding periods did not create any statistical difference except numerical differences on performance ($P > 0.05$) when all study periods were taken into account. Spleen and bursa of Fabricius were not observed to be affected by various dietary valine levels ($P > 0.05$), but there was significant statistical difference in thymus development on either 0 to 21 days or 21 to 42 days in groups fed with ratio containing 1% valine level ($P<0.05$). In terms of humoral immunity, although the highest degree of vaccination antibody titers were achieved in groups fed with a ratio containing 1.1% valine level, this was not considered to be statistically significant. It is recommended as a result of this study that, the optimal value of valine in broiler ratios were evaluated to be 1% for 0 to 21 days in contrast to NRC (1994) recommendations and 0.82% for 21 to 42 days as recommended by NRC (1994).

Keywords: Amino acid, broiler performance, immune response, valine.

INTRODUCTION

Feed stuff is the biggest cost item in industrial broiler farming and the protein sources are the biggest cost item in feed stuff. Soybean meal and/or foodstuffs of animal origin are used to provide protein. Leaving off the protein sources to reduce the costs is possible by adding the synthetic derivatives of essential amino acids such as lysine, methionine and threonine to the rations. Studies for the limiting amino acids in broiler rations have been performed on lysine, methionine and threonine; however there is not sufficient number of studies performed on valine. Very few studies performed on valine in the world are such as to support that the idea that this essential amino acid was another limiting amino acid after threonine (Corzo *et al*., 2009; Dozier *et al*., 2011).

However, the minimum level of the ration valine content is critical to ensure optimal growth, feed conversion and carcass yield (Corzo *et al*., 2008).

There is not much deficiency of amino acids such as isoleucine, valine, arginine and tryptophane in corn, soy and poultry meal based rations. However, the deficiency of these amino acids in these rations based on vegetable protein sources can be more critical. Therefore, valine can be the next limiting amino acid besides or after threonine in rations unprovided with animal protein sources (Thornton *et al*., 2006). Feather and leg anomalies (Farran and Thomas, 1992a, b), affected antibody titers (Bhargava *et al.*, 1971) and failure lymphoid organ development (Konashi *et al.*, 2000) occur in valine deficiency. In a small number of studies performed on valine, performance is affected negatively in case its optimal rates cannot be provided (Corzo *et al*., 2008).

The number of studies where valine requirement is researched in broiler rations is very insufficient and there are discrepancies between the values found. Although NRC (1994) recommendations stated that the 0.9% for the first period (0 to 21 days) and 0.82% for the second period (21 to 42 days) were suitable for optimum body weight gain and feed conversion, it was stated that more studies should be performed on this subject (NRC, 1994). In studies performed related to the valine rate required to be available in the ration, it was determined as 0.74% for the 8 to 21 days (Baker *et al.*, 2002); as 0,81% for the 14 to 21 days (Rodehutscord and Fatufe, 2005); 0.90% for the 0 to 21 days (Farran and Thomas, 1990); 1% for the 0 to 14 days (Corzo *et al.*, 2008) and 0.95% for the 14 to 28 days (Corzo *et al.*, 2008) in the first feeding period and as 0.85% for the 28 to 42 days (Corzo *et al.*, 2008); 0.72% (Mendoca and Jensen, 1989), 0.73% (Thornton *et al*., 2006), 0.82% (Corzo *et al.*, 2007), 0,85% (Berres *et al*., 2011) and 0.90% (Duarte *et al.*, 2014) for the 21 to 42 days in the second feeding period; and as the valine/lysine rate, it was found as 77.5% (Baker *et al.*, 2002) for the 8 to 21 days, 81% (Mack *et al*., 1999), 78% (Corzo *et al*., 2007), and 78% (Berres *et al.*, 2011) for the 22 to 42 days.

The maximum increase of weight with minimum cost is the most important factor in broiler feeding. The biggest component in the ration cost is the protein inputs. While revealing optimal rates with studies to be performed on the ration amino acids reduces the costs, it is of critical importance also in terms of preventing the antagonism to occur between amino acids. The purpose of the present study is to research the appropriate levels of use of valine in broiler rations and the effects of different levels of its use in different feeding periods on performance, immune system organ weights and vaccination antibody titers.

MATERIALS AND METHODS

The present study was conducted in Ankara University Veterinary Faculty Training Research and Application Farm - Broiler Test Poultry Farm. The study was found appropriate by Ankara University Animal Tests Local Ethics Committee's decisions no 2013-9-64 of 24.04.2013.

Animal husbandry

One control (C) and four test (D_1, D_2, D_3, D_4) groups were constituted on the purpose of researching the use of different levels of valine amino acid in broiler rations and the effects on the performance, that is, feed consumption, body weight gain, feed conversion ratio, carcass yield, and mortality rate. As well as immune response, and immune system organ weights including thymus, bursa of Fabricius and spleen. Also vaccination antibody titers were performed. A total of 245 Ross 308 male broiler chicks were used in the study. The birds were divided into 5 treatments each consisting of 49 chicks was organized for the test. Each group was separated into 7 replicates each consisting of 7 chicks and the trial was conducted in 35 divisions (each division was approximately 0.72 m^2).

Feed and water were given in feeders and waterers as *ad libitum* to the chick's implemented group feeding at the amounts that they could consume daily. The feeds were given in special plastic feeders one available in each divisions. The feeders were raised in parallel with the growth of animals. Feeders with 25 cm diameter weighing 12 kg capacity used in the first two weeks of age, while feeders were replaced by 35 cm diameter weighing 15 kg in the following weeks. Water was supplied by two drop type (nipple) waterers available in each division. The chicks were provided with fresh and clean water with regular controls over the water tank. The waterer lengths were raised in parallel with the growth of the animals. The poultry farm and the equipments used were disinfected before the study and hygiene and biosafety rules were obeyed.

Animals were placed into 90 cm long, 80 cm wide and 80 cm height 35 special divisions. 7 broiler chicks were placed into each division. This number was calculated considering animal welfare and the EU directives. 25 kg live weight is suggested for animal welfare. 18 kg live weight is appropriate for the divisions used in the study (each division is approximately 0.72 m^2) in terms of animal welfare (0.72 $m^2 \times 25$ kg). The chicks were estimated to reach 2.2 to 2.5 kg live weight as a result of the study. The estimated total weight per division as a result of the study is 15.4 to 17.5 kg and the divisions were made considering it would be appropriate to put 7 chicks to each division when this number was divided into the live weight of an average animal (17.5/2.5 = 7).

The ambient temperature was controlled hanging a thermometer into the poultry farm. The ambient temperature was kept at 32 to 35°C in the first two weeks and gradually decreased to reach about 20 to 24°C

Table 1. Ingredients and compositions of the experimental diets (g/kg).

Ingredients	0 – 21 Days					21 – 42 Days				
	C	D_1	D_2	D_3	D_4	C	D_1	D_2	D_3	D_4
Yellow corn	580.5	580.5	580.5	578.5	576.4	624.2	622.3	620.2	624.2	624.2
Hazelnut kernel meal	194.7	194.7	194.7	195.0	195.3	168.5	168.8	169.0	168.5	168.5
Soybean meal	165.0	165.0	165.0	165.0	165.1	140.0	140.0	140.2	140.0	140.0
Vegetable oil	19.1	19.1	19.1	19.8	20.5	27.5	28.1	28.8	27.5	27.5
Limestone	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Dicalcium phosphate	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Sodium chloride	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin premix[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
L-Valine	0.0	0.0	0.0	1.0	2.0	0.0	1.0	2.0	0.0	0.0
DL-Methionine	2.0	2.0	2.0	2.0	2.0	1.0	1.0	1.0	1.0	1.0
L-Lysine	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
L-Threonine	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Mineral premix[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

[1]Vitamin premix contains (per kg) 14 000 000 IU vitamin A, 4 000 000 IU vitamin D_3, 80 g vitamin E, 30 g vitamin K_3, 3 g vitamin B_1, 8 g vitamin B_2, 40 g niacin, 12 g pantothenic acid, 6 g vitamin B_6, 0.03 g vitamin B_{12}, 2 g folic acid, 0,15 g biotin, 50 g vitamin C.
[2]Mineral premix contains (per kg) 150 g mangan, 120 g iron, 150 g zinc, 14 g copper, 0,4 g cobalt, 3 g selenium.

Table 2. Calculated nutrient composition.

Ingredients	0-21 days					21-24 days				
	C	D_1	D_2	D_3	D_4	C	D_1	D_2	D_3	D_4
Valine, %	0.9	0.9	0.9	1.0	1.1	0.82	0.92	1.02	0.82	0.82
Metabolizable energy, kcal/kg			3000					3100		
Crude protein, %			22					20		
Calcium, %			1.01					1		
Phosphorus, %			0.7					0.67		
Lysine, %			1.1					1		
Methionine, %			0.5					0.38		
Threonine, %			0.8					0.74		

thereafter. The illumination was ensured with day light in the day and through normal bulbs at night. 24-hour illumination plan was implemented in the study. The ventilation was provided through 40 × 40 cm 3 ventilators and 70 × 90 cm 4 windows. Wood wool (medium thickness) was used in the poultry farm base. The bases were checked during the study and they were cleaned when they were wet, clean wood wool was placed again.

Experimental design

In the study, each group was given the rations including 22% crude protein (CP) and 3000 kcal/kg ME in the chick period (0 to 21 days) and 20% CP and 3100 kcal/kg ME in then chicken period (21 to 42 days). Corn, hazelnut kernel meal and soybean meal underlie the rations used in the study. In the first period of the study, the rate of valine for the control group was set as 0.9%, the value reported in the NRC (1994), no L-Valine was added to the D_1 and D_2 groups in the first feeding period, and L-Valine was added at the rates of 0.1 and 0.2% respectively to the D_3 and D_4 groups. For the second trial period, the rate of valine of the control group was set as 0.82%, the value reported in the NRC (1994), and L-Valine was added at the rates of 0.1 and 0.2% respectively to the D_1 and D_2 groups, no L-Valine was added to the D_3 and D_4 groups in the second feeding period. The rates of feed raw materials used in the test were shown in Table 1. The animals were vaccinated with Newcastle live vaccine on the 7th and 21st day.

Measurement

The raw ingredient compositions of experimental diets were determined according to methods reported in AOAC (1990). The formula suggested by TSE (1991) was used

Table 3. Effects of varying dietary valine amino acid levels on performance[1].

	Experimantal groups										P
	Control		D_1		D_2		D_3		D_4		
	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	
0-21 Days											
Body weight gain (g)	516.62	15.42	521.98	10.40	515.90	15.84	526.31	11.18	518.76	16.78	0.984
Feed consumption (g)	816.24	24.65	830.12	16.00	806.03	19.74	835.66	20.60	810.89	20.84	0.822
FCR	1.58	0.01	1.59	0.02	1.56	0.01	1.59	0.03	1.57	0.02	0.717
21-42 Days											
Body weight gain (g)	1420.70	33.09	1431.97	24.05	1416.88	64.27	1455.97	64.65	1407.77	61.77	0.972
Feed consumption (g)	2678.03	63.66	2720.07	43.82	2609.40	118.80	2743.37	115.08	2657.92	89.22	0.852
FCR	1.89	0.02	1.90	0.01	1.84	0.02	1.89	0.01	1.90	0.03	0.356
0-42 Days											
Body weight gain (g)	1937.31	44.14	1953.95	27.67	1932.78	74.14	1982.28	73.38	1926.53	73.66	0.969
Feed consumption (g)	3494.27	85.77	3550.19	48.37	3415.43	133.87	3579.03	128.25	3468.81	107.62	0.821
FCR	1.80	0.02	1.82	0.01	1.77	0.01	1.81	0.02	1.80	0.02	0.284

[1] The values indicate that the averages (x) and standart errors ($S\bar{x}$) of the 7 replicates in each group (n = 7).

in the calculation of metabolizable energy levels. The body weight and feed consumptions were weighted on the 1, 7, 14, 21, 28, 35 and 42 days and the body weight gain, feed consumptions, feed conversion ratio (feed consumption/live weight gain) were found over the differences between the weighing results. All animals were weighed individually on the 21st and 42nd days of the study and one animal from each subgroup, 35 × 2 = 70 animals in total, were randomly separated and slaughtered.

Their organs were separated with appropriate methods on the purpose of determining the relative organ weights of the immune system organs of thymus, bursa of Fabricius and spleen and evaluating the carcass parameters. Blood samples were received from each animal to determine vaccination antibody titers (Hemagglutination Inhibition-HI test) (OIE, 2012).

Statistical model

Data analysis was performed using General Linear Models (GLM) procedure of SPSS software program package (SPSS, 2010), version 16.0. All data were analyzed based on a completely randomized design using one way ANOVA. Duncan test was applied for the significance control of the difference between the groups. The rates of death were stated as percentage due to the number of deaths was not sufficient for the Chi-Square test.

RESULTS

In the first period of the study, no statistical difference was found between the BW, BWG, FC and FCR data obtained from D_3 (1% valine) and D_4 (1.1% valine) groups and the control groups (0.9% valine). In the second feeding period, no statistical difference was found between the BW, BWG, FC and FCR data obtained from D_1 (0.92% valine) and D_2 (1.02% valine) groups and the control groups (0.82% valine) (Table 3). Furthermore, the carcass weight and carcass performance were not affected by the addition of different levels of valine amino acid in different periods of experiment (Table 4).

No statistical difference was found between weight of spleen and bursa Fabricius and relative weight of the same organs ($P > 0.05$). But however, the organ weights related to thymus development obtained both at the end of the first period and at the end of the second period and the relative organ weights were found statistically significant between the groups ($P < 0.05$) (Table 5). The highest thymus weight obtained with the birds fed diet contains

Table 4. Average body weight, carcass weight and carcass performance (%) of the groups[1].

Parameter	N	Experimantal groups: Control $\bar{x}$	Control $S\bar{x}$	D_1 $\bar{x}$	D_1 $S\bar{x}$	D_2 $\bar{x}$	D_2 $S\bar{x}$	D_3 $\bar{x}$	D_3 $S\bar{x}$	D_4 $\bar{x}$	D_4 $S\bar{x}$	P
Body weight before slaughtering (g)	7	2066.00	99.60	2057.43	194.43	2068.00	162.48	2112.00	141.99	2074.86	161.31	0.970
Carcass weight (g)	7	1427.14	28.47	1447.29	60.92	1431.00	39.41	1455.14	33.47	1437.14	46.42	0.990
Carcass performance (%)	7	69.09	0.68	70.26	0.72	69.23	0.40	68.93	0.50	69.24	0.47	0.500

[1] The values indicate that average live weights before slaughtering, carcass weights (g) and carcass performances (%) (x) and their standart errors ($S\bar{x}$) of 7 broilers that randomly selected from each group (n=7).

Table 5. Average lymphoid organs weight of the groups[1].

Organ type	n	Experimantal groups: Control $\bar{x}$	Control $S\bar{x}$	D_1 $\bar{x}$	D_1 $S\bar{x}$	D_2 $\bar{x}$	D_2 $S\bar{x}$	D_3 $\bar{x}$	D_3 $S\bar{x}$	D_4 $\bar{x}$	D_4 $S\bar{x}$	P
21. Day												
Spleen, g	7	0.48	0.04	0.46	0.07	0.45	0.03	0.43	0.03	0.44	0.03	0.937
Spleen, % BW	7	0.08	0.01	0.08	0.01	0.08	0.01	0.07	0.01	0.07	0.01	0.961
Bursa of Fabricius, g	7	1.52	0.09	1.34	0.15	1.46	0.14	1.27	0.09	1.41	0.05	0.567
Bursa of Fabricius, % BW	7	0.26	0.01	0.23	0.03	0.25	0.02	0.22	0.02	0.24	0.01	0.629
Timus, g	7	1.70^{b}	0.04	1.72^{b}	0.05	1.71^{b}	0.06	2.08^{a}	0.14	2.02^{ab}	0.16	0.021
Timus, % BW	7	0.28^{c}	0.01	0.29^{bc}	0.01	0.29^{bc}	0.01	0.35^{a}	0.02	0.34^{ab}	0.02	0.016
42. Day												
Spleen, g	7	2.35	0.15	1.93	0.17	2.08	0.15	2.14	0.17	2.26	0.20	0.454
Spleen, % BW	7	0.11	0.01	0.10	0.01	0.10	0.01	0.10	0.01	0.11	0.01	0.525
Bursa of Fabricius, g	7	4.90	0.45	4.08	0.51	3.75	0.37	3.63	0.26	4.67	0.47	0.162
Bursa of Fabricius, % BW	7	0.24	0.02	0.20	0.03	0.18	0.02	0.17	0.01	0.23	0.03	0.176
Timus, g	7	9.48^{bc}	0.87	8.99^{c}	0.55	10.03^{abc}	0.85	11.91^{a}	0.49	11.41^{ab}	0.46	0.018
Timus, % BW	7	0.45^{bc}	0.04	0.43^{c}	0.03	0.48^{abc}	0.04	0.56^{a}	0.02	0.55^{ab}	0.03	0.038

a, b, c; Differences between mean values with different letters in the same line are statistically significant (*P* < 0.05).
[1] The values indicate average lenfoid organ weights (x) and standart errors ($S\bar{x}$) of 7 broilers that randomly selected from each group (n = 7).

1.0% valine (D_3) at 21 and 42 days of age. When both periods of the study were evaluated together, the highest thymus weights were obtained in the D_3 and D_4 groups.

The numeral differences between antibody titers obtained against the Newcastle virus vaccine was not found statistically significant (Table 6). During the study period, no sign of disease was observed in birds. The mortality rate during the study were followed up and recorded daily. The mortality numbers were found as 1, 0, 2, 0 and 1 and the mortality rates were found as 2.04, 0, 4.08, 0 and

Table 6. Average antibody titers against newcastle vaccine of the groups[1].

Days	n	Experimantal groups										P
		Control		D_1		D_2		D_3		D_4		
		$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	
Maternal antibody	7	9.00	0.30	9.00	0.21	9.00	0.21	8.85	0.34	9.28	0.18	0.835
21. Day	7	6.29	0.29	6.29	0.18	6.86	0.34	6.29	0.36	6.43	0.48	0.724
42. Day	7	7.71	0.87	7.71	0.89	6.14	0.34	7.14	0.55	8.29	0.52	0.237

[1]The values indicate average antibody titers (x) and standart errors ($S\bar{x}$) of 7 broilers that randomly selected from each group (n=7).

2.04% respectively in the C, D_1, D_2, D_3 and D_4 groups, respectively.

DISCUSSION AND CONCLUSION

Absence of statistical differences between the D_3 and D_4 groups which were fed by valine excessively in the first period of the study and the control groups in terms of performance shows that the rate of 0.9% recommended by NRC (1994) was enough in the first period and it had no positive effect. The study results contradict with some literature data (Baker et al., 2002; Rodehutscord and Fatufe, 2005) about researching the first period valine requirements. The difference in question is thought to be due to the study by Baker *et al*. (2002) covered the 8th to 21st day of the feeding period and the study by Rodehutscord and Fatufe (2005) covered the 14th and 21st days of the feeding period, difference of environment conditions and the conditions of poultry farms. The rate of 0.9% recommended by Farran and Thomas (1990) as well as NRC (1994) seems to be conforming to the results of the present study.

Absence of statistical difference between the D_1 and D_2 groups which were fed by valine excessively in the second period of the study and the control groups in terms of performance shows that the rate of 0.82% recommended by NRC (1994) was enough in the second period and it had no positive effects. Similarly, the findings are in accordance with the results of Corzo *et al*. (2007). The study results contradict the findings of Thornton *et a*l. (2006) and Berres *et al*. (2011) about researching the second period valine requirements. The value of 0.73% found in the study by Thornton *et al*. (2006) could not be compared because it was lower than the sublimit value of 0.82%. However, when the study by Thornton *et al*. (2006) is evaluated in itself, it was shown that increasing the valine rates in the rations from 0.72 to 0.82% did not have any positive effect on the performance, and the increasing the valine rate from 0.64 to 0.87% was shown to create a linear increase on BWG and FCR. The optimal rates were obtained with the value of 0.73% (Thornton *et al*., 2006). The carcass weights are in accord with the studies performed on the effect of valine amino acid on the carcass development (Corzo *et al*., 2007; Corzo *et al*., 2008).

When both periods of the study are evaluated together, the highest thymus weights were obtained in the D_3 and D_4 groups. In other words, amounts of valine of 0.1 and 0.2% given excessively in the first period of the study increased the organ development and relative organ weights. Konashi *et al*. (2000) have compared the NRC (1994) requirements and 16 and 50% lacking values. In this context, while a complete comparison could not be made with the study performed, the view of increasing the rate of valine in the ration, despite it did not affect the spleen organ weight and the relative weight, to affect the thymus in both studies and contribute to the development of immune system organs to be parallel shows that both studies were relatively are compatible. The study by Thornton *et al*. (2006) covers the different utilizations of valine between the 21st and 42nd days which are in tune with the results of the effect of different valine rates on the immune system in the second period researched on the D_1 and D_2 groups. In both studies, different valine rates in the second period do not increase the development of lymphoid organ. In the study by Bhargava *et al*. (1971), while the antibody titers increase with the increase of valine in the ration. As the causes and periods of antibody titers were different, marginal comparison was not been able to. When compared with the results of the study by Thornton *et al*. (2006), the results obtained in both studies were not found statistically significant.

In the present study, about adding different levels of valine to the broiler rations in different periods; because of the obtaining both the highest BW and statistically importance of thymus development, it is considered that the valine rate required to be in the ration of the first period feeding to be 1% would be appropriate generally. Because of the differences not found between the groups in the data obtained in the second period, it is considered that the rate of valine required to be in rations could be 0.82% the level recommended by NRC (1994), and the excessive amount could be unnecessary due to absence of any direct influence on either performance or immune system.

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