Effect of different digestible isoleucine/lysine ratios for broiler chickens

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ABSTRACT - Two experiments were conducted to evaluate different digestible isoleucine/lysine ratios on diets for broiler chickens in the starter (7 to 21 days) and finishing (30 to 43 days) phases. For the tests, the experimental design was of randomized blocks with seven treatments (six different digestible isoleucine/lysine ratios and a control treatment) and eight repetitions of 25 and 20 birds (COBB males) per experimental unit in the starter and finishing phases, respectively. The diets met the requirements, except for isoleucine and lysine. To avoid excess lysine in the experimental diets, the digestible lysine content was calculated to be 87% and 89% of the recommended for the starter and finishing phases, respectively. The control treatment was adequate in lysine and isoleucine. Feed intake, weight gain, feed conversion and carcass yield in the two phases were evaluated. There was quadratic effect of different ratios on feed intake in the finishing phase and on weight gain and feed conversion rate in both phases. There was quadratic effect on breast meat yield and breast fillets in the starter phase, but there was no significant effect on carcass yield in the finishing phase. The digestible isoleucine/lysine ratio recommended for broilers in the starter phase (7 to 21 days) is 66% and for the finishing phase (30 to 43 days), it is 68%.

Key Words: isoleucine, lysine, ratio, requirement

Introduction

The accurate knowledge of the nutritional requirements of essential amino acids and the nutritional composition of raw materials is essential for the correct balancing of diets and, in light of this, the constant update of the ideal protein profile is indispensable.

In the ideal protein, lysine is viewed as a reference to the other essential amino acids, and several studies have been conducted with the first three limiting amino acids to broiler chickens. Isoleucine is one of the limiting amino acids in diets with low protein for broiler chickens, and few studies have been conducted on the digestible isoleucine/lysine ratio (Park & Austic, 2000).

Several institutions related to research, in Brazil and around the world, perform experiments for the determination of nutritional requirement of birds; however, because of the differences in methodology, genetic variance and ambience, it is possible to observe different requirement values in the literature.

According to Baker et al. (2002), the complexity to establish accurate ratios for amino acids lies in the possibilities of response variables to be utilized (such as weight gain and feed conversion) and the statistical analyses that may be conducted, in which the quadratic one is known for overestimating the ratios, whereas the linear response plateau (LRP), for underestimating them. These authors have utilized the quadratic one as the plateau and showed it as a possibility to the determination of amino acid ratios, once the values achieved were close to 90% of the values obtained with the quadratic analysis. According to Leclercq (1998a) the lack of knowledge of the requirements of non-essential amino acids is also a factor that affects the determination of essential amino acid ratios.

In many studies, the only criterion utilized for the determination of the requirement or the ratio between amino acids is weight gain, which can lead to an underestimate of requirements, once this parameter usually presents the lowest values, when compared with feed conversion, breast yield and abdominal fat (Leclercq, 1998b).

Thus, the objective of this study was to evaluate the digestible isoleucine/lysine ratios in the diet on performance and carcass yield of male COBB broilers in the period from 7 to 21 and 30 to 42 days of age.

Material and Methods

Two experiments were conducted to determine the digestible isoleucine/lysine for male COBB 500 broilers in the phases from 7 to 21 days and 30 to 43 days. In the starter phase, the experimental design lasted 14 days (7 to 21 days of age), in which diets were supplied *ad libitum*. The experimental design was in completely randomized blocks, with seven treatments and eight replicates of 25 birds per experimental unit. Six different digestible isoleucine/lysine ratios (58.0; 61.5; 65.0; 68.5; 72.0 and 75.5%) were assessed. Diets were formulated to meet the requirements according to Rostagno et al. (2005), except for isoleucine and lysine. In order to avoid excess of lysine, the digestible lysine value of experimental diets was calculated to meet 87% of the requirements according to Rostagno et al. (2005) (1.000%). A positive control (C+) treatment adequate in lysine (1.146%) and isoleucine (0.755%) was included in the experimental design.

In the finishing phase, the experimental period lasted 13 days (30 to 43 days of age), when diets were supplied *ad libitum*. The experimental design was in completely randomized blocks with seven treatments and eight replicates of 20 birds per experimental unit. Six different digestible isoleucine/lysine ratios (58.0; 62.0; 66.0; 70.0; 74.0 and 78.0%) were assessed. Diets were formulated to meet the requirements according to Rostagno et al. (2005), except for isoleucine and lysine. In order to avoid the excess of lysine, the digestible lysine value of experimental diets was calculated to meet 89% of the requirements according to Rostagno et al. (2005) (0.900%). A positive control (C+) treatment adequate in lysine (1.010%) and isoleucine (0.725%) was included in the experimental design.

With the inclusion of industrial amino acids to provide the ratios tested, the starch varied between diets to compose 100% (Table 1). The analyses of amino acids (Tables 2 and 3) were conducted through EVONIK.

In the two phases of the experiment, birds were housed in masonry shed subdivided in 1.0×2.0 m pens (provided with bedding of wood shavings, nipple drinker and tubular feeder) with ceiling height of 3 m, covered with asbestos tile, with ridge vents; cemented floor; side walls comprised of 0.40 m walls and the rest with mesh wire; and plastic curtains for the control of temperature and air currents. The management of drinkers, feeders, curtains and birds followed the recommendations of the manual for managing COBB-500 broiler chickens (Cobb Vantress, 2008). Water and feed were supplied *ad libitum* during the experimental periods. Before the beginning of each experimental period, birds were fed branny commercial diet. The continuous lighting

Table 1 - Ingredients and calculated composition of basal diets of starter (7 to 21 days) and finishing (30 to 43 days) phases

Ingredients	7 to 21 days	30 to 43 days
Corn	70.67	76.69
Soybean meal (45%)	9.17	6.28
Gluten meal (60%)	6.00	5.00
Spray-dried plasma	3.73	4.00
Soybean oil	0.57	0.54
Dicalcium phosphate	1.856	1.524
Limestone	0.981	0.893
Salt	0.160	0.160
K_2CO_3	0.364	0.423
$NaHCO_3$	0.300	0.300
L-lysine HCl (79%)	0.425 (0.610)[1]	0.365 (0.505)[1]
L-isoleucine (98.5%)	0.000	0.000
DL-methionine (99%)	0.253	0.215
L-threonine (98%)	0.147	0.100
Glycine	0.439	0.248
L-arginine (98.5%)	0.413	0.358
L-tryptophan (98%)	0.030	0.040
L-valine (98.5%)	0.071	0.097
Choline chloride (70%)	0.100	0.100
Vitamins[2]	0.050	0.050
Minerals[3]	0.100	0.100
Anticoccidial	0.055	0.055
Antioxidant[4]	0.010	0.010
Glutamic acid	3.000	2.000
Inert	0.450	0.000
Starch[5]	0.660	0.450
Total	100.00	100.00
Calculated composition		
Crude protein, %	19.99	17.78
Metabolizable energy, kcal/kg	3150	3225
Digestible isoleucine, %	0.580	0.522
Digestible lysine, %	1.000 (1.146)[1]	0.900 (1.010)[1]
Digestible met + cys, %	0.825	0.761
Digestible threonine, %	0.745	0.661
Digestible tryptophan, %	0.183	0.180
Digestible arginine, %	1.203	1.070
Digestible valine, %	0.824	0.800
Ca, %	0.884	0.764
Available P, %	0.442	0.380
Na, %	0.214	0.223

[1] Control diet (C+).
[2] Vitamin mix (kg of product): vit. A - 15,000,000 UI; vit. D3 - 1,500,000 UI; vit. E - 15,000 UI; vit. B1 - 2.0 g; vit. B2 - 4.0 g; vit. B6 - 3.0 g; vit. B12 - 0.015 g; nicotinic acid - 25 g; pantothenic acid - 10 g; vit. K3 - 3.0 g; folic acid - 1.0 g; zinc bacitracin - 10 g; selenium - 250 mg; antioxidant BHT - 10 g; excipient q.s. - 1.000 g.
[3] Mineral mix (kg of product): manganese - 80 g; iron - 80 g; zinc - 50 g; copper - 10 g; cobalt - 2 g; iodine - 1 g; excipient q.s. - 1.000 g.
[4] Antioxidant: BHT (butylated hydroxytoluene).
[5] Variable with inclusion of L-isoleucine and L-lysine.

program (24 hours of natural + artificial light) was adopted during the whole experimental period and the heating of chicks was done with infrared light bulbs of adjustable height, set to promote the most comfort possible to birds. Records of the internal temperature of the shed were obtained with the installation of three maximum and minimum thermometers placed at different places of the facility, at the height of birds. The average temperatures recorded during the experiment were: from 7 to 21 days, 24 °C (20 and 29 °C

Table 2 - Total isoleucine of experimental diets of starter phase (7 to 21 days of age)

Isoleucine		Digestible isoleucine/lysine ratio (%)						
		58.0	61.5	65.0	68.5	72.0	75.5	C+
Total, %	Calculated	0.635	0.671	0.705	0.741	0.775	0.811	0.811
	Determined	0.620	0.670	0.720	0.720	0.760	0.790	0.790
Free, %	Calculated	0.000	0.035	0.070	0.105	0.140	0.175	0.175
	Determined	<0.010	0.036	0.073	0.108	0.141	0.167	0.173

C+ - positive control.

Table 3 - Total isoleucine of experimental diets of finishing phase (30 to 43 days of age)

Isoleucine		Digestible isoleucine/lysine ratio (%)						
		58.0	62.0	66.0	70.0	74.0	78.0	C+
Total, %	Calculated	0.573	0.609	0.644	0.681	0.716	0.753	0.753
	Determined	0.570	0.610	0.650	0.680	0.710	0.750	0.750
Free, %	Calculated	0.000	0.036	0.072	0.108	0.144	0.180	0.180
	Determined	<0.010	0.042	0.072	0.110	0.134	0.187	0.187

C+ - positive control.

minimum and maximum, respectively); and from 30 to 43 days, 22 °C (17 and 26 °C minimum and maximum, respectively).

Birds and diets were weighed at the beginning and end of each experimental period (7 to 21 days and 30 to 43 days) for the calculation of the following parameters: feed intake, weight gain and feed conversion. Mortality was recorded daily for the feed intake in the two phases to be corrected. Feed conversion was corrected to mortality and represented in grams of feed ingested per all live birds present in the pen divided by grams of weight per pen. In the starter phase, three birds with the average weight of every pen were slaughtered at 21 days for evaluation of carcass characteristics such as breast and breast yield in relation to live weight. In the finishing phase, three birds with the average weight of each pen were slaughtered at 44 days for evaluation of carcass yield, abdominal fat, breast, breast yield, thigh and drumstick.

Statistical analyses were conducted through the software SAEG (Sistema para Análises Estatísticas e Genéticas, version 9.1). Variance analysis and Dunnett test (P<0.05) were utilized for the contrast between treatment control and the different ratios. Regression analysis was conducted for the different ratios. For the response variables which presented quadratic behavior, the inflection point was calculated, as the best ratios and 95% of these values as confidence (Sakomura & Rostagno, 2007). The Linear Response Plateau (LRP) was utilized for response variables that presented significant quadratic effect in the regression analysis. The data were also adjusted by the quadratic model associated to the plateau for the variables which presented significant quadratic effect, inserting the plateau (y) in the quadratic equation. The recommendations of best ratio for each phase were obtained through the average of the different response variables in face of the different analyses, in function of the good adjustment of the data to them.

Results and Discussion

Analyzing the results (Table 4), one can observe that broiler chickens fed the diet containing digestible isoleucine/lysine ratio of 58% showed worse (P<0.05) weight gain and feed conversion when compared with treatment control, and broilers which received the ratio of 75.5%, only weight gain. The other ratios were significantly similar (P>0.05) to the treatment control, indicating the possibility of meeting the digestible isoleucine/lysine ratio between the values of 61.5% and 72%.

Table 4 - Feed intake (FI), weight gain (WG) and feed conversion (FC) of broiler chickens fed diets containing different digestible isoleucine/lysine ratios during the starter phase (7 to 21 days)

Ratio (%)	FI (g/bird)	WG (g/bird)	FC
C+	1000	707a	1.416a
58.0	984	653b	1.506b
61.5	1002	695a	1.441a
65.0	998	696a	1.434a
68.5	1011	707a	1.431a
72.0	998	698a	1.432a
75.5	998	686b	1.455a
		ANOVA - regression	
Quadratic	ns[1]	0.001	0.001
CV (%)	2.58	3.20	1.55

a,b - means with different letters in the same row are significantly different from treatment positive control (C+) by the Dunnett test (P<0.05).
[1] Non-significant (P>0.05).

For the regression analysis in the starter phase, there was no significant difference (P>0.05) in feed intake; however, quadratic effect (P<0.05) was observed for weight gain (WG - 1368.53 + 60.6357Iso − 0.443013Iso^2; R^2 = 0.90) (Figure 1) and for feed conversion (FC = 4.6459 − 0.0941626Iso + 0.000687838Iso^2; R^2 = 0.91) (Figure 2); optimum ratios were of 68 and 68%, respectively. By applying a confidence limit of 95% for the response of the quadratic equation for weight gain and for feed conversion one gets the optimum relation of 65% for both. At the LRP analysis for weight gain, best ratio of 62% was obtained with plateau at 697 g (WG = - 43.0 + 12.0Iso; R^2 = 1.00) (Figure 1) and for feed conversion, the ratio of 62% with plateau at 1.438 (FC = 2.583143 – 0.018571Iso; R^2 = 1.00) (Figure 2). By applying the quadratic analysis with plateau, one obtains best ratio of 64% for both parameters.

Table 5 - Breast (B), breast yield (BY), breast fillet (F), breast fillet yield (FY) of broiler chickens fed diets containing different digestible isoleucine/lysine ratios during the starter phase (7 to 21 days) and slaughtered at 21 days of age

Ratio (%)	B (g)	BY (%)	F (g)	FY (%)
C+	183a	20.71a	147a	16.69a
58.0	150b	18.30b	118b	14.37b
61.5	168b	19.49b	132b	15.37b
65.0	168b	19.55b	135b	15.73b
68.5	173b	19.70b	138b	15.74b
72.0	170b	19.73b	135b	15.72b
75.5	168b	19.70b	134b	15.75b
	ANOVA - regression			
Quadratic	0.001	0.005	0.001	0.002
CV (%)	4.78	3.34	5.03	3.87

a,b Means with different letters in the same column are significant different from treatment positive control (C+) by the Dunnett test (P<0.05).
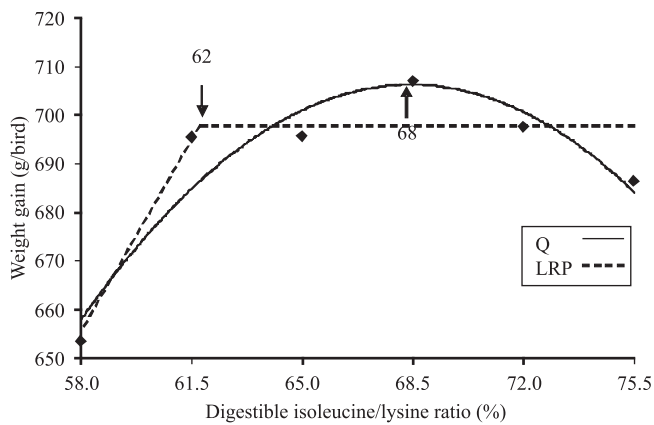

Figure 1 - Effect of different digestible isoleucine/lysine ratio in the diet on the weight gain of broiler chickens in the phase from 7 to 21 days.
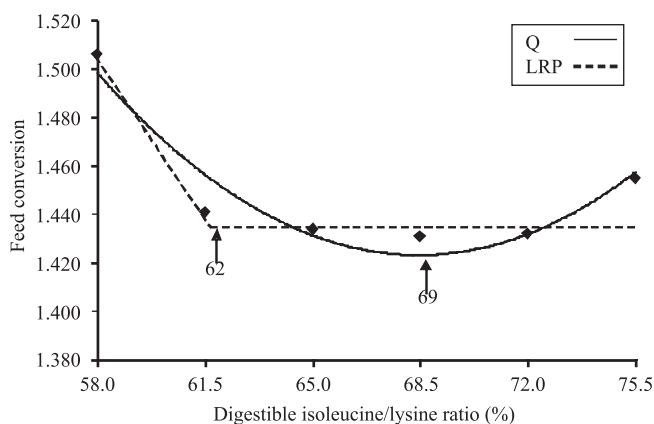

Figure 2 - Effect of different digestible isoleucine/lysine ratio in the diet on the feed conversion of broiler chickens in the phase from 7 to 21 days.

Due to the meeting of the digestible lysine requirement for the starter phase (Table 5), birds fed the diet control presented better (P<0.05) weight and breast yield and fillet yield when compared with birds fed diets containing different digestible isoleucine/lysine ratios and sub-optimum lysine level.

For the regression analysis, quadratic effect was observed (B<0.05) for breast weight (B = - 592.795 + 22.0915Iso − 0.159439Iso^2; R^2 = 0.88) (Figure 3), breast yield (BY = - 24.9433 + 1.27411Iso − 0.00906046Iso^2; R^2 = 0.88) (Figure 4), breast fillet weight (F = - 563.471 + 20.235Iso − 0.145955Iso^2; R^2 = 0.92) (Figure 5) and breast fillet yield (FY = - 30.8103 + 1.33092Iso − 0.00948617Iso^2; R^2 = 0.92) (Figure 6), with optimum ratios of 69, 70, 69 and 70%, respectively. By applying confidence limit of 95% for the response of the quadratic equation to weight and yield of both parameters evaluated, one obtains optimum ratios of 66 and 67%, respectively. At the LBY analysis for breast weight, best ratio of 62% was obtained with plateau at 169 g (B = 5.142857 – 148.285714Iso; R^2 = 1.00) (Figure 3), for breast yield, best ratio of 62% was obtained with plateau at 19.67% (BY = - 1.42 + 0.34Iso; R^2 = 1.00) (Figure 4), for breast fillet weight, 62% with plateau at 135 g (F = 4.0 – 114.0Iso; R^2 = 1.00) (Figure 5) and for breast fillet yield, ratio of 63% with plateau at 15.71% (FY = -2.201429 + 0.285714Iso; R^2 = 1.00) (Figure 6). By applying quadratic analysis with plateau, the best ratios for weight and yield of both parameters assessed are 65 and 66%, respectively.

In the literature, it is possible to observe different recommendations of digestible isoleucine/lysine ratios for broilers at the starter phase. According to Baker et al. (2002), the complexity in establishing accurate ratios for amino acids lies in the possibilities of variable responses to be utilized (such as weight gain and feed conversion) and the
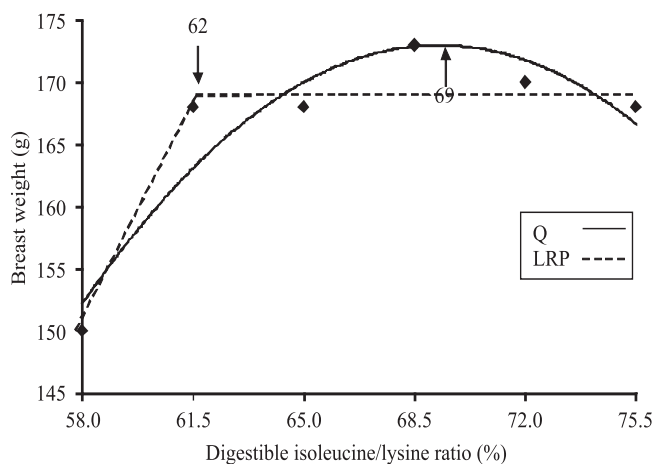
Figure 3 - Effect of different digestible isoleucine/lysine ratios in the diet on the breast weight of broiler chickens slaughtered at 21 days of age.
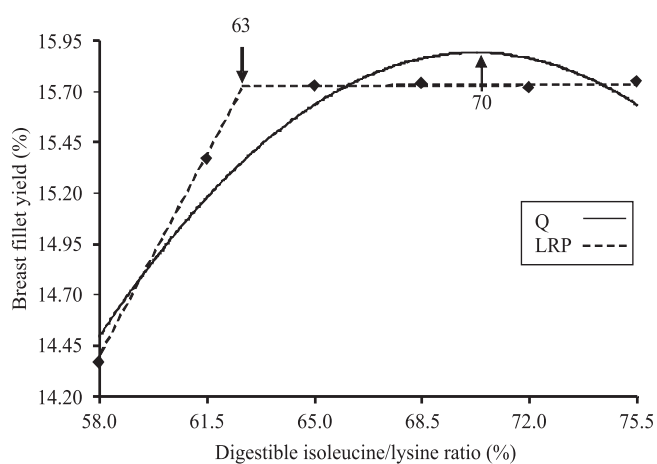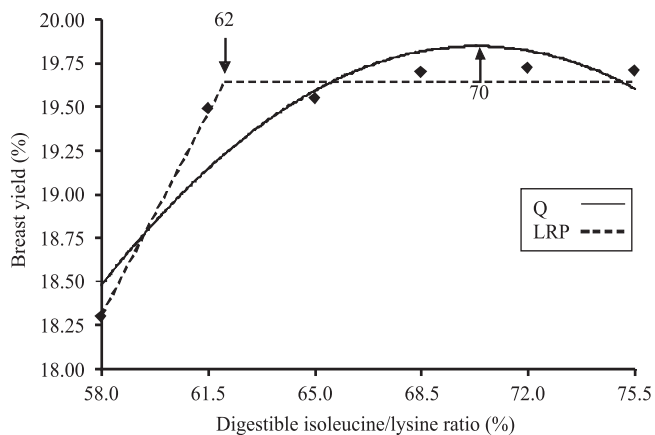

Figure 4 - Effect of different digestible isoleucine/lysine ratios in the diet on the breast yield of broiler chickens slaughtered at 21 days of age.
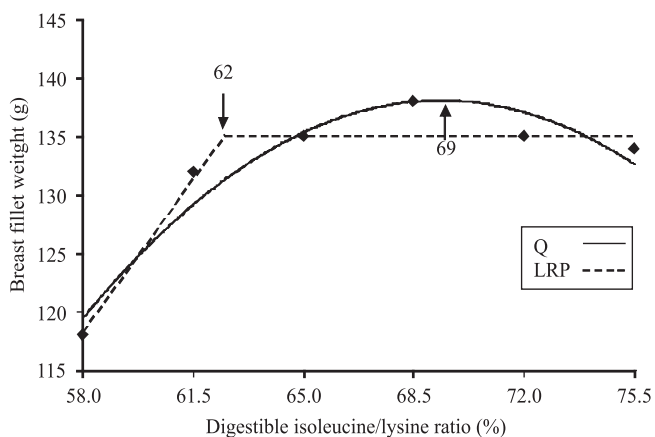

Figure 5 - Effect of different digestible isoleucine/lysine ratios in the diet on the breast fillet weight of broiler chickens slaughtered at 21 days of age.

Figure 6 - Effect of different digestible isoleucine/lysine ratios in the diet on the breast fillet yield of broiler chickens slaughtered at 21 days of age.

statistical analyses that can be carried out. The quadratic one is known for overestimating the ratios, which represents damage and increase in the environmental pollution due to greater nitrogen excretion by animals, whereas the LRP is known for underestimating them, so it can reduce animal performance. Baker et al. (2002) utilized the quadratic analysis as plateau and demonstrated it as a possibility at the determination of the ratios for amino acids, once the values obtained were close to the confidence limit of 90% of the values obtained in the quadratic analysis, although most researchers adopt 95% of the quadratic one (Sakomura & Rostagno, 2007).

Through the results obtained from the different variables herein studied, the recommendation for the digestible isoleucine/lysine ratio, which meets the main productive parameters of broilers in the phase from 7 to 21 days, is 66%, which is a value close to the recommended by Rostagno et al. (2011), of 67%. However, Baker et al. (2002) concluded that 61.4% is the best isoleucine/lysine ratio for the starter phase (08 to 21 days) based on the LRP of weight gain and feed conversion, which is inferior to the value recommended by Baker (1997), of 67%. In a literature review, Sá & Nogueira (2010) observed as average of several studies, the ratio of 66.6% digestible isoleucine/lysine for broiler chickens from 08 to 21 days of age.

Kidd et al. (2004) recommended 6.7 to 7.1 g/kg isoleucine for broiler chickens from 18 to 30 days of age, which are values inferior to the 8.10 g/kg isoleucine in the diet obtained with the conversion of the result from the present study.

Analyzing the results for the finishing phase (Table 6), one can observe that broilers fed diets containing different digestible isoleucine/lysine ratios presented worse (P<0.05) feed conversion when compared with those fed the diet

Table 6 - Feed intake (FI), weight gain (WG) and feed conversion (FC) of broiler chickens fed different digestible isoleucine/lysine ratios during the finishing phase (30 to 43 days)

Ratio (%)	FI (g/bird)	WG (g/bird)	FC
C+	2499	1366a	1.830a
58	2403	1210b	1.988b
62	2499	1290b	1.939b
66	2499	1304b	1.917b
70	2515	1330a	1.892b
74	2476	1314a	1.884b
78	2482	1317a	1.886b
		ANOVA – regression	
Quadratic	0.021	0.006	0.007
CV (%)	3.15	4.13	1.75

Means with different letters on the same column are significantly different from treatment positive control (C+) by the Dunnett test (P<0.05).

control, so it was possible to infer that this is an effect of the sub-optimum level of lysine employed; nevertheless, for weight gain, the ratios of 70, 74 and 78% were significantly similar (P>0.05) to control, indicating the possibility of meeting of the best digestible isoleucine/lysine ratio close to these values.

For the regression analysis, quadratic effect (P<0.05) was observed for feed intake (FI = - 795.78 + 94.7991Iso – 0.679058Iso^2; R^2 = 0.76) (Figure 7), weight gain (WG = - 1592.88 + 81.1666Iso – 0.563571Iso^2; R^2 = 0.93) (Figure 8) and feed conversion (FC = 3.825832 – 0.0516222Iso + 0.000343034Iso^2; R^2 = 0.99) (Figure 9); optimum ratios were 70, 72 and 75%, respectively. By utilizing the confidence limit of 95% for the response of the quadratic equation to feed intake, weight gain and feed conversion, the optimum ratios are 66, 68 and 72%, respectively. At the LRP analysis for feed intake, best ratio of 62% was obtained with plateau at 2493 g (FI = 1011.0 + 24.0Iso; R^2 = 1.00) (Figure 7), for weight gain, it was 63% with plateau at 1316 g (WG = 50.0 + 20.0Iso; R^2 = 1.00) (Figure 8) and for feed conversion, the ratio of 69% with plateau at 1.888 (FC = - 0.00883493 + 2.49557XIso; R^2 = 0.91) (Figure 9). By applying quadratic analysis with plateau, the best ratios for feed intake, weight gain and feed conversion were 64, 68 and 72%, respectively.

For the carcass parameters evaluated in the finishing phase (Table 7), there was no significant effect (P>0.05) of the different digestible isoleucine/lysine ratios evaluated. Through the present study, it is possible to infer that the isoleucine requirement for the carcass parameters assessed at 44 days is lower than the requirement for performance.

Through the results obtained in the different variables studied in the present study, 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%, and this is the same value recommended by
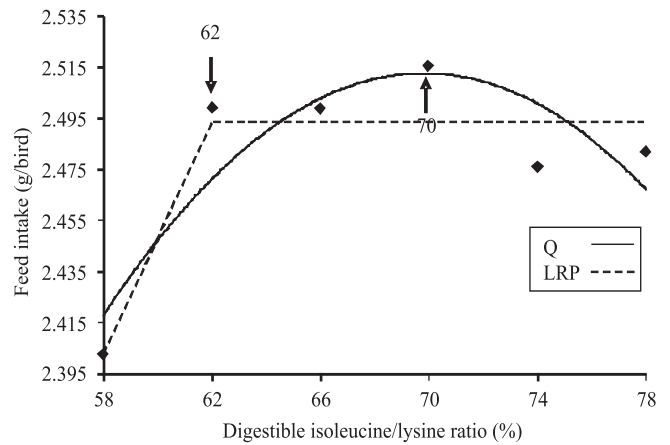

Figure 7 - Effect of different digestible isoleucine/lysine ratios in the diet on the feed intake of broiler chickens in the phase from 30 to 43 days of age.
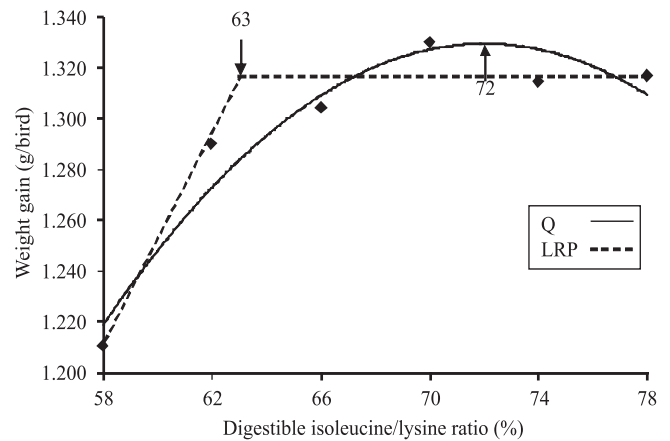

Figure 8 - Effect of different digestible isoleucine/lysine ratios in the diet on the weight gain of broiler chickens in the phase from 30 to 43 days of age.
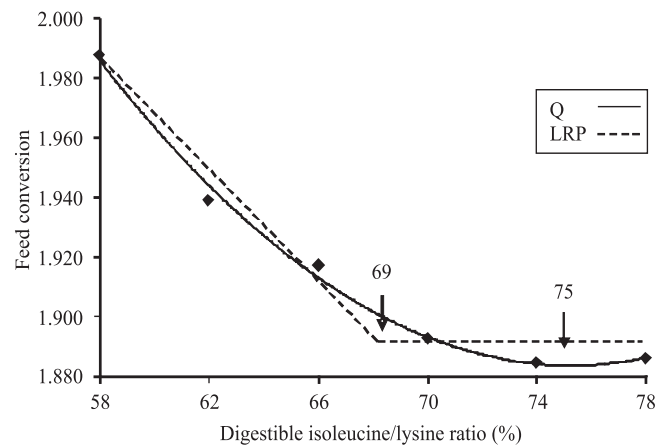

Figure 9 - Effect of different digestible isoleucine/lysine ratios in the diet on the feed conversion of broiler chickens in the phase from 30 to 43 days of age.

Table 7 - Effect of different digestible isoleucine/lysine ratios on carcass (C), abdominal fat (AF), breast (B), breast fillet (F) and thigh and drumstick (TD) of broilers slaughtered at 44 days of age

Ratio (%)	C		AF		B		F		TD	
	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
C+	2156a	72.49	40	1.87	775a	35.95	604a	28.03a	617	28.65
58	2023b	71.75	41	2.04	702b	34.73	537b	26.53b	593	29.35
62	2076b	71.82	44	2.13	729b	35.11	562b	27.07a	604	29.12
66	2107a	72.49	41	1.94	744a	35.33	571b	27.08a	615	29.20
70	2109a	71.96	45	2.16	741a	35.13	571b	27.10a	605	28.72
74	2125a	73.11	51	2.40	745a	35.08	569b	26.81a	614	28.90
78	2106a	72.15	47	2.24	752a	35.71	579a	27.53a	607	28.83
					ANOVA					
Regression	ns[1]	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	2.57	1.79	18.00	18.89	4.56	3.21	4.84	4.26	3.67	3.05

Means with different letters in the same column are significantly different from treatment positive control (C+) by the Dunnett test ($P<0.05$).
[1] Non-significant ($P>0.05$).

Rostagno et al. (2011). Campos et al. (2009) concluded that 70% is the best digestible isoleucine/lysine ratio for this phase. In a literature review, Sá & Nogueira (2010) observed the ratios of 67.1% and 69.5% digestible isoleucine/lysine for broiler chickens in the phases from 22 to 35 and 35 to 42 days of age as average of different studies.

Conclusions

The isoleucine requirement for the carcass parameters evaluated at 44 days is lower than the requirement for performance. The digestible isoleucine/lysine ratio recommended for broiler chickens at the starter phase (7 to 21 days) is 66% and at the finishing phase (30 to 43), 68%.

Acknowledgements

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