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Digestible Valine Requirements in Low-Protein Diets for Broilers Chicks

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ABSTRACT

Three experiments were carried out to evaluate the levels of digestible valine in diets with reduced crude protein on the performance, carcass yield and muscle fiber diameter of male broilers during the pre-starter (1 to 8 d of age), starter (9 to 21 d of age) and grower phases (21 to 42 d of age). A total of 1,080 chickens in the pre-starter phase, 900 in the starter phase and 864 in the grower phase were distributed in a completely randomized design with six treatments, consisting of a control positive diet (C+) and diets with 2 percentage points (p.p.) reduction of crude protein level and five graded levels of digestible valine (Val), with six replicates of 30, 25 and 24 birds per experimental unit, respectively. The recommended level of TID Val in the low-protein diets for broilers in the pre-starter, starter and grower stages were 1.028; 0.905 and 0.789%, respectively. The reduction of 2 percentage points of the crude protein level in diets based on corn and soybean meal impaired ($p \leq 0.05$) the feed conversion ratio in the starter and grower stages. Likewise, the reduced-protein diets decreased ($p \leq 0.05$) muscle fiber diameter, but did not affect ($p > 0.05$) carcass and parts yields, or abdominal fat percentage at 42 days.

INTRODUCTION

Poultry production is as one of most important agribusinesses in Brazil. The continuous progress of this industry is a result of advances in scientific knowledge and technologies, such as nutrition, which is essential to obtain the best poultry performance (Campos *et al.*, 2012).

Changes in the international market related to the ban of the inclusion of animal products in animal feeds, and feed formulation manipulations aiming at reducing the excretion of potential pollutants, such as nitrogen, have led to formulation of broiler diets with reduced protein content. Reduced-protein diets may change amino acid availability and promote negative interactions among amino acids, resulting in poor performance. One of such interactions is that among valine, isoleucine, and leucine, are branched-chain amino acids (BCAA). Corn and soybean meal proteins contain more leucine than valine and isoleucine, which may increase the requirement of the last two amino acids in broiler diets, particularly during the grower phase (Berres *et al.*, 2010) and when reduced-protein diets are fed to broilers. Valine is more susceptible than isoleucine to the increase in dietary leucine levels (D'Mello, 2003), and it is considered the fourth limiting amino acid in broilers diets based on corn and soybean meal with no inclusion of animal feedstuffs (Thornton *et al.*, 2006; Tavernari *et al.*, 2013). Valine requirements increase with broiler age, when dietary crude protein content is reduced (Corzo *et al.*, 2007; Corzo *et al.*, 2010; Duarte *et al.*, 2014).

The objective of this experiment was to evaluate the effects of different digestible valine levels in pre-starter, starter, and grower diets with reduced crude protein on the performance, carcass yield, and muscle fiber diameter of broilers.

MATERIAL AND METHODS

All procedures used in this study were approved by the Ethics and Research Committee of State University of Maringá, under protocol CEUA/UEM 1361/2014.

Three experiments were carried out in the poultry sector of the Experimental Farm of Iguatemi of the State University of Maringá. In the three experiments, all birds were reared in floor pens (2.0 × 1.0 m) with fresh wood shavings as litter. Each pen was equipped with one tube feeder and six nipple drinkers. Temperature was maintained at 32°C at placement and was gradually reduced to ensure comfort using a heater controlled by a thermostat, exhaustion fans, and cool cells. Ventilation was accomplished by negative air pressure. The lighting program throughout the study consisted of 23 h of light and 1 h of dark. Feed and water were available to the birds *ad libitum*.

In experiment I, 1,080 one-day-old male Cobb 700 broilers, with 50.66g ± 0.89 average initial body weight, were distributed according to a completely randomized design, into six treatments with six replicates of 30 birds per pen (density of 0.76 kg.m^{-2}). The treatments consisted of a control positive (C+) diet based on corn and soybean meal, containing22% crude protein, as recommended by Rostagno *et al.* (2011), and five different levels of digestible valine (0.866, 0.935, 1.009, 1.084, and 1.158%) in the diet formulated with 2% reduction of crude protein (CP) level. The dietary levels of valine were obtained by supplementing the reduced-protein diet with L-valine at the expense of inert filler, kaolin. The digestible valine levels (TID Val) corresponded the recommendations for the pre-starter diet (1.009% TID Val), according to Rostagno *et al.* (2011), and two levels below and two levels above of that recommendation.

In experiment II, 900 9-d-old male Cobb 700 broilers, with 209.45 g ± 2.93 average body weight, were distributed according to a completely randomized design into six treatments with six replicates of 25 birds per pen (density of 2.618 kg.m^{-2}). The birds were fed a conventional diet from one to eight days of age, prior to the experimental period. The treatments consisted of a control positive (C+) diet based on corn and soybean meal, containing 20% crude protein, as recommended by Rostagno *et al.* (2011), and five different levels of digestible valine (0.755; 0.830; 0.904; 0.979 and 1.053%) in the diet formulated with 2% reduction of crude protein (CP) level.

In experiment III, 864 21-d-old male Cobb 700 broilers, with 924 g ± 18.6 average body weight, were used. The reduction in bird number in order to maintain rearing density (9.24 kg.m^{-2}). Birds were distributed according to a completely randomized design into six treatments with six replicates of 20 birds each per pen. The birds were fed a conventional diet from one to 21 days of age, prior to the experimental period. The treatments consisted of a control positive (C+) diet based on corn and soybean meal, containing 18.5% crude protein, as recommended by Rostagno *et al.* (2011), and five different levels of digestible valine (0.695; 0.755; 0.815; 0.875 and 0.935%) in the diet formulated with 2% reduction of crude protein (CP) level.

The positive control (C+) diets and the basal diet with reduced crude protein (CP) levels are shown in Table 1.

Broilers and feed were weighed at the beginning and end of each experimental period (1 to 8 d, 9 to 21 d and 21 to 42 d of age) for performance evaluation (feed intake, weight gain, and feed conversion ratio). At 42 days of age, at the end of the grower period (Experiment III), carcass yield and muscle fiber diameter were determined.

Two birds per experimental unit (12 birds per treatment), with the average body weight of the experimental unit (±5%), were selected and fasted for 06 hours. Birds were sacrificed by intravenous administration of sodium thiopental (70mg/kg), followed by section of the jugular vein. Carcasses were plucked, eviscerated, and weighed to determine carcass yield, calculated as carcass weight relative to live body weight. Breast, leg (thigh and drumstick), back, and wing yields were calculated as the weight of these parts relative to eviscerated carcass weight. The abdominal fat surrounding the cloaca, gizzard, proventriculus, and adjacent abdominal muscles was removed, weighed, and expressed as a percentage of eviscerated carcass weight.

Two other birds per experimental unit (12 birds per treatment), with the average body weight of the experimental unit (± 5%), were then selected to measure the diameter of the muscle fibers of the *pectoralis major*. Birds were sacrificed as described above. A tissue block of the *pectoralis major* muscle (1 cm x 1 cm x 2.5 cm) of two broilers per pen was cross-sectioned perpendicularly to the direction of

Table 1 – Ingredients and calculated composition of the control diet (C+) and of the reduced-protein diet (RP) fed during pre-starter, starter, and grower phases.

Ingredients	Pre-starter		Starter		Grower	
	C+	RP	C+	RP	C+	RP
Corn	55.15	64.12	59.08	69.44	64.98	72.53
Soybean meal 45%	38.04	29.17	34.77	24.68	28.60	21.01
Soybean oil	2.18	0.60	2.20	0.38	2.92	1.62
Limestone	0.96	0.96	0.97	0.97	0.86	0.86
Dicalcium phosphate	1.91	1.99	1.51	1.59	1.18	1.25
Salt	0.50	0.50	0.48	0.48	0.45	0.45
Vit.+ Min. supplement [1,2]	0.40[1]	0.40[1]	0.40[1]	0.40[1]	0.40[2]	0.40[2]
DL-Methionine 99%	0.361	0.437	0.287	0.373	0.255	0.321
L-Lysine HCl 78,5%	0.294	0.566	0.217	0.526	0.240	0.473
L-Threonine 98%	0.108	0.225	0.058	0.191	0.055	0.156
L-Arginine 99%	0.022	0.278	-	0.252	0.038	0.217
L-Tryptophan 99%	-	0.023	-	0.024	-	0.043
L-Valine 98%	0.079	-	0.024	-	0.038	-
L-Isoleucine 98%	-	0.168	-	0.150	0.028	0.136
Inert material[3]	-	0.560	-	0.540	-	0.530
Calculated Values						
Crude Protein, %	22.0	20.0	20.0	18.0	18.5	16.5
Metabolizable energy, Mcal/kg	2950	2950	3000	3000	3125	3125
Calcium, %	0.92	0.92	0.82	0.82	0.69	0.69
Available phosphorus, %	0.47	0.47	0.391	0.391	0.320	0.320
Chlorine, %	0.406	0.458	0.388	0.441	0.367	0.367
Sodium, %	0.217	0.217	0.210	0.210	0.198	0.198
Potassium, %	0.856	0.719	0.788	0.653	0.711	0.595
TID met+cys, %	0.944	0.944	0.846	0.846	0.762	0.762
TID Lysine, %	1.310	1.310	1.174	1.174	1.044	1.044
TID Threonine, %	0.852	0.852	0.763	0.763	0.679	0.679
TID Arginine,%	1.415	1.415	1.268	1.268	1.268	1.268
TID Valine, %	1.009	-	0.904	-	0.815	0.695
TID Tryptophan,%	0.248	0.223	0.224	0.224	0.198	0.198
TID Isoleucine, %	0.878	0.878	0.787	0.787	0.710	0.710
TID Leucine, %	1.709	1.508	1.610	1.401	1.497	1.323
TID Phenylalanine, %	0.928	0.854	0.936	0.770	0.845	0.705
TID Histidine, %	0.498	0.461	0.502	0.420	0.457	0.388

1 - Starter vitamin supplement (amount per kg of premix): Vit. A 2,916.67 IU; Vit. D3 583.33 IU; Vit.E 8,750.00 mg; Vit. K3 433.33 mg; Vit. B1 408.33 mg; Vit. B2 1,333.33 mg, Vit. B12 4,166.67 µg; Niacin 8,983.33 mg; Pantothenic Acid 3,166.67 mg; Folic Acid 200.00 mg; Biotin 25.00 mg; Choline 67,500 mg; Mineral supplement (amount per kg of premix): Iron 12,600.00 mg; Copper 3,072.00 mg; Iodine 248.00 mg; Zinc 12,600.00 mg; Manganese 15,004.00 mg; Selenium 61.200 mg; Cobalt 50.400 mg.

2 - Grower vitamin supplement (Amount per kg of premix): Vit. A 2,250.000 IU/kg; Vit. D3 450,000 IU/kg; Vit. E 7,000 IU/kg; Vit. K3 418 mg/kg; Vit. B1 300 mg/kg; Vit. B2 1,000 mg/kg, Vit. B12 3,000 mcg/kg; Niacin 7,000 mg/kg; Pantothenic acid 2,500 mg/kg; Folic Acid 140 mg/kg; Biotin 14 mg/kg; Mineral supplement(amount per kg of premix):Iron 12.5 g/kg; Copper 3,000 mg/kg; Iodine 250 mg/kg; Zinc 12.5 g/kg; Manganese 15 g/kg; Selenium 75 mg/kg; Cobalt 50 mg/kg.

3 - L-Valine 98% addition in substitution of inert material (kaolin), according to the digestible valine levels in diet.

the myofibers and stored in liquid nitrogen. Next, the samples were transferred to cryostat chamber (Leica CM1850, Leica Microsystems GmbH, Wetzlar, Germany) at -23ºC, where they remained for 40 min for thermal adaptation. Serial cryostat sections (10-µm; –20°C) were made and captured with glass slides for histology (Pullen, 1977). Slides were stained with hematoxylin and eosin, and analyzed under a light microscope at 10Xmagnification, coupled to a digital camera attached. Ten images were captured per bird and the obtained images were analyzed using the software Motic® Images. All fibers were measured, using the small fiber diameter measuring method, according to Dubowitz & Brooke (1973).

Data were submitted to analysis of variance. The F-test ($p<0.05$) was applied to the compare two treatments, the control positive (C+) treatment and the recommended TID Val levels in the treatments with reduced crude protein (1.009% in the pre-starter phase; 0.904% in the starter phase and 0.815% in the grower phase, separately in each phase). The data obtained with five digestible valine levels in the reduced CP diet were also submitted to analysis of regression. A significance level of $p\leq0.05$ was adopted. The statistical analyses were carried out using the software Statistical and Genetic Analysis System (SAEG, 2005).

RESULTS AND DISCUSSION

In the pre-starter phase (Experiment I), dietary TID Val levels had a quadratic effect ($p \leq 0.05$) on weight gain and feed conversion ratio, with the highest weight gain estimated at 1.013% and the best feed conversion ratio at 1.028% TID Val level.

Birds fed the reduced-protein diet with1.009% TID Val presented higher feed intake ($p \leq 0.05$) and higher weight gain ($p \leq 0.05$) compared with those fed the C+ diet, containing the same TID Val level, during the pre-starter phase (Table 2). The higher feed intake observed in treatments with reduced protein may be explained by the supplementation of industrial amino acids, which may have caused an excess of essential amino acids, stimulating feed intake in order to compensate a possible deficiency of nitrogen for the synthesis of non-essential amino acids.

There were no effects of the evaluated TID Val levels ($p>0.05$) on feed intake or weight gain in the starter phase (Experiment II) and grower phase (Experiment III). However, in both phases, there was a quadratic effect ($p \leq 0.05$) of TID Val levels on feed conversion ratio, with the best levels estimated at 0.905% and 0.853% for the starter and grower phases, respectively. The best estimated level for the starter phase (0.905% of TID Val) is close to that recommended by Rostagno

Table 2 – Effect of dietary TID Val levels on broiler performance from1 to 8 (Exp I), 9 to 21 (Exp II) and 21 to 42 days (Exp III).

TID Valine (%)	Feed intake (g/bird)	Weight gain (g/bird)	Feed conversion ratio
1 to 8 days			
C+	135.561 ± 4.44	112.740 ± 3.02	1.202 ± 0.03
0.866	141.332 ± 4.37	109.067 ± 3.94	1.296± 0.06
0.935	137.919 ± 5.75	110.285 ± 4.19	1.250± 0.05
1.009	138.680 ± 5.75	114.875 ± 1.97	1.207 ± 0.06
1.084	139.101 ± 7.14	114.001 ± 6.75	1.221± 0.05
1.158	135.655 ± 4.12	107.626 ± 1.46	1.268 ± 0.01
p-value	0.017	0.005	0.001
Regression	NS	[1]Q = 1.013	[2]Q= 1.028
C+ *vs.* 1.009	≤0.001	≤0.001	NS
CV (%)	2.78	3.49	3.01
9 to 21 days			
C+	924.378 ± 21.74	622.208 ± 16.22	1.486 ± 0.01
0.755	909.304 ± 9.43	566.075 ± 6.40	1.606± 0.01
0.830	895.261 ± 24.37	564.757 ± 7.60	1.585± 0.03
0.904	912.042 ± 19.53	584.990 ± 14.59	1.562± 0.03
0.979	920.325 ± 15.06	579.136 ± 6.18	1.588 ± 0.03
1.053	922.602 ± 14.24	575.643 ± 4.86	1.603 ± 0.03
p-value	0.315	0.234	0.034
Regression	NS	NS	[3]Q= 0.905
C+ *vs.* 0.904	NS	NS	≤0.001
CV (%)	4.81	4.33	3.69
21 to 42 days			
C+	3913 ± 0.02	1951 ± 0.02	2.006± 0.02
0.695	3969 ± 0.04	1861 ± 0.03	2.133 ± 0.02
0.755	3961 ± 0.02	1899 ± 0.02	2.087 ± 0.02
0.815	4034 ± 0.03	1973 ± 0.02	2.045± 0.01
0.875	3934 ± 0.01	1913 ± 0.01	2.056 ± 0.01
0.935	3952 ± 0.02	1910 ± 0.01	2.070 ± 0.01
p-value	0.451	0.112	0.007
Regression	NS	NS	[4]Q = 0.853
C+ *vs.* 0.815	NS	NS	≤0,001
CV (%)	2.27	3.25	2.86

NS – Not Significant; Q –Quadratic effect.

[1]$Y = 176 + 572.57$ TID Val-282.42 TID Val^2($R^2 = 0.79$). P = 0.014;

[2]$Y = 4.715 – 6.809$ TID Val+ 3.3085 TID Val^2 (R^2=0.97). P = 0.001;

[3]$Y = 2.848 – 2.821$ TID Val+ 1.558 TID Val^2 ($R^2 = 0.85$). P = 0.021;

[4]$Y = 4.567 – 5.587$ TID Val+ 3.432 TID Val^2 ($R^2 = 0.97$), P = 0.001.

et al. (2011), of 0.904%. The reduced protein in the diet not affect the recommended TID Val level, possibly due the lower digestible leucine (TID Leu) level and to the supplementation of isoleucine in the RP diet in the starter phase (Table 1). High dietary leucine level increases the requirements of valine and isoleucine (D'Mello & Lewis, 1970), and therefore, the lower TID Leu level did not require valine supplementation.

The TID Val levels estimated for the grower phase (21 to 42 days) was 0.853% for feed conversion ratio in the 16.5% crude protein diet, differently that recommended by Tavernari *et al.* (2013), who estimated 0.790% TID Valin diets with 18.2% CP for 30- to 42-d-old broilers. Despite the small difference in crude protein levels (16.5 and 18.2%), the difference in the requirement estimates is probably due to the inclusion of animal byproducts in the study of Tavernari *et al.* (2013), which reduces valine requirement.

In the starter and grower phases, the C+ diet promoted better feed conversion ratio ($p \leq 0.05$) when compared with those fed the reduced-CP diet with same TID Val level (Table2), which may be explained by the difference in essential to nonessential ratios (EAA:NEAA). In the starter phase, the control diet had a ratio of 45:55 and was increased to 48:52 in the reduced-CP diet, while the grower phase ratio was 45:55 to 49:51 when the dietary protein was reduced in 2 p.p. According to Jiang *et al.* (2005), the EAA:NEAA recommended ratio is 46:54 in reduced-protein diets with for broilers. Corzo *et al.* (2011) evaluated valine supplementation to conventional or reduced-protein diets (17.6% CP) fed to 28- to 42-d-old broilers found worse feed conversion ration when birds fed the low CP diet, and attributed this result to arginine and isoleucine deficiency. Considering that in the present study, arginine and isoleucine levels did not differ between the experimental diets, the worse feed conversion ratio may be attributed to inadequate EAA:NEAA ratios. The reduced-CP diet contained less nonspecific nitrogen for AANE synthesis, directing AAE for the synthesis of AANE, which may cause AAE deficiency, therefore limiting protein synthesis (Heger, 2003).

There was no effect ($p > 0.05$) of the dietary treatments on carcass and parts yields (back, thighs, drumsticks and wings) or abdominal fat percentage of 42-d-old broilers (Table 3) Dozier *et al.* (2012) found similar results, when feeding broilers diets supplemented with valine from 4 to 6 weeks of age. However, there was a quadratic effect ($p \leq 0.05$) of TID Val levels on breast yield, with the highest value estimated at 0.826% of TID Val (Figure 5). Tavernari *et al.* (2013) estimated 0.730% TID Val for 30- to 43-old broilers fed diets containing animal by products in order to obtain maximum breast yield. The higher estimated level of TID Val the present (0.826%) compared with that of Tavernari *et al.* (2013) may be ascribed to the dietary inclusion of only plant-derived products, which may increase valine requirements in reduced-protein diets (Berres *et al.*, 2010).

Based on the result presented on Table 3, the TID Val level required to maximize breast yield (0.826%) was lower than that required to optimize feed conversion ratio (0.853%) in 21- to 42-d-old. Valine is a branched-chain amino acid that is mainly metabolized in the skeletal muscle, and it is responsible for body protein balance (Rogero & Tirapegui, 2008). Considering previous findings (Berres *et al.*, 2011; Tavernari *et al.*, 2013), it may be concluded that valine requirements for protein accretion are lower than those required for feed conversion in broilers. Therefore, the TID Val levels estimated for feed conversion ratio are recommended to be applied in broiler feed formulation, as they are

Table 3 – Effect of dietary TID Val levels on carcass and parts yields, and abdominal fat percentage of 42-d-old broilers.

TID Valine (%)	Carcass (%)	Breast (%)	Thigh and drumstick (%)	Wing (%)	Back (%)	Abdominal Fat (%)
C+	71.79	40.32	31.08	10.41	18.25	1.79
0.695	72.13	39.11	30.92	10.48	18.59	2.27
0.755	71.43	39.67	31.07	10.63	18.57	2.91
0.815	72.31	39.94	31.43	10.28	18.24	2.40
0.875	72.17	39.57	31.54	10.29	18.39	2.13
0.935	71.66	39.42	31.15	10.23	19.29	2.74
SEM	0.43	0.48	0.35	0.20	0.35	0.16
p-value	0.215	0.021	0.335	0.416	0.115	0.084
Regression	NS	[1]Q = 0.826	NS	NS	NS	NS
C+ *vs.* 0.815	NS	NS	NS	NS	NS	NS
CV (%)	2.24	4.39	4.09	7.11	7.58	23.78

NS – Not significant; Q – quadratic effect ($p < 0.05$).

[1]$Y = 11.981 + 67.49$ TID Val– 40.873 TID Val^2 ($R^2 = 0.94$). P=0.002.

higher than those estimated for other production parameters.

The muscle fiber diameter of the *pectoralis major* in 42-d-old broilers linearly increased ($p \leq 0.05$) with dietary TID Val values (Table 4). Increasing dietary Val content stimulates muscle development, increasing muscle fiber diameter due to the deposition of myofibril proteins (Petracci & Cavani, 2012), which contributed to increase the breast yield of the broilers fed TID Val levels higher than 0.755%.

Table 4 – Effect of dietary TID Val levels on the diameter of the muscle fiber of the *pectoralis major* in 42-d-old broilers.

TID Valine (%)	Muscle diameter of the *Pectoralis major* (µm)
C+	49.874± 0.266
0.695	45.875 ± 0.241
0.755	46.396 ± 0.249
0.815	46.339 ± 0.277
0.875	47.711 ± 0.251
0.935	49.595± 0.299
p-value	0.001
Regression	[1]L
C+ *vs.*0.815	≤0.001
CV (%)	19.21

L – Linear effect ($p<0.05$).

[1]$Y = 35.291 + 14.592x$ ($R^2 = 0.84$). $p=0.015$.

The broilers fed the reduced-protein with 0.815% of TID Val presented lower ($p \leq 0.05$) muscle fiber diameter of the *pectoralis major* compared with to birds fed the C+ diet in the grower phase (Table 4). This effect may be related to the imbalance between AAE and AANE, as part of the AAE may have been used to synthesize AANE, reducing AAE availability for myofibril protein deposition.

CONCLUSION

The recommended digestible valine levels to achieve maximum feed conversion ratio in broilers during pre-starter, starter, and grower phases are 1.028%, 0.905%, and 0.853%, respectively, whereas for maximum breast yield, it is 0.826% for broilers between 21 and 42 days of age.

Feeding low-protein diets during the grower phase reduced muscle fiber diameter, but did not affect carcass and parts yields or abdominal fat percentage in 42-d-old broilers.

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