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Digestible isoleucine requirements for 22- and 42-day-old broilers

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ABSTRACT. Current experiment established different criteria to evaluate the requirements of digestible isoleucine for 22- and 42-day-old broilers by different regression models (quadratic, exponential and Linear Response Plateau) and, in the case of statistical significance, the comparison of means by Duncan's test at 5% probability. A total of 1,920 Cobb 500 male broilers were used in an entirely randomized experimental design, with 6 treatments (6 digestible isoleucine levels: 0.6118; 0.6655; 0.7192; 0.7729; 0.8265 and 0.8802%) and 8 replications, with 40 broilers each. Data on performance and carcass characteristics were evaluated. Level 0.7192% of digestible isoleucine was considered standard. The digestible isoleucine level recommended for 22- and 42-day-old broilers is 0.7729%, corresponding to a digestible isoleucine:lysine ratio of 72%.

Keywords: digestible amino acids, ideal protein, performance, regression analyses.

Exigências em isoleucina digestível para frangos de corte de 22 a 42 dias de idade

RESUMO. Um experimento foi realizado com o objetivo de estabelecer critérios de avaliação das exigências de isoleucina digestível para frangos de corte de 22 a 42 dias de idade utilizando-se diferentes modelos de regressão (quadrático, exponencial e de retas segmentadas ou Linear Response Plateau) e em caso de significância estatística, as médias foram comparadas pelo teste de Duncan a 5 % de probabilidade. Foram utilizados 1.920 frangos de corte machos com 22 dias de idade, em delineamento inteiramente ao acaso, com seis tratamentos (seis níveis de isoleucina digestível: 0,6118; 0,6655; 0,7192; 0,7729; 0,8265 e 0,8802%) e oito repetições de 40 aves. Utilizou-se como padrão o nível de 0,7192% de isoleucina digestível. O nível de isoleucina digestível recomendado para frangos de corte de 22 a 42 dias de idade é 0,7729%, correspondente à relação isoleucina:lisina digestível de 72%.

Palavras-chave: aminoácidos digestíveis, proteína ideal, desempenho, análises de regressão.

Introduction

Formulating diets according to the ideal protein concept which expresses the need for critical amino acids relative to lysine theoretically allows for the most efficient utilization of protein by maximizing nitrogen efficiency and retention and, in turn, minimizes nitrogen excretion (Baker, 1997). One of the several benefits of ideal protein is that once a ratio or percentage relative to a reference amino acid (lysine) is established for a certain age, the requirement of all other amino acids under a variety of conditions based on the lysine requirement may be calculated (Mack et al., 1999). Matching the dietary amino acid profile to that required by fowls is crucial for maximizing their performance (Baker, 1997).

Although isoleucine is one of the limiting amino acids for broilers in commercial diets (Fernandez, Aoyagi, Han, Parsons & Baker, 1994), it is less limiting than valine in low protein corn- and soybean-based diets for broilers (Edmonds, Parsons & Baker, 1985; Fernandez et al., 1994; Han, Suzuki, Parsons & Baker, 1992). Isoleucine is potentially limiting in low protein diets for laying hens that have been supplemented with lysine, methionine and tryptophan (Keshavarz, 1998). Therefore, it is highly relevant to develop a nutritional model of isoleucine imbalance and to determine which amino acids might be most likely to precipitate the imbalance.

It has been previously demonstrated that in most practical diets, isoleucine and valine are often limiting next to threonine, which is the third limiting amino acids after TSAA (Met) and lysine (Baker, Batal, Parr, Augspurger & Parsons, 2002; Corzo, Loar & Kidd, 2009). Several research reports have addressed the digestible isoleucine needs of broilers throughout the grow-out period of broilers (Burnham & Gous, 1992;

Corzo, Dozier, Kidd & Hoehler, 2008) although minimum recommendations for low-cost formulation are varied.

The optimal ratio of isoleucine to lysine may affect the broilers' metabolic efficiency and growth performance coupled to the utilization of valine and other limiting amino acids (Corzo et al., 2009). Few reports have addressed the optimal digestible isoleucine ratio relative to digestible lysine for age brackets close to slaughter, with regard to carcass and breast meat yield optimization (Kidd, Burnham & Kerr, 2004; Mack et al., 1999).

Current research evaluates diets with different digestible isoleucine levels from 22 to 42 days on live performance and carcass characteristics of commercial high-yielding broilers.

Material and methods

Animals and experimental procedures

The experiment was conducted between the 22nd and 42nd days of the broilers. A total of 1,920 one-day-old male Cobb 500 chicks were housed in a masonry poultry house with 80 boxes. The housing and experimental procedures reported herein were approved by the Institutional Animal Care and Use Committee (CEBEA 001771-09) of the State University of São Paulo, Brazil.

The initial heating derived from 250-watt infrared lamps so that temperature between 28 and 30ºC could be maintained during the first two weeks. The chicks were vaccinated in the hatchery against Marek, Gumboro and Bouba disease, followed by vaccination on the 5th and 21st days against Gumboro disease, and on the 8th day against Newcastle disease.

The litter was made from wood shavings and the amount placed in each box was 1.2 kg of dry matter/bird housed, so that all treatments had the same initial amount of material used as litter, at a height of 5 cm. Lighting schedule was 24 hours of light throughout the experimental period and broilers received feed and water *ad libitum* throughout the entire experimental period.

During the initial period (1-21 days), the birds were reared in an experimental shed, fed on diets with 3,005 kcal ME kg^{-1} and 21.6% CP to reach their nutritional requirements, according to recommendations by Rostagno et al. (2005) for phases 1 to 7 and 8 to 21 days of age. At the end of the 21st day of age, the broilers were weighed, selected according to the criterion of average weight of each box (750 ± 35 grams) and distributed in a completely randomized design involving six treatments (levels of digestible isoleucine), with eight replications of 40 birds each.

Nutritional recommendations of crude protein, metabolizable energy, calcium, available phosphorus and digestible amino acids used in the experimental diets were established by Rostagno et al. (2005), following recommenddations for stages 22-33 and 34-42 days of age.

Chemical analyses of the ingredients in the experimental diets (Table 1) were performed according to methodology described by Silva & Queiroz (2002). The metabolizable energy (ME) and digestibility coefficients were established by Rostagno et al. (2005).

Treatments consisted of a isoleucine-deficient basal diet, formulated with digestible amino acids, according to the ideal protein concept, or rather, from 22 to 42 days of age, supplemented with five different digestible isoleucine levels, using 0.7642% of digestible threonine, 0.1919% of digestible tryptophan and 0.8802 of digestible valine, following Duarte et al. (2014); Duarte et al. (2012); Duarte et al. (2013), respectively.

The basal diet (Table 2) was formulated to contain 0.6118% digestible isoleucine, representing 57% of 1.0735 digestible lysine level and the other diets with increasing levels of 2% in relation to the basal diet. The latter contained 0.6655, 0.7192, 0.7729, 0.8265 and 0.8802, or rather, two levels below and three levels above the 0.7192% level recommended by Rostagno et al. (2005), as the levels of lysine, methionine + cystine and other amino acids in the diet formulation.

Table 1. Chemical analyses and composition in total (TAA) and digestible (DAA) amino acids of ingredients in the experimental diets.

	Corn grain		Soybean meal	
Dry matter (%)	88.90		89.10	
Crude protein (%)	8.11		44.40	
Metabolizable energy (kcal kg^{-1})	3381		2256	
Ether extract (%)	3.61		1.66	
Crude fiber (%)	1.73		5.41	
Calcium (%)	0.03		0.24	
Available phosphorus (%)	0.08		0.18	
Sodium (%)	0.02		0.02	
%	TAA[1]	DAA[2]	TAA	DAA
Total alanine	0.59	0.55	1.94	1.90
Total arginine	0.36	0.33	3.19	3.06
Total glycine	0.31	0.30	1.89	1.88
Total isoleucine	0.27	0.24	2.01	1.83
Total leucine	0.97	0.92	3.42	3.12
Total lysine	0.23	0.20	2.72	2.50
Total cystine	0.18	0.16	0.62	0.60
Total methionine	0.17	0.16	0.60	0.54
Total meth + cys	0.35	0.32	1.22	1.06
Total phenylalanine	0.39	0.35	2.32	2.15
Total tyrosine	0.24	0.21	1.50	1.47
Total threonine	0.29	0.24	1.74	1.53
Total tryptophan	0.06	0.05	0.58	0.52
Total valine	0.39	0.34	2.13	1.90
Total histidine	0.24	0.22	1.16	1.10
Total serine	0.39	0.34	2.29	2.24

[1]Total amino acids, determined by Degussa Laboratory - Animal Nutrition Service - São Paulo, São Paulo State, Brazil. [2]Digestible amino acids, based on digestibility coefficients of *Tabelas Brasileiras para Aves e Suínos* (Rostagno et al., 2005).

Table 2. Percentage composition of experimental diets for 22-42-day-old broilers.

Ingredients	Digestible isoleucine levels					
	0.6118	0.6655	0.7192	0.7729	0.8265	0.8802
Corn	69.285	69.285	69.285	69.285	69.285	69.285
Soybean meal	22.450	22.450	22.450	22.450	22.450	22.450
Soybean oil	3.060	3.060	3.060	3.060	3.060	3.060
Dicalcium phosphate	1.600	1.600	1.600	1.600	1.600	1.600
Limestone	1.080	1.080	1.080	1.080	1.080	1.080
L-Lysine (78.5%)	0.380	0.380	0.380	0.380	0.380	0.380
DL-Methionine (99%)	0.240	0.240	0.240	0.240	0.240	0.240
L-Tryptophan (98%)	0.080	0.080	0.080	0.080	0.080	0.080
L-Threonine (98%)	0.270	0.270	0.270	0.270	0.270	0.270
L-Arginine (99%)	0.170	0.170	0.170	0.170	0.170	0.170
L-Valine (96.5%)	0.210	0.210	0.210	0.210	0.210	0.210
L-Isoleucine (99%)	0.000	0.054	0.107	0.161	0.215	0.269
L-Glutamic acid (99%)	0.325	0.260	0.195	0.130	0.065	0.000
Inert (washed sand)	0.000	0.011	0.023	0.034	0.045	0.057
Vit. + min. supplement[1]	0.400	0.400	0.400	0.400	0.400	0.400
Salt	0.450	0.450	0.450	0.450	0.450	0.450
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values						
Crude protein	16.6712	16.6712	16.6712	16.6712	16.6712	16.6712
Metabolizable energy (kcal kg^{-1})	3.1750	3.1750	3.1750	3.1750	3.1750	3.1750
Calcium	0.8740	0.8740	0.8740	0.8740	0.8740	0.8740
Sodium	0.2100	0.2100	0.2100	0.2100	0.2100	0.2100
Total phosphorus	0.6185	0.6185	0.6185	0.6185	0.6185	0.6185
Available phosphorus	0.4060	0.4060	0.4060	0.4060	0.4060	0.4060
Digestible lysine	1.0735	1.0735	1.0735	1.0735	1.0735	1.0735
Digestible methionine	0.4663	0.4663	0.4663	0.4663	0.4663	0.4663
Digestible met. + cyst.	0.7730	0.7730	0.7730	0.7730	0.7730	0.7730
Digestible threonine [2]	0.7642	0.7642	0.7642	0.7642	0.7642	0.7642
Digestible tryptophan [3]	0.1919	0.1919	0.1919	0.1919	0.1919	0.1919
Digestible arginine	1.1270	1.1270	1.1270	1.1270	1.1270	1.1270
Digestible valine [4]	0.8802	0.8802	0.8802	0.8802	0.8802	0.8802
Digestible isoleucine	0.6118	0.6655	0.7192[5]	0.7729	0.8265	0.8802

[1] Enrichment per kilogram of diet: 8,000 IU vitamin A, 1,800 IU vitamin D3, 12 mg vitamin E, 2 mg vitamin K3, 1 mg vitamin B1, 4 mg vitamin B2, 1 mg Vitamin B6, 10 mcg Vitamin B12, 0.40 mg folic acid, 0.04 mg biotin, 28 mg niacin, 11 mg of calcium pantothenate, 6 mg Cu, 0.10 mg Co, 1 mg I, 50 mg Fe, 65 mg Mn, 45 mg Zn, 0.21 mg Se, 500 mg choline chloride 50%, 60 mg coccidiostat, 12 mg antioxidant. [2]Duarte et al. (2012). [3]Duarte et al. (2013). [4]Duarte et al. (2014), [5]Rostagno et al. (2005).

L-Isoleucine was added to the basal diet to replace L-Glutamic Acid so that all treatments would have the same nitrogen level and to eliminate any effects related to differences in their concentration. In the broilers' metabolism, the supply of a non-specific nitrogen source plus essential amino acids is required for the synthesis of non-essential amino acids and other nitrogen compounds. Thus, diets with low levels of isoleucine contained higher levels of L-Glutamic acid.

Growth performance and carcass yield traits

The birds were weighed at the beginning and at the end of the experiment to determine weight gain, or rather, the difference between weights at 21 and 42 days. Likewise, feed intake was obtained by the difference between the feed provided and feed left over in the troughs. Subsequently, feed conversion was calculated by the ratio between feed intake and bird's weight gain. Mortality was collected daily and the dead weight was recorded and used to adjust feed conversion rates. Viability (VC) was obtained from the total number of birds housed minus dead birds, represented by dead or removed experimental unit, divided by the total number of birds housed (multiplied by 100).

To calculate the production efficiency index (PEI = [average daily weight gain (g) x VC (%)] / (feed conversion x 10), average weight gain, feed intake and feed conversion of the birds were considered at that age. The above averaging was performed due to the fact that all birds received the same diet during the first 22 days of age when they were weighed and selected for the early experimental period.

At the end of the experimental period, eight birds per experimental unit, with body weight near plot average, were selected and submitted to a fasting period of 6 hours. They were then killed by jugular bleeding, plucked and eviscerated. After weight of carcass, they were cut to evaluate carcass yield (excluding head, neck and feet), breast yield, thigh + drumstick yield, wing yield and back yield.

Statistical analysis

Statistical analyses were performed by GLM procedure of SAS (2004) software (Statistical Analysis System). In the case of statistical significance, means were compared by Duncan's test at 5% probability. Three regression models were used to determine digestible isoleucine requirements, namely, the quadratic model described by Robbins, Norton & Baker (1979), the exponential model described by Noll & Waibel

(1989) and the Linear Plateau Response (LRP) with 90% maximum square, according to the best fit obtained for each variable under analysis.

Results and discussion

Growth performance from 22 to 42 days of age

Table 3 shows the average performance of broilers between 22 and 42 days of age and the summary of the statistical analyses for the different variables. An appropriate fit was not obtained by LRP and by quadratic models for birds' performance data, since these models were not significant ($p > 0.05$) by regression analysis of variance. However, feed intake allowed a proper fit for the linear model ($FI = 3.7321 – 0.4017 * Iso$; $R^2 = 63.48$; $p < 0.05$). Feed intake decreased linearly as the digestible isoleucine level increased from 0.6118 to 0.8802%. Feed intake was depressed at the highest level of digestible isoleucine. These results disagree with those by Campos et al. (2012) who reported no significant effects of isoleucine levels on the feed intake of broilers from 28 to 40 days old.

It was not possible to describe the behavior of weight gain, feed conversion ratio, viability and productive efficiency index by the regression models proposed. Due to the non-significance effect ($p > 0.05$) of digestible isoleucine levels on the variables, the means were compared using Duncan's test at 5% of probability. No differences were observed between treatments for weight gain and viability (Table 3). Significant effect of digestible isoleucine levels occurred on feed conversion and productive efficiency index ($p < 0.05$), where the level of 0.7192% digestible isoleucine produced the worst result. Feed conversion was worse at 0.7192% digestible isoleucine level, corresponding to isoleucine: lysine ratio of 67%. These results disagree with Rostagno et al. (2005) who concluded that for optimum performance of broilers between 22 and 42 days of age, the isoleucine: lysine ratio should be 67% (0.70% digestible isoleucine). Moreover, Mejia, Zumwalt, Kim, Tillman & Corzo (2011) reported the best result of feed conversion in 4 - 6-week-old broilers when 67% isoleucine: lysine ratio was employed. Klain, Scott & Johnson (1960) recommended the range between 0.60 and 0.80% of digestible isoleucine for the same period. However, Hale, Barber, Corzo & Kidd (2004) registered levels of 0.67; 0.66 and 0.68% digestible isoleucine in broilers diets for better results of weight gain, feed intake and feed conversion, respectively, during 30-42 days of age and recommended digestible isoleucine levels ranging between 0.59 and 0.64%. Other levels studied did not differ statistically from the performance results, corroborating results by Rostagno et al. (2005) who recommended 0.73% of digestible isoleucine level in the diet of broilers between 22 and 42 days of age, corresponding to isoleucine: lysine ratio of 68%.

Campos et al. (2012) analyzed the effect of isoleucine: lysine ratio on broiler performance and observed that 69% isoleucine: lysine ratio was recommended for maximum performance of broilers from 28 to 40 days old. According to Tavernari et al. (2012), the best isoleucine: lysine ratios for feed intake, weight gain and feed conversion were 64, 68 and 72%, respectively.

The productive efficiency index behaved similarly to feed conversion (Table 3) where 0.7192% digestible isoleucine level, corresponding to isoleucine: lysine ratio of 67% showed the worst results, with no statistical differences of 0.8802 digestible isoleucine level (isoleucine: lysine ratio of 82%). According to Tavernari et al. (2012), the recommendation for the digestible isoleucine/lysine ratio which meets the main productive parameters for broilers in the phase from 30 to 43 days is 68%. It is actually the same value recommended by Rostagno et al. (2005). Campos et al. (2012) concluded that 70% is the best digestible isoleucine/lysine ratio for this phase.

Effect on the weight of several parts in broilers

There was no significant effect ($p > 0.05$) for the carcass parameters evaluated with regard to the different digestible isoleucine levels (Table 4). Current analysis shows that the isoleucine requirement for the carcass parameters assessed at 42 days is lower than the requirement for performance. These results agreeing with findings by Corzo et al. (2008) who tested 0.58; 0.66 and 0.68% of digestible isoleucine levels in broilers' diets and Tavernari et al. (2012) who evaluated different digestible isoleucine/lysine ratios (58.0; 62.0; 66.0; 70.0; 74.0 and 78.0%) on diets for broilers in the finishing (30 to 43 days) phase. Berres et al. (2010) likewise evaluated broilers fed on diets with increased Val- and Ile-to-Lys ratios from 14 to 35 days and registered no differences in the treatment for carcass evaluation at 35 days.

Mack et al. (1999) recommended feeding a digestible isoleucine: lysine ratio of 71% for improved live performance and carcass characteristics. Kidd, Kerr, Allard, Rao & Halley (2000) evaluated the response of male Ross × Arbor Acres broilers fed on diets with adequate or deficient in isoleucine levels (based on NRC (2012)) and found that birds fed on 90% of requirements estimated had a decrease in breast meat yield. These authors reported an improvement in breast meat yield of male Ross × Arbor Acres broilers when l-Isoleucine was used to supplement a marginal diet in this amino acid.

Table 3. Feed intake (FI), weight gain (WG), feed conversion (FCR), viability (VC) and productive efficiency index (IEP) of broilers, between 22 and 42 days and between 1 and 42 days, fed on diets with different digestible isoleucine levels.

Digestible isoleucine levels (g 100 g^{-1})	Performance				
	FI[1] (kg)	WG (kg)	FCR[2] (kg kg^{-1})	VC (%)	IEP[2] 1-42 days of age
0.6118	3.481	1.818	1.915 a	99.112	348.954 a
0.6655	3.434	1.787	1.923 a	98.871	343.423 a
0.7192	3.493	1.749	1.999 b	97.838	325.896 b
0.7729	3.410	1.818	1.877 a	98.730	352.881 a
0.8265	3.422	1.802	1.900 a	98.004	345.287 a
0.8802	3.354	1.745	1.923 a	99.698	341.167 ab
P-value	0.046*	0.103[NS]	0.004*	0.491[NS]	0.024*
F-value	4.19	1.97	4.02	0.90	2.92
CV(%)	3.751	3.886	3.489	2.093	4.934

Values have been adjusted for mortality weight. VC = viability (number of finished birds); IEP = [average daily WG (g) x VC (%)] / (FC x 10). NS = not significant; *$p < 0.05$; [1]Linear effect; [2]Means in the column followed by different letters differ ($p < 0.05$) by Duncan's test.

Table 4. Carcass yield (CY), breast yield (BrY), thigh + drumstick yield (TH+DR), back yield (BY) and wing yield (WY) of broilers fed on diets containing different digestible isoleucine levels from 22 to 42 days of age.

Digestible isoleucine levels (g 100 g^{-1})	Carcass characteristics[1]				
	CY (%)	BrY (%)	TH + DR (%)	BY (%)	WY (%)
0.6118	75.384	36.990	29.848	23.118	10.044
0.6655	75.074	36.714	30.042	22.748	10.497
0.7192	75.225	37.000	30.083	22.773	10.144
0.7729	75.153	37.771	29.781	22.117	10.331
0.8265	75.221	37.362	30.028	22.403	10.207
0.8802	74.438	36.972	30.207	22.633	10.188
P-value	0.794[NS]	0.451[NS]	0.937[NS]	0.300[NS]	0.152[NS]
F-value	0.50	0.96	0.25	1.26	1.72
CV(%)	1.767	2.897	2.836	3.873	3.469

NS = not significant, [1]eviscerated carcass without feet, head and neck.

Hale et al. (2004) found that the total isoleucine requirement for broilers from 30 to 42 days of age was 0.63% for breast yield and 0.68% for feed conversion (0.59 to 0.64% digestible isoleucine), corresponding to 62 to 67% of a digestible isoleucine: lysine ratio.

Based on these data, it may be concluded that supplemental Isoleucine at specific ratios affected feed intake, feed conversion and productive efficiency index during growing phases, but no effects were observed on body component yields. When determining AA requirements for different measurements, those for maximal growth are the lowest, followed by those for breast meat yield and feed conversion, and finally are highest for minimizing abdominal fat percentage (Leclercq, 1998). The order of response may, however, be dependent on the specific AA studied, as shown by Schutte & Pack (1995) with sulfur-containing amino acid. In contrast to a previous work (Leclercq, 1998), feed intake and feed conversion in the present study was the measure that was most affected by diet, whereas body component yields were not affected by it.

Conclusion

Isoleucine requirement for the carcass parameters evaluated at 42 days is lower than the requirement for performance. The digestible isoleucine level recommended for broilers between 22 and 42 days of age is 0.7729%, corresponding to a digestible isoleucine: lysine ratio of 72%.

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