The optimum valine: lysine ratios on performance and carcass traits of male broilers based on different regression approaches

P. S. Agostini,[*,1] R. R. Santos,[*] D. R. Khan,[†] D. Siebert,[†] and P. van der Aar[*]

[*]*Schothorst Feed Research, PO Box 533, 8200 AM, Lelystad, The Netherlands; and* [†]*CJ Europe GmbH, Schwalbach, 65824, Taunus, Germany*

ABSTRACT Three different regression approaches were applied to determine the optimal digestible (d.) and analyzed Val:Lys ratios for broiler performance and carcass yield. One-day-old male Cobb 500 broilers ($n =$ 960) were assigned to 1 of 8 diets, with 6 pens/diet and 20 birds/pen, for 42 days. The negative control consisted of the basal diet with a d.Val:d.Lys ratio of 0.63 and with 93% of the required d.Lys. The positive control consisted of the basal diet with a d.Val:d.Lys of 0.80, with no reduction in d.Lys content. The other (test) diets contained a range of d.Val:d.Lys ratios, all with 93% of the required d.Lys. Data on feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were submitted to regression analysis, applying quadratic polynomial (QP), exponential asymptotic (EA), and linear response plateau (LRP) models. Since Val did not affect carcass or breast meat yield, no regression was performed. Digestible and analyzed Val:Lys ratios were similar based on the regression models. The intercept between the QP and LRP models was used to determine the optimum Val:Lys ratio. Overall, the ideal d.Val:d.Lys ratio will vary according to the main goal of poultry production, i.e., BWG or FCR. For BWG, the ideal ratio was found to be 0.78 (0 to 12 d), 0.73 (0 to 28 d), and 0.76 (0 to 35 or 0 to 42 d). For FCR, the optimum d.Val:d.Lys was found to be 0.80 (0 to 12 d), 0.75 (0 to 28 d), and 0.78 (0 to 35 or 0 to 42 d). The optimum analyzed Val:Lys ratio was slightly higher. For instance, for BWG the optimum ratio was 0.80 (0 to 12 d), 0.76 (0 to 28 d), and 0.79 (0 to 35 or 0 to 42 d). For FCR, the optimum Val:Lys was 0.81 (0 to 12 d), 0.79 (0 to 28 d), and 0.81 (0 to 35 or 0 to 42 d). Valine did not affect carcass or breast meat yield.

Key words: amino acid, quadratic polynomial, exponential asymptotic, linear response plateau, Cobb 500

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INTRODUCTION

For several reasons, there is a trend towards the reduction of the crude protein content in broiler diets. One such reason is that protein is the second most expensive component of a diet, after energy. In addition, excess crude protein can impair gut health and has a negative impact on the environment due to increased nitrogen excretion through the feces (Corzo et al., 2009). Diets that are low in crude protein must have appropriate essential amino acid levels to meet the birds' requirements, which can be fulfilled through the supplementation of synthetic amino acids such as lysine (Lys), methionine (Met), threonine (Thr), tryptophan (Trp), and valine (Val). This is supported by the fact that growth performance is not compromised by a marginal reduction in crude protein as long as the essential amino acid requirements are met (Holsheimer and Janssen, 1991; Han et al., 1992; Sigolo et al., 2017).

In the past, the use of L-Val in broiler feeds was limited due to its high price and relatively low availability. However, L-Val is becoming more available for the feed industry, resulting in a more viable price, leading to its inclusion in feed formulas. Moreover, to decrease the dietary content of crude protein, which is linked to lower levels of soybean meal, the use of L-Val in broiler diets is becoming more attractive.

Various studies have been carried out to determine the optimal ratio of Met or Thr to Lys in broilers, whereas the optimal Val to Lys ratio has been little investigated. Valine is considered the fourth limiting amino acid after Met, Lys, and Thr in vegetable diets when considering live weight gain in birds (Rostagno et al., 2011). When diets are formulated with low protein levels, isoleucine becomes co-limiting with Val, notwithstanding feed supplementation with Lys, Met, and Thr (Corzo et al., 2007; Kumar et al., 2015).

Several factors like gender, production stage, health status, and animal genetics may have a direct impact on the Lys requirement. In addition, interactions among

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[1]Corresponding author: pagostini@schothorst.nl

Val, isoleucine, and leucine contribute to differences in the digestible Val to digestible Lys ratio (d.Val:d.Lys) (Torres et al., 1995; Corzo et al., 2009; Berres et al., 2010; Corzo et al., 2010; Wiltafsky et al., 2010). For example, Tuttle and Balloun (1976) concluded that high dietary leucine increases the requirements for Val and isoleucine in growing chickens and turkeys. Such factors could be responsible for the variable results of studies that addressed the optimum d.Val:d.Lys in broiler chickens. Corzo et al. (2007) reported an ideal d.Val:d.Lys ratio of 0.78 in male Ross 308 birds from 21 to 42 d of age. In a study carried out by Dozier et al. (2012), there was no significant effect on broiler performance or carcass characteristics when male Ross 708 broilers were fed a d.Val:d.Lys ratio of either 0.74, 0.78, or 0.82, indicating that the 0.74 d.Val:d.Lys ratio could be adequate. Later on, Tavernari et al. (2013) concluded that the optimal d.Val:d.Lys ratio for male Cobb 500 broilers is 0.77 in the starter phase (8 to 21 d) and 0.76 in the finisher phase (30 to 43 d).

The type of statistical regression model used to determine the optimum absolute or relative (based on the concept of ideal protein) requirement of Val in growing animals may result in different dietary recommendations. The choice of the statistical model depends on the shape of the data or on how much of the total variability of a particular studied parameter is explained by a given regression model. In this type of study, non-linear models such as the linear response plateau (**LRP** or broken line), quadratic polynomial (**QP**), or exponential asymptotic (**EA**) are normally used to estimate amino acid requirements (Simongiovanni et al., 2012).

The present study was designed to determine the optimal digestible and analyzed Val:Lys ratios for maximum growth, best feed efficiency, and maximum breast meat weight and yield in male Cobb broilers from 0 to 42 d of age, by taking different regression approaches into account.

MATERIALS AND METHODS

Ethics

All research was approved and carried out according to the restrictions of the Animal and Human Welfare Codes in the Netherlands.

Birds and Housing

One-day-old male Cobb 500 broilers, purchased from a local commercial hatchery, were used in this study, with 8 dietary treatments of 120 chicks each (divided among 6 pens with 20 chicks each). The birds were housed in 48 floor pens (20 birds/pen; 6 pens/treatment) with wood shavings as bedding material in the broiler facilities of Schothorst Feed Research (SFR), Lelystad, the Netherlands. Each pen (2 m^2) had one feeder and three drinking nipples. Birds were housed until the end of the experiment, i.e., at day 42. Each pen was considered a replicate. The ambient temperature was gradually decreased from 34.5°C at arrival of the birds to 18.0°C at 42 days of age. Room temperature and relative humidity were recorded daily. Light was provided continuously for the first 24 h to give birds the opportunity to find the feed and water readily. After that, the light schedule was 22L:2D for 1 day, and then 8L:4D:10L:2D for the remaining experimental period, complying with EU legislation of a minimum of 6 h of darkness from the second day onwards. Birds were vaccinated against Newcastle Disease at day 10 and against Infectious Bursal Disease at day 20 of the trial. The health status of the flock was monitored by the poultry veterinarian. Temperature and relative humidity were monitored and recorded on a daily basis. All animals were monitored for abnormalities such as abnormal behavior, clinical signs of illness, and mortality throughout the experiment.

Diets and Experimental Design

Eight low protein starter, grower, and finisher diets based on corn, wheat, soybean meal, peas, and rapeseed meal were prepared based on supplementation of L-Val at different levels (Table 1). The negative control (**NC**) had a low Val content and consisted of a basal diet containing a d.Val:d.Lys ratio of 0.63. This diet was formulated to contain 93% of the required amount of d.Lys in order to make Lys the second limiting amino acid in this experiment and, therefore, to determine the optimum d.Val:d.Lys ratio. The other essential amino acids were formulated to be around 5% above the required values. In order to avoid negative interactions between branched chain amino acids, maximum d.Leu:d.Lys and d.Ile:d.Lys ratios were set in the treatment diets (1.09 and 0.67, respectively). A positive control (**PC**) diet was formulated to contain a d.Val:d.Lys ratio of 0.80, with no Lys deficiency. The other 6 dietary treatments are summarized in Table 2. The test diets were fed during the starter (0 to 12 d), grower (12 to 28 d), and finisher (28 to 42 d) phases. All test diets contained phytase and non-starch polysaccharides enzyme. The starter and grower diets also contained a coccidiostat. The pellet diameter was 2.3 mm in the starter and 3.0 mm in the grower and finisher diets. Initially, a batch of the most important feedstuffs (corn, wheat, soybean meal, and rapeseed meal) was reserved and a sample of each feedstuff was analyzed by NutriControl (Veghel, the Netherlands) to determine the complete amino acid profile. Subsequently, diets were re-formulated and a large batch of basal diet was produced (NC diet) per feed phase. Subsequently, this batch was split into 8 sub-batches to which the L-Val and L-Lys (for the PC diet) were added at the expense of L-Glutamate during the mixing process, according to the treatment schedule shown in Table 2. Afterwards, the sub-batches were pelleted. During manufacture, a

Table 1. Composition and calculated nutrients content of starter, grower, and finisher diets with different d.Val:d.Lys levels.

Ingredients (%)	Starter, 1 to 12 d		Grower, 12 to 28 d		Finisher, 28 to 42 d	
	Negative Control (NC)	Positive Control (PC)	Negative Control (NC)	Positive Control (PC)	Negative Control (NC)	Positive Control (PC)
Corn	34.50	34.50	34.74	34.74	33.95	33.95
Wheat	20.11	20.11	22.50	22.50	29.47	29.47
Soybean meal	16.27	16.27	11.06	11.06	8.00	8.00
Peas	10.00	10.00	12.00	12.00	12.00	12.00
Rapeseed meal	4.00	4.00	2.40	2.40	3.25	3.25
Wheat middlings	3.40	3.40	3.12	3.12	3.00	3.00
Sunflowerseed meal	–	–	2.70	2.70	–	–
Poultry fat	4.13	4.13	4.99	4.99	4.97	4.97
Limestone	1.42	1.42	1.13	1.13	0.91	0.91
Mono-Ca-phosphate	0.84	0.84	0.47	0.47	0.22	0.22
$NaHCO_3$	0.50	0.50	0.52	0.52	0.48	0.48
Salt	–	–	–	–	0.01	0.01
L-Lys (79%)	0.51	0.62	0.50	0.60	0.45	0.54
DL-Met (98%)	0.39	0.41	0.35	0.38	0.32	0.34
L-Thr (98%)	0.27	0.29	0.25	0.27	0.23	0.24
L-Trp (98%)	0.05	0.06	0.05	0.06	0.05	0.05
L-Val (99%)	–	0.26	–	0.24	–	0.21
L-Arg (99%)	0.29	0.33	0.25	0.28	0.28	0.28
L-Ile (99%)	0.15	0.17	0.15	0.16	0.15	0.17
Gly (99%)	0.44	0.48	0.40	0.44	0.36	0.40
L-glutamate	0.98	0.46	0.76	0.28	0.92	0.52
Phytase	0.33	0.33	0.33	0.33	0.33	0.33
NSP enzyme (Glu-Xyl)	0.25	0.25	0.25	0.25	0.25	0.25
Salinomycin	0.58	0.58	0.58	0.58	–	–
Premix broiler	0.60	0.60	0.50	0.50	0.40	0.40
Nutrient composition						
Moisture (g/kg)	119.70	119.68	119.88	119.86	120.88	120.87
Ash (g/kg)	52.34	52.53	43.62	43.79	35.84	35.99
Crude protein (g/kg)	190.0	191.5	175.0	176.4	160.0	161.0
Crude fat (g/kg)	67.95	67.95	76.62	76.62	76.87	76.87
AMEn (kcal/kg)	2900	2899	3000	3000	3075	3075
d.Lys (g/kg)	11.16	12.00	10.23	11.00	9.11	9.81
d.Val d.Lys	0.63	0.80	0.63	0.80	0.63	0.80

Table 2. Experimental treatments in the starter, grower, and finisher phases.

Treatment	d.Val:d. Lys ratio	Starter (0 to 12 d)		Grower (12 to 28 d)		Finisher (28 to 42 d)	
		d.Val (g/kg)	d.Lys (g/kg)	d.Val (g/kg)	d.Lys (g/kg)	d.Val (g/kg)	d.Lys (g/kg)
1 (Negative control, NC)	0.63	7.03	11.16[1]	6.44	10.23[1]	5.74	9.11[1]
2	0.68	7.59	11.16	6.96	10.23	6.19	9.11
3	0.73	8.15	11.16	7.46	10.23	6.65	9.11
4	0.78	8.70	11.16	7.98	10.23	7.11	9.11
5	0.83	9.26	11.16	8.49	10.23	7.56	9.11
6	0.88	9.82	11.16	9.00	10.23	8.02	9.11
7	0.93	10.38	11.16	9.51	10.23	8.47	9.11
8 (Positive control, PC)	0.80	9.60	12.00[2]	8.80	11.00[2]	7.85	9.81[2]

[1]d.Lys was limiting (around 7%) and the other essential amino acids were around 5% above their normal requirements.
[2]d.Lys level was close to the values recommended by Cobb Vantress (2015).

composite sample was taken from each test diet. Three sub-samples were taken for each sample. One sample of each test diet was sent to NutriControl for amino acid profile determination in the NC and PC diets and for Val content in the other diets (with added L-Val). One sample of the NC and PC diets was used for proximate analyses by SFR and one sample of all test diets was stored frozen at −20°C at SFR. The test diets were analyzed for moisture, ash, crude protein, and crude fat to verify dietary nutrient composition. Feeds were stored in a cool and dry place until fed to the chickens. The animals had ad libitum access to feed and water. Treatments were randomly allocated per block to the pens. Animals were identified by a unique pen number.

Broiler Performance and Carcass Traits

Broilers were weighed on a per pen basis on day 0, 12, 28, 35, and 42, and feed consumption and mortality were recorded throughout the experiment. Body weight gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) were determined in the grow-out phases from 0 to 12, 12 to 28, 28 to 35, and 28 to 42 d of age. The European Poultry Efficiency Factor (**EPEF**) was calculated from 0 to 28, 0 to 35, and 0 to 42 d as follows:

$$\text{EPEF} = ((10 \times \text{BWG}/\text{number of days}) \times (1 - \text{mortality rate})/100)/\text{FCR}$$

Two chicks from each pen (96 in total) were processed at 42 d. Birds were weighed individually and were fasted for 8 h before slaughter, which was performed by unilateral neck cutting followed by manual evisceration. Carcasses were chilled and stored for approximately 4 h. Carcass weight (kg) was defined as the weight of the plucked, bled, and eviscerated carcass without head, neck, and feet. Carcass yield was expressed as % of BW at 42 d. Breast meat weight included both the musculus pectoralis major and minor (without skin). Breast meat yield was also expressed as % of BW at 42 d.

Statistical Analysis

Three regression models were used to determine the optimal dietary d.Val:d.Lys (or analyzed total Val:Lys) ratios based on BWG, FCR, carcass, and breast yields. For this set of analyses, the PC treatment was not included. All individual observations were taken into account for the regression models. The three models used are as follows:

Model 1: QP

$$Y = \beta 0 + \beta 1 \times X + \beta 2 \times X^2,$$

where

Y	Response variable
X	Val:Lys ratio
$\beta 0$	Intercept
$\beta 1$	Linear coefficient
$\beta 2$	Quadratic coefficient

Maximum responses were calculated as follows: $X = -\beta 1/(2 \times \beta 2)$.

Model 2: EA

$$Y = \beta 0 + \beta 1 \times (1 - \text{EXP}(-\beta 2 \times (X - \beta 3)))$$

where

Y	Response variable
X	Val:Lys ratio
$\beta 0$	Response variable estimated for the lowest Val:Lys ratio
$\beta 1$	Difference between the maximum and minimum response obtained by the increasing Val:Lys ratio
$\beta 2$	Slope of the exponential curve
$\beta 3$	Lowest Val:Lys ratio

Maximum responses were considered at 95% of the plateau and calculated as follows:

$X = \ln(0.05)/\beta 2 + \beta 3$

Model 3: LRP

$$Y = \beta 0 + \beta 1 \times (\beta 2 - X)$$

where

$$(\beta 2 - X) = 0 \text{ for } X > \beta 2,$$

Y	Response variable
X	Val:Lys ratio
$\beta 0$	Value at the plateau
$\beta 1$	Slope
$\beta 2$	Val:Lys ratio at the break point (maximum response)

Raw data were evaluated for outliers per statistical model (analysis of variance or regression) and per measurement period. Significant outliers (outside the range of mean ± 2.5 times SD) were marked as such in the raw data file and excluded from the data set before statistical analysis.

RESULTS

Diet Analysis

The expected (calculated) and analyzed total Lys and total Val of the NC and PC diets as well as the analyzed free Val levels in the other diets (with added L-Val) are shown in Table 3. In general, the analytical results across nutrients were similar to the calculated ones. The analyzed crude protein levels in all NC and PC diets were higher than the expected values (up to 18 g/kg diet) (data not shown). The addition of different levels of L-Val to the NC diets was confirmed in the present study (analyzed vs expected values), although with some variations when considering the dose levels between the lowest and highest d.Val:d.Lys ratios. Therefore, it was decided to use the expected digestible values of Lys and Val as a reference for further regression analyses.

Bird Performance and Carcass Traits

The description of the results will be focused on the results obtained through regression analyses since the results obtained through analysis of variance are of less importance in this study. The latter are shown in Tables 4 and 5 for performance and carcass traits respectively.

Three different approaches, i.e., QP, EA, and LRP, were applied to assess the relationship between d.Val:d.Lys ratios for BWG (Figure 1) and FCR (Figure 2). The PC was not considered in the regression analysis.

Table 3. Expected (calculated) and analyzed amino acid composition of the test diets.

		Expected (calculated) values (g/kg)						Analyzed values (g/kg)					
Feed period	d.Val:d.Lys ratio	Total Lys	Total Val	Total M + C	Total Thr	Total Arg	Val: Lys	Total Lys	Total Val	Total M + C	Total Thr	Total Arg	Val: Lys
Starter	0.63 (Negative control, NC)	12.29	8.19	9.22	8.68	13.26	0.67	13.20	9.10	9.10	9.20	14.30	0.69
	0.68	12.29	8.74	9.22	8.68	13.26	0.71	13.20	9.70	9.10	9.20	14.30	0.73
	0.73	12.29	9.31	9.22	8.68	13.26	0.76	13.20	10.30	9.10	9.20	14.30	0.78
	0.78	12.29	9.86	9.22	8.68	13.26	0.80	13.20	11.20	9.10	9.20	14.30	0.85
	0.83	12.29	10.43	9.22	8.68	13.26	0.85	13.20	11.10	9.10	9.20	14.30	0.84
	0.88	12.29	10.98	9.22	8.68	13.26	0.89	13.20	11.90	9.10	9.20	14.30	0.90
	0.93	12.29	11.54	9.22	8.68	13.26	0.94	13.20	12.60	9.10	9.20	14.30	0.95
	0.80 (Positive control, PC)	13.14	10.76	9.42	8.88	13.58	0.82	14.00	11.40	9.40	9.40	14.20	0.81
Grower	0.63 (NC)	11.27	7.50	8.57	7.98	12.18	0.66	12.20	8.10	8.40	8.30	12.50	0.66
	0.68	11.27	8.01	8.57	7.98	12.18	0.71	12.20	8.70	8.40	8.30	12.50	0.71
	0.73	11.27	8.52	8.57	7.98	12.18	0.76	12.20	9.70	8.40	8.30	12.50	0.79
	0.78	11.27	9.03	8.57	7.98	12.18	0.80	12.20	9.80	8.40	8.30	12.50	0.80
	0.83	11.27	9.55	8.57	7.98	12.18	0.85	12.20	10.20	8.40	8.30	12.50	0.84
	0.88	11.27	10.05	8.57	7.98	12.18	0.89	12.20	10.80	8.40	8.30	12.50	0.88
	0.93	11.27	10.57	8.57	7.98	12.18	0.94	12.20	11.30	8.40	8.30	12.50	0.93
	0.80 (PC)	12.05	9.86	8.87	8.15	12.45	0.82	12.80	10.40	8.70	8.50	13.10	0.81
Finisher	0.63 (NC)	10.04	6.77	7.85	7.13	11.08	0.67	10.40	6.80	7.50	7.10	11.00	0.65
	0.68	10.04	7.27	7.85	7.13	11.08	0.72	10.40	7.30	7.50	7.10	11.00	0.70
	0.73	10.04	7.66	7.85	7.13	11.08	0.76	10.40	7.80	7.50	7.10	11.00	0.75
	0.78	10.04	8.16	7.85	7.13	11.08	0.81	10.40	8.20	7.50	7.10	11.00	0.79
	0.83	10.04	8.55	7.85	7.13	11.08	0.85	10.40	8.60	7.50	7.10	11.00	0.83
	0.88	10.04	9.05	7.85	7.13	11.08	0.90	10.40	9.10	7.50	7.10	11.00	0.87
	0.93	10.04	9.54	7.85	7.13	11.08	0.95	10.40	9.40	7.50	7.10	11.00	0.90
	0.80 (PC)	10.74	8.87	8.03	7.29	11.14	0.83	11.20	9.00	7.90	7.50	11.20	0.80

Table 4. Effect of dietary d.Val:d.Lys (or analyzed Val:Lys) ratios (0 to 12, 0 to 28, 0 to 35, and 0 to 42 d) on body weight gain (BWG; g), feed intake (FI; g), and feed conversion ratio (FCR; g/g), as well as the European Poultry Efficiency Factor (EPEF).

	Calculated d.Val:d.Lys ratio (analyzed Val:Lys ratio[1])									
Items	0.63 (*0.67*) (Negative control, NC)	0.68 (*0.72*)	0.73 (*0.78*)	0.78 (*0.81*)	0.83 (*0.83*)	0.88 (*0.89*)	0.93 (*0.93*)	0.80 (*0.81*) (Positive control, PC)	LSD[2]	*P*-value
0 to 12 d										
BWG (g)	344^{a}	360^{c,d}	358^{b,c,d}	367^{d}	362^{c,d}	354^{a,b,c}	350^{a,b}	364^{c,d}	9.9	<0.001
FI (g)	418	426	429	430	422	427	417	430	12.2	0.17
FCR (g/g)	1.214^{e}	1.183^{b,c}	1.199^{c,d,e}	1.173^{a,b}	1.164^{a}	1.207^{d,e}	1.193^{c,d}	1.183^{b,c}	0.018	<0.001
0 to 28 d										
BWG (g)	1,524^{a}	1,655^{b,c}	1,686^{b,c}	1,687^{b,c}	1,694^{b,c}	1,699^{c}	1,642^{b}	1,690^{b,c}	56.9	<0.001
FI (g)	2,204^{a}	2,306^{b}	2,307^{b}	2,306^{b}	2,306^{b}	2,322^{b}	2,255^{a,b}	2,306^{b}	70.4	0.025
FCR (g/g)	1.446^{c}	1.393^{b}	1.368^{a}	1.367^{a}	1.361^{a}	1.367^{a}	1.374^{a,b}	1.365^{a}	0.021	<0.001
EPEF	370^{a}	413^{b}	440^{c}	434^{c}	441^{c}	439^{c}	427^{b,c}	443^{c}	18.8	<0.001
0 to 35 d										
BWG (g)	2,086^{a}	2,319^{b}	2,368^{b,c}	2,404^{c}	2,366^{b,c}	2,389^{b,c}	2,371^{b,c}	2,386^{b,c}	74.5	<0.001
FI (g)	3,268^{a}	3,471^{b}	3,492^{b}	3,474^{b}	3,475^{b}	3,499^{b}	3,438^{b}	3,466^{b}	105.8	0.002
FCR (g/g)	1.567^{d}	1.497^{c}	1.475^{b,c}	1.445^{a}	1.469^{a,b}	1.465^{a,b}	1.450^{a}	1.453^{a,b}	0.024	<0.001
EPEF	362^{a}	431^{b}	455^{c}	446^{b,c}	448^{b,c}	456^{c}	460^{c}	458^{c}	22.0	<0.001
0 to 42 d										
BWG (g)	2,595^{a}	2,919^{b}	3,078^{c}	3,019^{c}	3,040^{c}	3,035^{c}	3,053^{c}	3,080^{c}	88.5	<0.001
FI (g)	4,356^{a}	4,654^{b}	4,804^{c}	4,683^{b,c}	4,740^{b,c}	4,751^{b,c}	4,697^{b,c}	4,753^{b,c}	135.1	<0.001
FCR (g/g)	1.679^{d}	1.594^{c}	1.561^{a,b}	1.552^{a,b}	1.559^{a,b}	1.566^{b}	1.539^{a}	1.543^{a,b}	0.024	<0.001
EPEF	347^{a}	433^{b,c}	457^{c}	422^{b}	451^{c}	452^{c}	457^{c}	459^{c}	25.9	<0.001

[a–e]Means in a row for a given major effect not sharing a common superscript differ significantly ($P \leq 0.05$). Each mean represents values from 6 replicates (20 birds/replicate), using the pen as the experimental unit.

[1]Calculated as the weighted average of each treatment across the starter, grower, and finisher phases by taking the number of days per phase as weight criteria (Table 2).

[2]LSD: least significant difference.

Italic values in brackets refer to analyzed Val:Lys ratio.

Table 5. Effect of dietary d.Val:d.Lys (or analyzed Val:Lys) ratios on carcass weight (g), carcass yield (%), breast weight (g), and breast yield (%) at day 42 of 2 selected birds per pen.

	Calculated d.Val:d.Lys ratio (analyzed Val:Lys ratio[1])									
Items	0.63 (*0.67*) (Negative control, NC)	0.68 (*0.72*)	0.73 (*0.78*)	0.78 (*0.81*)	0.83 (*0.83*)	0.88 (*0.89*)	0.93 (*0.93*)	0.80 (*0.81*) (Positive control, PC)	LSD[2]	*P*-value
Carcass weight (g)	1,889^{a}	2,117^{b,c}	2,157^{b,c}	2,066^{b,c}	2,178^{c}	2,124^{b,c}	2,028^{a,b}	2,108^{b,c}	144.0	0.008
Carcass yield (%)	68.4	67.9	67.7	68.3	68.1	68.4	67.4	68.4	1.62	0.88
Breast weight (g)	560^{a}	620^{b,c,d}	573^{a,b}	580^{a,b,c}	637^{d}	613^{b,c,d}	572^{a,b}	628^{c,d}	51.5	0.021
Breast yield (%)	20.2	19.9	18.8	18.9	20.0	19.7	19.0	20.4	1.38	0.15

[a–d]Means in a row for a given major effect not sharing a common superscript differ significantly ($P \leq 0.05$). Each mean represents values from 6 replicates (2 birds/replicate), using the pen as the experimental unit.

[1]Calculated as the weighted average of each treatment across the starter, grower, and finisher phases by taking the number of days per phase as weight criteria (Table 2).

[2]LSD: least significant difference.

Italic values in brackets refer to analyzed Val:Lys ratio.

The details of the equations used to determine the optimal d.Val:d.Lys ratios for BWG and FCR in the different periods are presented in Table 6. The data used when the analyzed Val:Lys ratios were considered for the aforementioned parameters are presented in Table 7.

Body Weight Gain

The dose–response relationships between the different d.Val:d.Lys ratios and BWG determined by the QP, EA, and LRP regressions are depicted in Figure 1. Since the Val:Lys ratios followed a similar pattern (Table 7), no extra figure was prepared. The optimum d.Val:d.Lys ratio for BWG in the period between 0 and 12 d of age was estimated to be 0.78 ($R^2 = 0.39$) based on the QP approach. The optimal Val:Lys ratio for the same period was 0.80 ($R^2 = 0.38$). For both the d.Val:d.Lys and Val:Lys ratios, the estimates based on the EA and LRP approaches were not selected due to the much lower R^2 compared with that obtained using the QP approach. From 0 to 28 d of age, the optimum d.Val:d.Lys ratio for BWG was estimated to be 0.81 ($R^2 = 0.52$), 0.71 ($R^2 = 0.52$), and 0.69 ($R^2 = 0.52$) based on the QP,

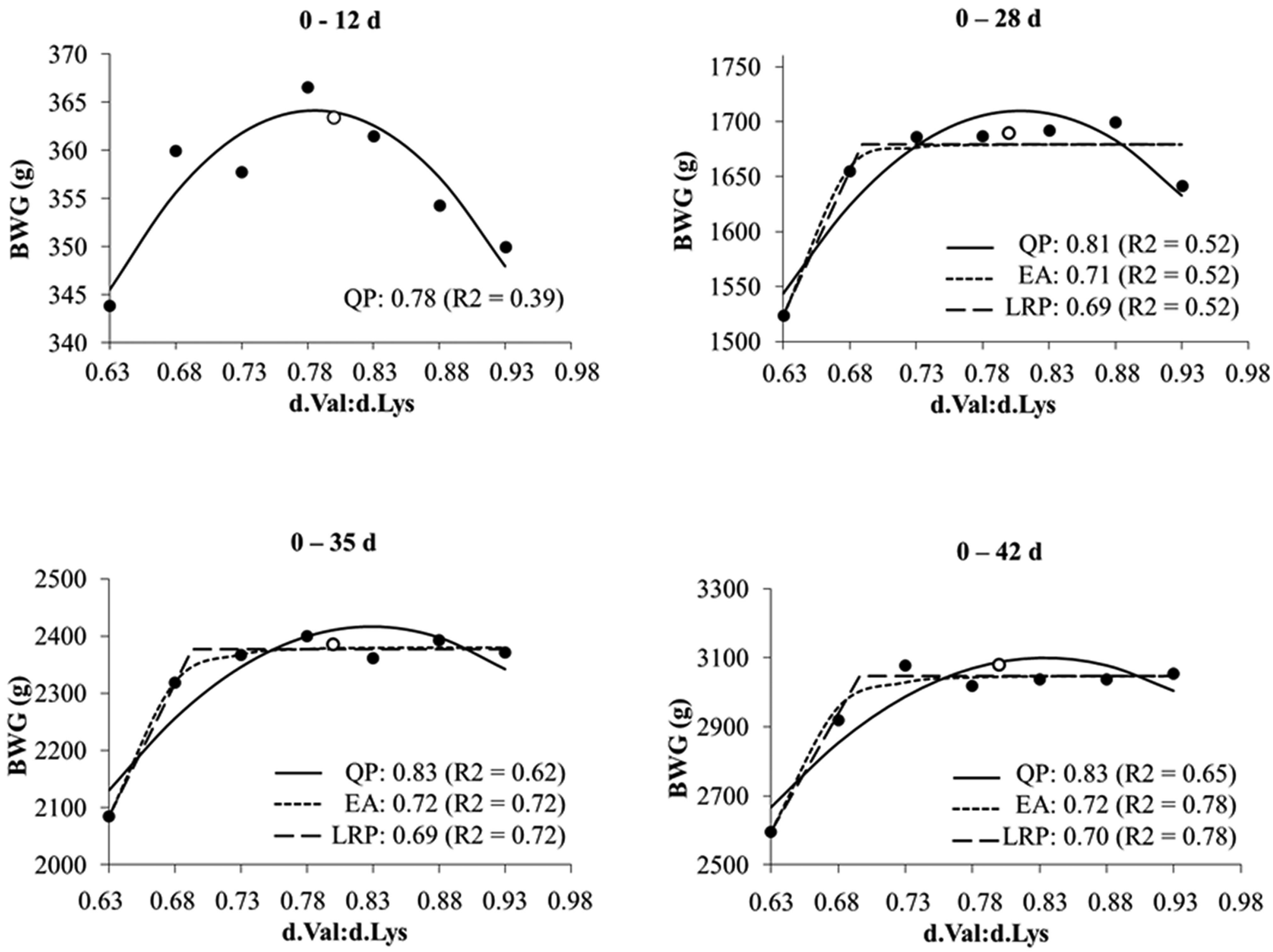

Figure 1. Effect of d.Val:d.Lys ratio on body weight gain (BWG) (g) of broilers from 0 to 12, 0 to 28, 0 to 35, and 0 to 42 d of age. The white circle represents the positive control (PC) diet, which is not included in the regression models.

EA, and LRP approaches, respectively. The optimum Val:Lys ratio was 0.83 (R^2 = 0.53), 0.74 (R^2 = 0.52), and 0.72 (R^2 = 0.52) based on the QP, EA, and LRP approaches, respectively. From 0 to 35 d of age, the optimum d.Val:d.Lys ratio for BWG was 0.83 ($R^2 = 0.62$), 0.72 ($R^2 = 0.72$), and 0.69 ($R^2 = 0.72$) based on the QP, EA, and LRP approaches, respectively, while the optimum Val:Lys ratio was 0.85 (R^2 = 0.64), 0.76 (R^2 = 0.71), and 0.73 (R^2 = 0.71) based on the QP, EA, and LRP approaches, respectively. Finally, from 0 to 42 d of age, the optimum d.Val:d.Lys ratio for BWG was estimated to be 0.83 ($R^2 = 0.65$), 0.72 ($R^2 = 0.78$) and 0.70 ($R^2 = 0.78$) based on the QP, EA and LRP approaches, respectively. The optimum Val:Lys ratio was 0.85 (R^2 = 0.69), 0.76 (R^2 = 0.78) and 0.73 (R^2 = 0.78) based on the QP, EA, and LRP approaches, respectively.

Feed Conversion Ratio

The dose–response relationships between the different d.Val:d.Lys ratios and FCR obtained using QP, EA, and LRP regressions are depicted in Figure 2. Since the Val:Lys ratios followed the same pattern (Table 7), no extra figure was prepared. The optimum d.Val:d.Lys ratio for FCR in the period from 0 to 12 d was estimated to be 0.80 ($R^2 = 0.26$) based on the QP approach, while the optimum Val:Lys ratio in the same period was 0.81 ($R^2 = 0.25$), also based on the QP approach. The estimates based on the EA and LRP approaches were not selected in this case either. From 0 to 28 d of age, the optimum d.Val:d.Lys ratio for the FCR was estimated to be 0.82 (R^2 = 0.71), 0.75 (R^2 = 0.73), and 0.71 (R^2 = 0.74) based on the QP, EA, and LRP approaches, respectively. The optimum Val:Lys ratio was 0.84 (R^2 = 0.73), 0.79 (R^2 = 0.73), and 0.74 (R^2 = 0.74) based on the QP, EA, and LRP approaches, respectively. From 0 to 35 d of age, the optimum d.Val:d.Lys ratio for the FCR was 0.85 (R^2 = 0.70), 0.78 (R^2 = 0.78), and 0.71 (R^2 = 0.77) based on the QP, EA, and LRP approaches, respectively, while the optimum Val:Lys ratio was 0.87 (R^2 = 0.73), 0.82 (R^2 = 0.78), and 0.74 (R^2 = 0.77) based on the QP, EA, and LRP approaches, respectively. Finally, from 0 to 42 d of age, the optimum d.Val:d.Lys ratio for the FCR in this period was estimated to be 0.85 (R^2 = 0.70), 0.76 (R^2 = 0.81), and 0.70 (R^2 = 0.81) based on the QP, EA, and LRP approaches, respectively. The optimum Val:Lys ratio was 0.87 (R^2 = 0.73), 0.79 (R^2 = 0.81), and 0.74 (R^2 = 0.81) based on the QP, EA, and LRP approaches, respectively.

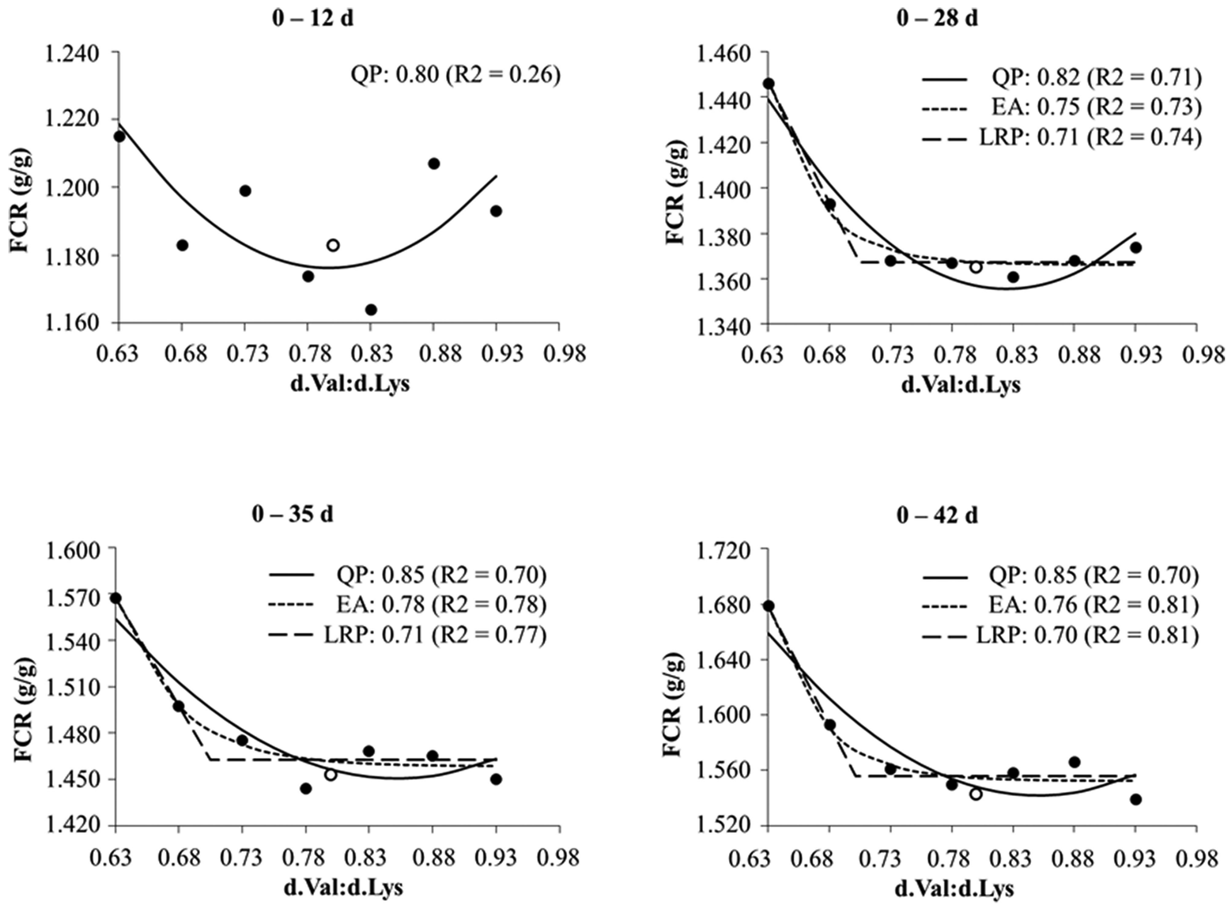

Figure 2. Effect of d.Val:d.Lys ratio on feed conversion ratio (FCR) (g/g) of broilers from 0 to 12, 0 to 28, 0 to 35, and 0 to 42 d of age. The white circle represents the positive control (PC) diet, which is not included in the regression models.

Carcass Traits

Since there was no clear dose–response relationship between the d.Val:d.Lys ratio and carcass traits, there were no regressions models that fitted the data particularly well.

DISCUSSION

The present study determined the optimum digestible and analyzed Val:Lys ratios needed to obtain the best performance and carcass traits in broilers from 0 to 42 d of age. For this, three regression models (QP, EA, and LRP) were applied. Based on the fact that the results for both digestible and total amino acids were similar when applying regression analysis, herein we discuss only the digestible Val:Lys ratios.

A PC group was fed with an optimal level of d.Lys and the expected ideal d.Val:d.Lys ratio of 0.80. Hence, it was expected that birds from the PC group would outperform birds from the other treatments, due to this higher d.Lys level. However, broilers from the PC group only outperformed under the d.Val:d.Lys treatments for EPEF from 0 to 28 d, and BWG and EPEF from 0 to 42 d. This may have occurred for different reasons. First, the d.Lys level in the NC diet was less limiting than expected for growth performance, as confirmed by the higher levels of analyzed compared with calculated total Lys levels. Second, the PC diet had a similar composition of the NC diet, differing only in the amount of L-Lys, DL-Met, L-Thr, L-Trp, L-Val, L-Arg, L-Ile, and Gly that were added (higher in the PC diet). In contrast, less L-Glu was added to the PC diet compared with the NC diet in order to keep a constant crude protein level between both diets, which could explain why the PC treatment did not outperform some of the other treatments, likely due to the lower d.Glu level of the PC diet compared with the NC diet (33.0 vs. 38.2 g/kg in the starter phase; 29.6 vs. 34.5 g/kg in the grower phase; and 29.9 vs. 33.9 g/kg in the finisher phase). Such differences in d.Glu levels have resulted in lower level of non-essential amino acids in the PC diet compared with the NC diet (85.3 vs. 90.0 g/kg in the starter phase; 77.4 vs. 81.9 g/kg in the grower phase; and 72.3 vs. 76.5 g/kg in the finisher phase), which would be necessary to compensate the depressed growth attributed to a diet with low crude protein levels (Awad et al., 2015). Importantly, Lys remained the second limiting amino acid in our study since the ratio of the other essential amino acids to Lys in the NC diet was above the bird's requirements. In addition, birds have responded with higher BWG and better FCR to graded increase

Table 6. Optimum d.Val:d.Lys ratios observed for the best performance responses in broiler chickens.

Parameter	Regression model[1]	Equation	Optimum d.Val:d.Lys ratio	Coefficient of determination (R^2)	Ideal[2] d.Val:d.Lys ratio
0 to 12 d					
BWG	QP	$Y = -112.5 + 1214 \times X - 773 \times X^2$	0.78	0.39	–
FCR	QP	$Y = 2.142 - 2.424 \times X - 1.521 \times X^2$	0.80	0.26	–
0 to 28 d					
BWG	QP	$Y = -1697 + 8427 \times X - 5211 \times X^2$	0.81	0.52	0.73
	EA	$Y = 1523 + 157 \times (1 - \exp(-40.135 \times (X - 0.63)))$	0.71	0.52	
	LRP	$Y = 1679 - 2629 \times (0.69 - X)$, when $X < 0.69$, if $X \geq 0.69$ Y = 1679 g	0.69	0.52	
FCR	QP	$Y = 2.85 - 3.623 \times X + 2.196 \times X^2$	0.82	0.71	0.75
	EA	$Y = 1.447 - 0.081 \times (1 - \exp(-24.687 \times (X - 0.63)))$	0.75	0.73	
	LRP	$Y = 1.367 + 1.062 \times (0.71 - X)$, when $X < 0.71$, if $X \geq 0.71$ Y = 1.367	0.71	0.74	
0 to 35 d					
BWG	QP	$Y = -2567 + 12{,}028 \times X - 7257 \times X^2$	0.83	0.62	0.76
	EA	$Y = 2085 + 295 \times (1 - \exp(-31.85 \times (X - 0.63)))$	0.72	0.72	
	LRP	$Y = 2377 - 4655 \times (0.69 - X)$, when $X < 0.69$, if $X \geq 0.69$ Y = 2377 g	0.69	0.72	
FCR	QP	$Y = 2.966 - 3.557 \times X + 2.087 \times X^2$	0.85	0.70	0.78
	EA	$Y = 1.567 - 0.108 \times (1 - \exp(-20.416 \times (X - 0.63)))$	0.78	0.78	
	LRP	$Y = 1.463 + 1.398 \times (0.71 - X)$, when $X < 0.71$, if $X \geq 0.71$ Y = 1.463	0.71	0.77	
0 to 42 d					
BWG	QP	$Y = -4111 + 17{,}286 \times X - 10{,}361 \times X^2$	0.83	0.65	0.76
	EA	$Y = 2596 + 452 \times (1 - \exp(-32.045 \times (X - 0.63)))$	0.72	0.78	
	LRP	$Y = 3046 - 6838 \times (0.70 - X)$, when $X < 0.70$, if $X \geq 0.70$ Y = 3046 g	0.70	0.78	
FCR	QP	$Y = 3.28 - 4.084 \times X + 2.399 \times X^2$	0.85	0.70	0.78
	EA	$Y = 1.679 - 0.126 \times (1 - \exp(-23.723 \times (X - 0.63)))$	0.76	0.81	
	LRP	$Y = 1.556 + 1.724 \times (0.70 - X)$, when $X < 0.70$, if $X \geq 0.70$ Y = 1.556	0.70	0.81	

[1]QP: quadratic polynomial; EA: exponential asymptotic; LRP: linear response plateau.
[2]Estimated at the first point with an intersection between QP curve and LRP models.

of d.Val:d.Lys ratio. Moreover, the ideal ratios observed for BWG and FCR are in line with studies performed by other research groups (Thornton et al., 2006; Tavernari et al. (2013).

Birds fed diets with the highest d.Val:d.Lys ratio (= 0.93) had a lower BWG and FCR during the starter and grower phases compared with the lower ratios, but in the finisher period (28 to 35 and 28 to 42 d), the highest BWG and lowest FCR were recorded when the d.Val:d.Lys ratio was 0.88 and 0.93, respectively. This is probably a consequence of the compensatory growth in the finisher period (28 to 42 d) rather than a result of the extra L-Val supply because of both the lower maintenance requirement and body weight of these birds during the starter and grower phase.

To determine the ideal d.Val:d.Lys ratio from 0 to 12, 0 to 28, 0 to 35, and 0 to 42 d, several curve fitting models were applied, given that different regression models will fit properly or not depending on the different evaluated parameters. Birds fed different d.Val:d.Lys ratios responded with a gradual increase in growth or improvement in feed efficiency up to a certain ratio, regardless of the period tested, which indicates that Val was limiting in this study. However, the absence of a relationship between the d.Val:d.Lys ratio and carcass traits was observed, which means that the supplementation of Val was not critical in promoting higher carcass/breast weights or yields. Furthermore, no regression model was able to adequately fit the data on carcass traits (low R^2 values observed). Similarly, Thornton et al. (2006) observed that the growth and feed efficiency of Ross 508 broilers responded linearly to increasing L-Val supplementation, whereas no response was observed for carcass traits. This finding was more recently confirmed by Tavernari et al. (2013) who studied the effect of several d.Val:d.Lys ratios (from 0.70 to 0.85) on Cobb 500 broilers, in which Val had no significant effect on carcass and cut yields, but did affect growth and feed conversion.

Regardless of the measured response parameters, curve fitting was less accurate in the starter phase (0 to 12 d) than during 0 to 28, 0 to 35, and 0 to 42 d, based on the coefficients of determination (R^2). This was not caused by differences in calculated and analyzed Val:Lys and subsequent differences in Lys levels in diets, but by the larger variation in this early period, resulting in a less reliable optimum d.Val:d.Lys ratio. Birds fed high d.Val:d.Lys ratios showed worse performance responses especially in the starter phase (0 to 12 d) and from 0 to 28 d of age, indicating that these birds were more sensitive to high d.Val:d.Lys ratios. This probably occurred due to the greater energy expenditure needed to deaminate and excrete the excess Val. This can be explained by the fact that in the starter phase the optimum ratio could only be estimated using the QP model (unlike in other measured periods), which normally overestimates the ideal ratio.

The optimum d.Val:d.Lys ratio estimated by the QP and EA models is usually an overestimate (Sakomura and Rostagno, 2007), while the LRP model tends to underestimate it (Pack, 1996). In the present study, the difference in optimum d.Val:d.Lys ratio determined per

Table 7. Optimum analyzed total Val:Lys ratios observed for the best performance responses in broiler chickens.

Parameter	Regression model[1]	Equation	Optimum Val:Lys ratio	Coefficient of determination (R^2)	Ideal[2] Val:Lys ratio
0 to 12 d					
BWG	QP	$Y = -276 + 1592 \times X - 991 \times X^2$	0.80	0.38	–
FCR	QP	$Y = 2.469 - 3.181 \times X - 1.958 \times X^2$	0.81	0.25	–
0 to 28 d					
BWG	QP	$Y = -2807 + 10{,}931 \times X - 6621 \times X^2$	0.83	0.53	0.76
	EA	$Y = 1524 + 156 \times (1 - \exp(-41.298 \times (X - 0.67)))$	0.74	0.52	
	LRP	$Y = 1679 - 2753 \times (0.72 - X)$, when $X < 0.72$, if $X \geq 0.72$ Y = 1679 g	0.72	0.52	
FCR	QP	$Y = 3.306 - 4.629 \times X + 2.751 \times X^2$	0.84	0.73	0.79
	EA	$Y = 1.447 - 0.081 \times (1 - \exp(-25.289 \times (X - 0.67)))$	0.79	0.73	
	LRP	$Y = 1.367 + 1.112 \times (0.74 - X)$, when $X < 0.74$, if $X \geq 0.74$ Y = 1.367	0.74	0.74	
0 to 35 d					
BWG	QP	$Y = -4042 + 15{,}249 \times X - 9012 \times X^2$	0.85	0.64	0.79
	EA	$Y = 2085 + 295 \times (1 - \exp(-32.97 \times (X - 0.67)))$	0.76	0.71	
	LRP	$Y = 2377 - 4876 \times (0.73 - X)$, when $X < 0.73$, if $X \geq 0.73$ Y = 2377 g	0.73	0.71	
FCR	QP	$Y = 3.364 - 4.391 \times X + 2.552 \times X^2$	0.87	0.73	0.81
	EA	$Y = 1.567 - 0.109 \times (1 - \exp(-20.147 \times (X - 0.67)))$	0.82	0.78	
	LRP	$Y = 1.463 + 1.463 \times (0.74 - X)$, when $X < 0.74$, if $X \geq 0.74$ Y = 1.463	0.74	0.77	
0 to 42 d					
BWG	QP	$Y = -6361 + 22{,}206 \times X - 13{,}047 \times X^2$	0.85	0.69	0.79
	EA	$Y = 2595 + 454 \times (1 - \exp(-31.824 \times (X - 0.67)))$	0.76	0.78	
	LRP	$Y = 3046 - 7160 \times (0.73 - X)$, when $X < 0.73$, if $X \geq 0.73$ Y = 3046 g	0.73	0.78	
FCR	QP	$Y = 3.774 - 5.134 \times X + 2.956 \times X^2$	0.87	0.73	0.81
	EA	$Y = 1.678 - 0.126 \times (1 - \exp(-23.575 \times (X - 0.67)))$	0.79	0.81	
	LRP	$Y = 1.556 + 1.805 \times (0.74 - X)$, when $X < 0.74$, if $X \geq 0.74$ Y = 1.556	0.74	0.81	

[1]QP: quadratic polynomial; EA: exponential asymptotic; LRP: linear response plateau.
[2]Estimated at the first point with an intersection between QP curve and LRP models.

response parameter and per measured period seems to be variable across different regression models (Table 6). Therefore, the ideal d.Val:d.Lys ratio for the different parameters in the present study was determined according to Euclydes and Rostagno (2001), who used the QP model to establish the first point at which the quadratic response curve intersected the plateau value established by the LRP model. The intercept value of X can be calculated using the QP equation once the plateau value (Y) of the LRP is determined (Table 6).

Therefore the estimated ideal d.Val:d.Lys ratio in the present study was 0.73 and 0.75 from 0 to 28 d, 0.76 and 0.78 from 0 to 35 d, and 0.76 and 0.78 from 0 to 42 d for BWG and FCR, respectively. The ideal d.Val:d.Lys ratio in the starter phase could not be estimated since only the optimum ratio through the QP approach could be calculated in this period.

Tavernari et al. (2013) concluded that the optimal d.Val:d.Lys ratio for male Cobb 500 broilers is 0.77 in the starter phase (8 to 21 d) and 0.76 in the finisher phase (30 to 43 d). Corzo et al. (2007) stated that the ideal d.Val:d.Lys ratio for male Ross 308 birds from 21 to 42 d of age was 0.78. The findings from these authors are similar to the observations made in the present study.

The ideal d.Val:d.Lys ratio calculated in the studied periods (0 to 12, 0 to 28, 0 to 35, and 0.42 d) was lower than expected (≅ 0.80) in the present study. In a study carried out by Dozier et al. (2012), no significant effects on broiler performance or carcass characteristics were noted when male Ross 708 broilers were fed a d.Val:d.Lys ratio of either 0.74, 0.78, or 0.82, indicating that the 0.74 d.Val:d.Lys ratio could be adequate. One of the potential reasons for the low ideal d.Val:d.Lys ratio observed in the present study might be genetic selection that has taken place over the years, since broilers now produce more breast meat than earlier. This means that their Lys requirement has increased, together with the requirement for other essential amino acids such as Val. However, the increase in the requirement for essential amino acids might be lower than that for Lys, resulting in a reduced d.Val:d.Lys ratio.

In conclusion, the ideal d.Val:d.Lys ratio for broilers in the starter phase (0 to 12 d) was 0.78 (BWG) and 0.80 (FCR). From 0 to 28 d, the ideal d.Val:d.Lys ratio appears to be lower than in the starter phase, i.e., 0.73 (BWG) and 0.75 (FCR), and the ideal d.Val:d.Lys ratio from 0 to 35 and 0 to 42 d is higher than that from 0 to 28 d of age, i.e., 0.76 (BWG) and 0.78 (FCR) in both periods. However, when the data were correlated based on the analyzed values of the test diets, the ideal Val:Lys ratio for broilers in the starter phase (0 to 12 d) was 0.80 (BWG) and 0.81 (FCR). From 0 to 28 d, the ideal Val:Lys ratio appears to be lower than in the starter phase, i.e., 0.76 (BWG) and 0.79 (FCR), and the ideal Val:Lys ratio from 0 to 35 and 0 to 42 d is higher than that from day 0 to 28 of age, i.e., 0.79 (BWG) and 0.81 (FCR) in both periods. These data also show that supplementation of Val is not critical in promoting higher carcass/breast weights or yields.

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