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The Effects of Dietary Valine on Performance, Serum Antibody Titre and Bone Mineralization in Broiler Chicks

Research Article

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ABSTRACT

The present study was carried out to examine the effect of supplemental dietary valine in the starter diet of broilers (0-15 d of age) on performance, antibody response to Newcastle disease virus vaccination and bone mineralization. The experiment was conducted using 300 Ross 308 male broilers. One-day-old chicks were randomly allotted to 3 groups; each group consisted of 5 replicate pens with 20 birds per pen. Chickens were fed one of three dietary treatments with varying concentrations of valine; the first diet was adequate in valine (0% valine), the second and third diets had an excess in valine (0.08% and 0.16% synthetic valine in diet) because valine was added in place of inert filler. The results showed that supplemental dietary valine did not alter either feed intake, body weight gain or feed conversion ratio. Hemagglutination Inhibition titre was significantly increased when valine was added to the diet (P<0.05). The supplemental dietary valine had a significant effect on the calcium concentration in bone (P<0.05), but there was no significant effect on the phosphorus concentration in bone.

KEY WORDS antibody response, bone mineralization, broiler chicks, valine.

INTRODUCTION

A deficiency of dietary protein or amino acids has long been known to impair immune function and increase the susceptibility of animals and humans to infectious diseases (Li *et al.* 2007). Defining roles of individual amino acids in immune responses can aid in developing effective strategies to improve health. Bhargava *et al.* (1971) evaluated valine responses when chicks were inoculated with Newcastle disease virus; results indicated that valine requirement for antibody production was greater than that for growth (Bhargava *et al.* 1971). Importantly, branched-chain amino acids including leucine, isoleucine and valine provide the α-amino group for the synthesis of glutamine primarily in skeletal muscle, which has been considered as a part of the immune system (Newsholme *et al.* 1997). Problems associated with leg disorders persist in the broiler industry despite continued genetic selection for birds with improved skeletal conformation. Although calcium and phosphorus levels tend to be the primary focus of nutritional efforts to reduce the incidence or severity of leg disorders, other nutrients may also interact in this regard.

One nutritional aspect that is worthy of attention is the relationship that exists between dietary amino acids levels and calcium absorption and excretion (Coto *et al.* 2009). Farran and Thomas (1992) investigated the problem of leg abnormality associated with feeding a valine deficient diet as compared with a diet deficient in all of the branched chain amino acids or a valine supplemented diet. Bone ash and calcium content for valine-deficient birds were the lowest among the three groups studied. The results also indicated that valine deficiency per se increased calcium excretion in urine and induced leg abnormality in young chickens. Therefore, we examined responses to valine supplementation in Ross 308 male broiler chicks, at equal dietary crude protein levels, from 1 until 15 d post hatch. The goal of this study was to determine whether there is an effect on growth performance, humoral immune response and bone mineralization in broiler chickens fed diets with increased level of valine.

MATERIALS AND METHODS

Bird husbandry

Three hundred one-day-old Ross 308 male broiler chicks were used within 3 treatments and 5 replications with 20 birds per pen. Stocking density was 10 birds per 1 m^2 of floor space.

Light was provided 23 h daily. Birds and feed were weighed at placement on d 0 and at the end of the experimental phase (15 d) for the determination of body weight gain, feed consumption and feed conversion. Mortality data were collected and the weights of dead birds were recorded daily.

Mortality was low for all treatments; therefore, feed intake was adjusted for mortality to calculate feed efficiency. At d 10, chicks were vaccinated for Newcastle by eye drop. At d 15, blood from 2 birds per replicate was sampled for antibody titer against Newcastle disease by hemagglutination inhibition (HI) test and 3 birds per replicate were slaughtered to evaluate calcium and phosphorus concentrations of femur and tibia.

Diets

The experimental diet based on corn and soybean meal was formulated to meet or exceed all marginal amino acids (QuickChick, 2006) except for valine. Broiler chicks were fed diets with different concentrations of L-valine (0%, 0.08% or 0.16%) to provide recommendation or more than recommendation of valine amounts from 1 to 15 d of age. Before constructing least-cost experimental diets, the total amount of soybean meal and corn to be used for the study was acquired and analyzed for all amino acids (Liames and Fontaine, 1994) and crude protein (AOAC, 1995) (Table 1). Digestible coefficients were multiplied by the amino acid levels analyzed of the ingredients to calculate percentage digestible amino acid values. Ingredient and nutrient compositions of the feed mixtures are presented in Table 2. Chicks consumed feed mash and water on an *ad libitum* basis.

Hemagglutination inhibition (HI) test

Blood samples were allowed to sit overnight at room temperature to separate blood cells and serum.

Hemagglutination inhibition test was performed according to the method described (Thayer and Beard, 1998; Villegas, 1999).

Table 1 Amino acid and crude protein composition of ingredients (% as-fed)

Amino acid tested	Corn	Soybean meal
Met	0.16	0.6
Cys	0.18	0.67
Met + Cys	0.33	1.28
Lys	0.24	2.74
Thr	0.29	1.79
Try	0.06	0.62
Arg	0.37	3.34
Ils	0.26	2.09
Leu	0.92	3.50
Val	0.36	2.18
Crude protein	7.81	46.22

Table 2 Ingredients (g/kg as-fed basis) and calculated composition of experimental diets

Ingredient	0% valine	0.08% valine	0.16% valine
Corn	552	552	552
Soybean meal	386	386	386
Vegetable oil	14	14	14
DL-met	3.2	3.2	3.2
L-lys	2.2	2.2	2.2
L-thr	0.8	0.8	0.8
L-val	0	0.8	1.6
Dicalcium phosphate	20	20	20
Oyster shell	12	12	12
Salt	3.2	3.2	3.2
Vitamin-mineral premix[1]	5	5	5
Sand	1.6	0.8	0
ME, kcal/kg	2900	2900	2900
CP, %	22.30	22.35	22.40
Ca, %	1.03	1.03	1.03
Available P, %	0.49	0.49	0.49
Lys (SID), %	1.25	1.25	1.25
Met (SID), %	0.61	0.61	0.61
Met + Cys (SID), %	0.90	0.90	0.90
Thr (SID), %	0.79	0.79	0.79
Val (SID), %	0.92	1.00	1.08
Val / Lys (SID)	0.74	0.80	0.86

[1] Provided per kilogram of diet: vitamin A: 9000 IU; vitamin E: 18 IU; vitamin B_{12} 0.015 mg; Cholecalciferol: 2000 IU; Riboflavin: 6.6 mg; Niacin: 30 mg; Pantothenic acid: 25 mg; Menadione: 4 mg; Folic acid: 1 mg; Thiamin: 1.8 mg; Pyridoxine: 2.9 mg; Biotin: 0.15 mg; Se: 0.2 mg. Mn: 99 mg; Zn: 84 mg; Fe: 50 mg; Cu: 10 mg and I: 0.99 mg.

Briefly, 25 µL of each serum sample diluted in 100 µL of newcastle disease viruses (NDV) antigen was placed in the first column of 96 well plates. The rest of the wells in the plate contained 50 µL of NDV antigen. Then twofold serial dilutions followed by passing 50 µL from one column to the next to the end of the plate. The antigen used to dilute the serum contained 10 hemagglutination activities HA units. Then, 50 µL of prepared 0.5% chicken red blood were added into each well and allowed to incubate for 45 min at room temperature before HA was read. The HI titer was determined by the well of the highest serum dilution where a clear button was visible.

Ca and P determination

The femur and the tibiotarsus were collected from the right pelvic limb. The bones were freed of soft tissue, partially defattened with ether for at least 24 hours, ashed at 550 ˚C. Ca and P were assessed by ashing, extraction and titration under conditions outlined in AOAC (1995).

Statistical analysis

A completely randomized design was used in this experiment. Data collected were analyzed using general linear model of SAS (SAS, 2004) by following model:

$$Yij= \mu + Ai + eij$$

Where:
μ: the common mean.
Ai: the effect of the ith valine.
eij: random error.

Differences among treatments were compared using a Duncan's multiple range tests. All statements of significance are based on the 5% level of probability.

RESULTS AND DISCUSSION

The effect of increasing valine concentration on broiler performance for the 0-15 d period is shown in Table 3.

Table 3 Effects of dietary levels of valine supplementation on live performance of broiler chicks

Parameters	0% valine	0.08% valine	0.16% valine	SEM
Weight gain, g	274	277	286	13.81
Feed intake, g	511	532	521	9.35
FCR	1.88	1.96	1.83	0.07
HI titer, log_2	1.63[b]	1.75[b]	2.63[a]	0.18
Ca, mg/g of dried bone	63.9[b]	74.9[ab]	84.2[a]	5.50
P, mg/g of dried bone	9.9	9.7	8.9	0.43

FCR: feed conversion ratio.
HI: hemagglutination inhibition.
SEM: standard error of the means.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Increasing the recommended amount of valine in the diet led to slightly improved body weight gain although not significantly. There were no differences in feed intake among treatments (511, 532, 521 g for 0%, 0.08% or 0.16% valine treatments, respectively). Increasing the recommended amount of valine in starter period did not result in a significant effect in feed conversion ratio. Before the NDV vaccination, no significant difference in HI titer was evident between groups.

During the 0-15 d growth period, chicks showed a significant response to valine supplementation on HI test for antibody production against Newcastle virus. Antibody production against Newcastle virus increased numerically by adding 0.08% dietary valine, whereas 0.16% valine supplementation resulted in a significant greater antibody production against Newcastle virus (1.63, 1.75, 2.63 for 0%, 0.08% or 0.16% valine treatments, respectively, P<0.05, Figure 1). Furthermore, chicks responded to increasing dietary valine supplementation in calcium concentration of the femur and tibia; calcium concentration in femur and tibia increased numerically by adding 0.08% dietary valine, whereas 0.16% valine supplementation showed a significant increase in calcium concentration (63.9, 74.9, 82.2 mg/g of dried bone for 0%, 0.08% or 0.16% valine treatments, respectively, P<0.05, Figure 2); however, concentration of phosphorus in femur and tibia slightly decreased (P>0.05).

In the present study, there were no significant differences in growth rate, feed intake and feed conversion ratio among birds fed diets containing 0.92%, 1.00% or 1.08% of digestible valine (Table 3). These findings are consistent with the observations by Bhargava *et al.* (1971) who indicated that the valine requirement for antibody production was greater than that for growth. Chicks responded to increasing dietary valine concentration in antibody titer (P<0.05, Table 3). Dietary valine supplementation was found to exert an immunomodulatory effect on specific antibody production against Newcastle virus in the present study. This finding is consistent with Bhargava *et al.* (1971) that observed deficiencies of valine reduced antibody production against Newcastle virus in chickens. Increasing evidence shows that dietary supplementation of specific amino acids to animals and humans with malnutrition or infectious disease enhances the immune status, thereby reducing morbidity and mortality (Baker *et al.* 2002). The low HI titers in chicks fed low levels of valine *vs.* high titers in chicks fed high levels of valine can also be explained on the basis of the high requirement of valine for production of gamma globulin. In this study, chicks responded to increasing dietary valine levels in calcium concentration in femur and tibia (P<0.05, Table 3). Dietary valine supplementation was found to exert a greater calcification effect on femur and tibia. This finding is consistent with reports from Farran and Thomas (1992) that observed birds on valine deficient diet were lethargic and presented leg abnormalities. Bone ash and bone calcium for valine deficient birds were decreased, and plasma hydroxyproline was low in birds fed the valine deficient diet, indicating a reduction in bone collagen breakdown. Fractional excretion of calcium in valine deficient birds was three times higher than that of valine supplemented birds.

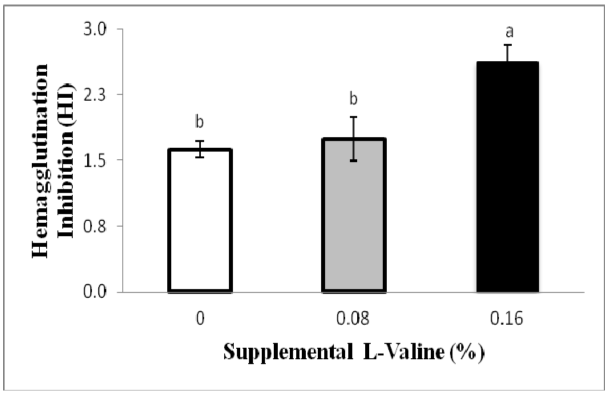

Figure 1 Effects of dietary levels of valine supplementation on hemagglutination inhibition test for antibody production against Newcastle disease virus of broiler chicks during 0-15 days (P<0.05)

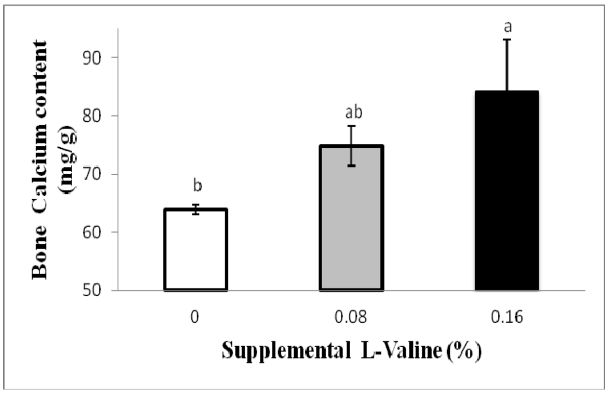

Figure 2 Effects of dietary levels of valine supplementation on calcium concentrations in femur and tibia of broiler chicks during 0-15 days, mg/g of dried bone (P<0.05)

Results reported by Farran and Thomas (1992) also indicated that valine deficiency per se increased calcium excretion in urine and induced leg abnormality in young chickens. Whereas no leg abnormality was observed, in the current study, calcium concentration of urine was not measured. Some of the variability in branched-chain amino acid estimates may be attributable to a lack of available nitrogen because diets have had reduced crude protein levels in an attempt to create branched-chain amino acid limitations (Burnham *et al.* 1992; Kidd *et al.* 2004). Improving the plane of nutrition in the starter phase diet to increase nutrient intake has received much attention and has been noted to be a valuable formulation strategy under certain feeding regimens (Kidd *et al.* 1998; Mozdziak *et al.* 2002).

Valine can be next-limiting to threonine in diets void of animal protein ingredients (e.g., diets containing supplemental methionine, lysine and threonine sources) (Corzo *et al.* 2007; Farran and Thomas 1990). The ideal protein concept has been developed, in which amino acid requirements are often expressed as ratio to lysine, leading to an ideal amino acid profile in which all indispensable amino acids are equally limiting. Using digestible levels of dietary amino acids eliminates differences in absorption and utilization from various sources (Emmert and Baker, 1997). The NRC (1994) recommendation for total valine-lysine ratio in the starter period is 0.82.

Farran and Thomas (1990) estimated valine requirements during the starter period (valine-lysine ratio: 0.82) which were later found to be in agreement with the NRC (1994) recommendation. However, research by Baker *et al.* (2002) estimated digestible valine-lysine ratio from 8 to 21 d of age to be 0.78 in diet.

The recommendation for digestible valine-lysine ratio for the starter diet is 0.75. In the present study, valine-lysine ratios in the starter diets were 0.74, 0.80 or 0.86. Dietary valine should be adequate to minimize amino acid excesses and provide a diet that closely meets the needs of the birds. This is particularly true in diets in which crude protein is being reduced but an adequate dietary valine is critical for supporting optimal growth, feed conversion, and carcass traits (Corzo *et al.* 2008).

CONCLUSION

Current trends in the broiler industry are to feed high levels of dietary essential amino acids in starter diets to enhance early growth rate. Our observations suggest that chicks fed valine levels slightly higher than the NRC (1994) recommendations can better support immune system and assimilate higher levels of calcium concentration in bone. Based on the present results, the valine-lysine ratio recommendation may need to reach 0.86 for high-yielding Ross 308 male broilers from 0 to 15 d of age.

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