The effect of gradually decreasing the dietary energy content, at constant or increased lysine:energy ratio on broiler performance, carcass yield, and body composition

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ABSTRACT Formulating diets with high AME, especially in the grower and finisher phases, hinders the inclusion of alternative ingredients that are usually cheaper and have lower AME. Moreover, as the chicken grows the feed intake capacity is greater and may be able to maintain BW over a wide range of AME. The objective of this study was to evaluate the performance of chickens fed diets with progressive AME reductions, at constant or increased standardized ileal digestible (**SID**) lysine: AME ratio (**Lys:AME**). Treatment 1 (control) was formulated following the SID lysine and AME recommendations for a 4-phase feeding program. Treatment 5 was formulated with −4, −8, and −12% AME in the grower-1, grower-2, and finisher phases, respectively, and with the same Lys:AME compared with the control. Treatment 9 had the same AME as treatment 5 but higher SID lysine, increasing the Lys:AME by 1.5, 3.5, and 5.0% compared with treatment 5. In the grower-1, grower-2, and finisher phases, the final 9 dietary treatments were prepared by mixing the control diet with either treatment 5 or 9 at different proportions (75:25, 50:50, or 25:75). All birds were fed the same starter control diet. Treatments were replicated in 10 pens with 31 male chickens each, and the growth performance of birds was monitored for 42 d. Final BW linearly decreased ($P < 0.05$) when lowering dietary AME, but it followed a positive quadratic response with higher Lys:AME ($P < 0.05$). Feed intake increased ($P < 0.05$) with low AME, independently of the Lys:AME; but the linear regression in the feed conversion ratio (**FCR**) had a lower slope when the Lys:AME increased. At the end of the study, there were no differences in carcass or breast meat yield ($P > 0.10$). Progressively reducing AME in the last feeding phases may be a viable nutritional strategy to increase the inclusion of alternative ingredients and potentially reduce feeding costs, despite increments in feed intake and FCR. Adjusting the Lys:AME in low AME diets may help maintain the final BW of birds.

Key words: broiler, alternative ingredients, AME, growth performance, standardized ileal digestible lysine

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INTRODUCTION

There is high pressure in the livestock sector to lessen the competition for ingredients that are used in the human food chain and for feeding animals (Ravindran, 2013; Tallentire et al., 2018). Such competition may promote price increments that would finally affect poultry producers. Thus, poultry nutritionists are eager to increase the usage of alternative cereals and co-products (El-Deek et al., 2020). The inclusion of alternative ingredients can prove difficult when targeting high dietary AME, especially in the last feeding phases. Common formulations of grower and finisher diets have around 2,950 and 3,100 AME kcal/kg (CVB, 2018) which significantly contributes to reducing the inclusion of low AME ingredients. Alternatively, forcing a minimum inclusion of these alternative ingredients, while maintaining the target AME, may further increase the final feed price.

Classen (2017) suggested that the capacity of the modern broiler chicken to adjust feed intake, based on dietary AME, has been affected by the continuous and aggressive genetic selection in the past decades. In response, Taylor et al. (2021) demonstrated that the capacity of the modern chicken to regulate feed intake remains viable; the maximum feed intake capacity is probably more related to maximal gut fill, and it depends on the kind and the amount of fiber included in the diet. Dilution of AME with oat hulls (insoluble fiber ingredient; Röhe and Zentek, 2021) did not impede feed

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intake, but when sugar beet pulp was added (soluble fiber ingredient), the chickens did not increase feed intake and reduced AME intake (Taylor et al., 2021).

After hatching, the broiler gastrointestinal tract is immature (Sklan, 2001), and exposure to high-fiber feed ingredients could permanently and negatively affect overall performance. After the first week to 10 d, the gastrointestinal tract significantly develops (Batal and Parsons, 2002; Khalil et al., 2022), and it may be better suited to digest greater quantities of alternative ingredients with a high-fiber content (e.g., barley, oats, sunflower meal, and canola meal). Then, it is reasonable to conclude that the opportunity to capitalize on the use of alternative ingredients is in the final feeding phases, where feed intake is the highest and when chickens have a better-developed gastrointestinal tract. This hypothesis of progressive AME reduction, parallel to the bird growth and throughout the production cycle, in conditions where results could be of use to poultry producers, is of interest. Furthermore, reducing the AME content of the diet, by increasing the inclusion of alternative high-fiber ingredients, would imply that the daily maintenance AME and amino acid requirements represent a higher proportion of the total nutrient intake, potentially leading to changes in the optimal standardized ileal digestible (**SID**) lysine (**Lys**) to AME ratio (**Lys:AME**). The objective of this study was to evaluate the growth performance, carcass traits, and carcass composition of chickens fed diets with progressive dietary AME dilutions, either with a constant or increased Lys:AME.

MATERIALS AND METHODS

Ethic Statement, Animals, and Management

The present study was approved by the Trouw Nutrition R&D Animal Care Ethical committee (Animal use protocol: 16-2019). Chickens were managed according to the Spanish Regulations of Usage of Animals in Research (Royal Decree 53/2013) in agreement with the European Parliament (2010).

A total of 2,790 one-day-old male Ross 308 chicks were used. Chicks were sourced from a local hatchery (Sada Group Inc., Seville, Spain) and were vaccinated against Marek and Gumboro diseases, infectious bronchitis, and coccidiosis before leaving the hatchery. Upon arrival, chicks were randomly distributed to 90 pens (1.48 m width × 2.10 m length) with 31 chickens per pen. The initial average BW of chickens was 43.5 ± 0.5 g (mean ± SD).

Two days before the arrival of the chicks, wood shavings (10 cm deep) were placed on each pen and the barn min temperature was set at 34°C. Temperature was then decreased to 32 (d 2), 31 (d 4), 30 (d 7), 28 (d 9), 26 (d 11), 24 (d 15), 22 (d 22) and 20°C on d 20 until the end of the study (d 43). The average relative humidity inside the barn was 36 ± 8 % (mean ± SD). During the first 3 d, the birds were exposed to continuous lighting (24 h) and 18 h:6 h light:dark schedule thereafter.

Each pen was equipped with 5 nipple drinkers, and feed was offered on a feeding plate (39 cm diameter) during the first 5 da. On d 3, a tower feeder was also added to each pen to ease the transition from the feeding plate to the tower feeder. During the whole study, chickens had ad libitum access to feed and fresh water.

Experimental Treatments

The 90 experimental pens were divided into 10 blocks of 9 consecutive pens. Within each block, pens were randomly assigned to 1 of 9 experimental treatments. Treatments were based on a 4-phase feeding program: starter (0−9 d), grower-1 (9−20 d), grower-2 (20−31 d), and finisher (31−42 d) phases. The starter feed was the same for all treatments and met the requirements for all nutrients (CVB, 2018). In each of the following feeding phases, the treatments were as follows: treatment 1 (control) diets were formulated to meet or exceed all nutrient recommendations (CVB, 2018). Treatment 5 diets had lower AME (−4, −8, and −12%) than Control in the grower-1, grower-2, and finisher phases, respectively, and maintained the Lys:AME compared to control. Treatment 9 diets had the same AME as treatment 5, but the SID Lys content was increased by 1.5, 3.5, and 5.0 %, increasing the Lys:AME in the same proportion when compared with treatments 1 or 5. In the grower-1, grower-2, and finisher phases, the 9 dietary treatments resulted from mixing the corresponding diets in treatments 1 and 5 or 1 and 9 at different proportions (75:25, 50:50, and 25:75) to make treatments 2, 3, and 4, or 6, 7, and 8 respectively. The ingredient composition and calculated nutrient contents of treatments 1, 5, and 9 are presented in Tables 1 and 2. All diets were formulated following the ideal protein concept and using the Bestmix least-cost formulation software (Wolfburg, Germany). The final mineral contents (Ca, digestible P, Na, K, Cl) were within the acceptable range in each feeding phase. All diets were fed as pellets with 2 mm diameter in the starter phase and 3 mm diameter in the grower-1, grower-2, and finisher phases.

Sampling and Calculations

On d 9, 20, 31, and 42 (the end of each feeding phase) and one block at a time, the collective weight of the birds and residual feed per pen were recorded for calculation of ADG, ADFI, and the feed conversion ratio (**FCR**). After weighing on d 20, 31, and 42, one random bird per pen, in treatments 1, 3, 5, 7, and 9, was electrically stunned (Storditore MV, Maino, Italy) and exsanguinated. The proventriculus and gizzard were removed, cleaned with water, and weighed individually. Due to logistical reasons, this sampling was not performed in all treatments. To maintain a similar stocking density across all the pens, one random bird was also removed from pens in treatments 2, 4, 6, and 8. The carcasses of birds sampled on d 42 were also de-feathered, individually bagged, and maintained at −20°C until further

Table 1. Ingredient composition of dietary treatments 1, 5, and 9 per feeding phase.

		Grower-1, 9–20 d			Grower-2, 20–31 d			Finisher, 31–42 d		
		Treatment			Treatment			Treatment		
Ingredient, %	Starter, 0–9 d	1	5	9	1	5	9	1	5	9
Wheat	30.87	33.96	33.96	33.96	35.28	35.28	35.28	36.60	36.60	36.60
Corn	23.86	25.63	19.74	19.44	28.36	16.17	15.64	28.60	9.45	8.75
Barley	-	-	4.50	4.50	-	10.00	10.00	-	17.50	17.41
Oats hulls	0.50	-	1.00	1.00	-	1.50	1.50	-	2.00	2.00
Soybean meal (48 %)	31.31	27.19	26.42	26.35	23.42	19.95	20.96	21.88	14.79	15.42
Soy protein concentrate	0.32	1.29	-	-	1.00	-	-	0.89	-	-
Rapeseed meal	2.00	2.50	3.50	3.50	3.00	5.50	5.38	3.00	6.70	6.31
Sunflower meal	-	-	2.00	2.00	-	4.50	4.00	-	6.50	6.50
Potato protein	1.50	1.00	0.55	0.94	1.00	-	0.08	0.90	-	0.57
Soya oil	5.47	5.07	4.80	4.80	5.01	4.20	4.20	5.47	3.85	3.85
Limestone	1.50	1.18	1.29	1.29	1.01	1.03	1.03	0.88	0.90	0.90
Salt	0.189	0.183	0.172	0.174	0.176	0.157	0.154	0.177	0.148	0.147
Sodium bicarbonate	0.240	0.213	0.298	0.288	0.225	0.240	0.244	0.224	0.294	0.251
Monocalcium phosphate	0.989	0.517	0.492	0.493	0.223	0.165	0.160	0.075	-	-
L-Lysine HCl	0.173	0.193	0.210	0.205	0.214	0.237	0.249	0.211	0.242	0.245
DL-Methionine	0.291	0.276	0.253	0.257	0.259	0.202	0.222	0.249	0.161	0.179
L-Threonine	0.070	0.073	0.087	0.083	0.085	0.101	0.111	0.090	0.095	0.097
L-Valine	0.024	0.025	0.027	0.023	0.040	0.027	0.039	0.044	0.016	0.019
L-Arginine	-	-	-	-	-	-	-	0.011	-	-
Vit Min premix[1]	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
NSPase, Axtra XB[2]	0.100	0.100	0.100	0.100	0.100	0.150	0.150	0.100	0.150	0.150
Phytase, Axtra PHY[3]	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]Added per kg of final feed: 10,000 IU, vitamin A (trans-retinyl acetate); 2,500 IU, vitamin D3 (cholecalciferol); 50 IU, vitamin E (all-rac-tocopheryl-acetate); 2.0 mg, vitamin B1 (thiamine mononitrate); 6 mg, vitamin B2 (riboflavin); 40 mg, vitamin B3 (niacin); 4.0 mg, vitamin B6 (pyridoxine HCl); 25 μg, vitamin B12 (cyanocobalamin); 2.0 mg, vitamin K3 (bisulfate menadione complex); 10 mg, pantothenic acid (d-Ca pantothenate); 1.0 mg, folic acid; 300 mg, choline (choline chloride); 150 mcg, d-biotin; 0.25 mg, Se (Na2SeO3);1.0 mg, I (KI); 15 mg, Cu ($CuSO_4 \cdot 5H_2O$); 65 mg, Fe ($FeCO_3$); 90 mg, Mn (MnO_2); 80 mg, Zn (ZnO); 2.25 mg/kg, butylated hydroxyanisole; 11.25 mg/kg, butylated hydroxytoluene.

[2]Added per kg of complete feed: 1,220 U endo-1,4 beta-xylanase and 152 U endo-1,3(4)-beta-glucanase, when added at 0.10%, and 1,830 U endo-1,4 beta-xylanase and 228 U endo-1,3(4)-beta-glucanase, when added at 0.15%.

[3]Added per kg of complete feed: 500 FTU valued at 0.13 % dP.

Table 2. Calculated and analyzed nutrient composition of treatments 1, 5, and 9 per feeding phase. Values are on an as-is basis.

		Grower-1, 9–20 d			Grower-2, 20–31 d			Finisher, 31–42 d		
		Treatment			Treatment			Treatment		
Nutrient	Starter, 0–9 d	1	5	9	1	5	9	1	5	9
Dry matter, g/kg	898 (908)[1]	898 (899)	900 (898)	900 (899)	897 (899)	902 (900)	902 (901)	897 (892)	906 (897)	906 (900)
Crude protein, g/kg	222 (225)	210 (207)	205 (208)	207 (200)	194 (191)	188 (183)	192 (189)	187 (190)	177 (177)	183 (186)
Ash, g/kg	60 (58)	52 (50)	54 (53)	54 (52)	46 (45)	49 (47)	49 (47)	42 (42)	47 (45)	47 (45)
Ether extract, g/kg	77 (75)	73 (70)	70 (70)	70 (64)	73 (66)	62 (58)	62 (57)	77 (73)	57 (56)	57 (57)
Crude fiber, g/kg	31 (29)	30 (28)	39 (36)	39 (39)	30 (27)	49 (48)	49 (50)	29 (29)	57 (54)	57 (54)
Starch, g/kg	336	365	351	349	391	365	362	400	368	363
NFE, g/kg[2]	503	529	528	526	551	551	548	559	565	560
TDF, g/kg	144	142	161	161	140	179	178	139	197	196
Soluble TDF, g/kg	12	12	14	14	12	16	16	12	19	19
Insoluble TDF, g/kg	119	116	133	133	116	150	149	115	165	164
AME, kcal/kg	2,850	2,900	2,784	2,784	2,950	2,714	2,714	3,000	2,640	2,640
SID Lys, g/kg	12.00	11.30	10.85	11.01	10.50	9.66	10.00	10.00	8.80	9.24
SID Lys:AME, g/Mcal	4.21	3.90	3.90	3.95	3.56	3.56	3.68	3.33	3.33	3.50
SID Met/Lys	0.49	0.50	0.49	0.49	0.50	0.48	0.49	0.51	0.47	0.48
SID Met+Cys/Lys	0.73	0.74	0.74	0.74	0.75	0.75	0.75	0.76	0.76	0.76
SID Thr/Lys	0.64	0.64	0.65	0.65	0.65	0.67	0.67	0.66	0.68	0.68
SID Trp/Lys	0.20	0.20	0.20	0.20	0.19	0.20	0.20	0.19	0.21	0.21
SID Arg/Lys	1.10	1.09	1.10	1.10	1.06	1.11	1.09	1.07	1.11	1.10
SID Ile/Lys	0.70	0.69	0.69	0.69	0.68	0.68	0.67	0.68	0.68	0.68
SID Val/Lys	0.78	0.78	0.78	0.78	0.79	0.79	0.79	0.80	0.80	0.80
SID Leu/Lys	1.28	1.28	1.26	1.26	1.28	1.24	1.22	1.29	1.23	1.22
SID Gly+Ser/Lys	1.44	1.44	1.44	1.44	1.43	1.45	1.43	1.44	1.47	1.46
Ca, g/kg	10.0 (9.6)	8.0 (7.8)	8.5 (8.4)	8.5 (8.4)	6.8 (6.8)	7.2 (6.9)	7.2 (7.2)	6.0 (6.1)	6.5 (5.9)	6.5 (6.4)
P, g/kg	5.8 (5.7)	4.7 (4.1)	4.9 (4.6)	4.9 (4.9)	4.0 (4.2)	4.3 (4.1)	4.3 (4.3)	3.6 (3.3)	4.0 (3.8)	4.0 (3.6)
dig P, g/kg	4.6	3.7	3.7	3.7	3.1	3.1	3.1	2.8	2.8	2.8
Na, g/kg	1.5	1.4	1.6	1.6	1.4	1.4	1.4	1.4	1.5	1.4
K, g/kg	9.5	9.0	9.0	9.0	8.3	8.4	8.5	8.0	7.9	8.0
Cl, g/kg	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

[1]Analyzed values are presented in parenthesis below the corresponding calculated value and are the average of the analysis performed in duplicate.

[2]Abbreviations: dig, digestible; NFE, nitrogen-free extract; SID, standardized ileal digestible; TDF, total dietary fiber.

analysis. At 1800 h on d 42, all feeders were raised, and 2 random birds per pen in treatments 1, 3, 5, 7, and 9 were individually wing-tagged. The next morning (d 43, ~0700 h), all birds in the study (about 2,300 birds) were sent to a commercial slaughterhouse (SADA Group Inc., Seville, Spain). Carcasses corresponding to tagged birds were sent back in a temperature-controlled truck (4°C) after chilling for 24 h at 4°C in the slaughterhouse. Upon arrival, these carcasses were weighed, and the breast meat was removed and weighed. Data were used for the calculation of carcass and breast meat yield. The total AME intake was calculated according to the following formula:

$$\text{Total AME intake, Mcal/bird} = \sum (\text{ADFI}_i \times \text{phase length}_i \times \text{diet AME}_i)$$

where $i =$ starter, grower-1, grower-2, and finisher; and ADFI, phase length, and diet AME are given in g.d^{-1}.bird^{-1}, days, and Mcal/g, respectively.

Feed and Carcass Analysis

All the experimental diets were analyzed for dry matter (procedure 930.15; AOAC, 2019), crude protein (procedure 968.06; AOAC, 2019), crude fiber (procedure 962.09; AOAC, 2019), ether extract (crude fat, procedure: 920.39; AOAC, 2019) in Trouw Nutrition (Toledo, Spain). The Ca and P contents were analyzed by spectrophotometry (Autoanalyzer 3 HR, Axflow) in MasterLab Trouw Nutrition (Madrid, Spain). The percentage content of fine particles, pellet durability, and pellet hardness of the experimental diets were also determined. For determination of fine particles and pellet durability, a sample of about 350 g was weighed, sieved, and weighed again; the weight difference, expressed as a percentage of the initial sample weight, was considered as the fine particle fraction. The square openings of the sieves were 1.4 or 2.4 mm depending on the pellet diameter (for 2 or 3 mm, respectively). The sieved sample was placed on a pFost tumbling can (30 cm × 30 cm × 12.5 cm) for 10 min at 50 rpm. After this process, the sample was sieved again, and the remaining feed, expressed as a percentage of the intact-pellet sample, was considered the percentage of pellet durability. Pellet hardness was determined with a tablet hardness tester (model: 8M, Dr. Schleuniger, Pharmatron AG, Switzerland) in 25 random pellets per experimental diet.

The frozen carcasses, from birds sampled on d 42, were left at room temperature overnight before grinding them using 2 commercial meat grinders (model: Cutter C-15, Cruells, Girona, Spain; model: Cutter T3N-3L, Bartscher, Salzkotten, Germany). A sample of approximately 180 g was taken from each bird. This sample was freeze-dried (LyoAlfa 15-85, Telstar, Spain) and ground (~1 mm; Model ZM-200, Retsch, Haan, Germany). Samples were then analyzed for dry matter, crude protein, and ether extract following the same procedures used for feed analysis. Results were expressed as a percentage of dry matter.

Statistical Analysis

Data were analyzed using SAS Studio (SAS Inst. Inc., Cary, NC). The pen was the experimental unit on performance data (BW, ADG, ADFI, FCR, mortality, and AME intake); for measurements taken on individual birds (organ weights, carcass and breast meat yield, and carcass composition), the bird was the experimental unit; for pellet hardness, individual pellets were considered the experimental unit. The normality of residuals was determined with the UNIVARIATE procedure of SAS, and outliers were determined using the INFLUENCE statement of the MIXED procedure of SAS. The main effect of the treatment was determined using the GLIMMIX procedure with a normal distribution, and the differences in the least-square means were determined using the SIMULATE statement. For all analyses, the block was considered a random effect, and the treatment was considered a fixed effect. Within each Lys:AME group, linear and quadratic covariate analyses were performed relating the different responses to dietary AME; the constant Lys:AME group included treatments 1 to 5, and the increased Lys:AME group included treatments 1 and 6 to 9. Mortality was analyzed using a binomial distribution in the GLIMMIX procedure. Significant differences between treatments were determined when $P \leq 0.05$ and a trend when $0.05 < P \leq 0.10$.

RESULTS

Feed Analysis and Feed Quality

During the present trial, the birds appeared healthy, and no outbreak of any apparent disease was registered. Table 2 shows the comparison of the calculated and analyzed values of crude protein, crude fiber, and ether extract. No major deviations from the intended values are reported.

The percentage of fine particles in the starter feed was 2.66% (Table 3). In the grower-1 phase, treatment 1 had the greatest quantity of fine particles with 8.39%, and the one with the lowest amount was treatment 3 (5.58%). In the grower-2 and finisher phases, the percentage of fine particles decreased when lowering dietary AME, independently of the Lys:AME. In treatments 1, 2, and 6, the treatments with the highest AME in each feeding phase, the fine particles accounted on average for 6.9% in the last two feeding phases, while in treatments with the lowest AME (treatments 5 and 9), the fine particles represented only 2.1%. Inversely, pellet durability was the lowest among treatments with the highest AME (91% average across grower-1, grower-2, and finisher phases), and it was the highest in treatments with the lowest AME (treatments 5 and 9; 94.4% average). Given that pellet hardness was repeated 25 times per treatment diet, statistical analysis was

Table 3. Analyzed fine particle content, pellet quality, and pellet hardness of the experimental diets per feeding phase.

	Treatment										
	Control	Reduced AME, constant Lys:AME				Reduced AME, increased Lys:AME					
	1	2	3	4	5	6	7	8	9	SEM	*P*-value
Fine particles, %[1]											
Starter[2]	2.66										
Grower-1	8.39	7.81	5.58	6.78	7.29	6.93	6.73	6.96	6.01		
Grower-2	7.89	6.92	8.11	6.06	2.65	8.77	7.39	5.01	2.12		
Finisher	6.65	3.77	5.12	3.44	1.91	7.45	8.11	6.57	1.94		
Pellet durability, %[1]											
Starter[2]	97.2										
Grower-1	89.8	92.2	92.8	91.7	93.2	92.3	91.6	93	93.2		
Grower-2	91.1	92.1	92.7	93.1	94.2	91.8	92.5	94.3	94.4		
Finisher	89.8	90.7	93.3	94.2	95.7	89.8	91.4	93.5	95.6		
Pellet hardness, kPa[3]											
Starter[2]	1.52									0.082	-
Grower-1	1.36[d]	1.51[bcd]	1.64[abc]	1.49[dc]	1.82[a]	1.69[abc]	1.56[abcd]	1.76[ab]	1.65[abc]	0.058	<0.001
Grower-2	1.53[cd]	1.63[bcd]	1.67[bcd]	1.90[ab]	1.80[bc]	1.46[d]	1.73[bcd]	1.88[ab]	2.10[a]	0.065	<0.001
Finisher	1.15[f]	1.56[de]	1.66[cd]	2.12[b]	2.60[a]	1.32[ef]	1.53[de]	1.84[c]	2.26[b]	0.070	<0.001

[a-f]Within a row, values with different superscripts are different ($P < 0.05$).
[1]Values are averages of the analyses performed in duplicate.
[2]Starter phase feed was common to all experimental treatments.
[3]Values are least-square means ± SEM; n = 25.

made. In each of the experimental feeding phases, the pellet hardness was the lowest in treatment 1 ($P < 0.05$), and it increased as AME in the diet decreased, independently of the Lys:AME.

Growth Performance

There were no differences in BW at the end of the starter (9 d) or grower-1 (20 d) phases ($P > 0.10$; Table 4). At the end of grower-2 (31 d), there was a tendency for lower BW in treatment 5 when compared with the control ($P = 0.063$). The final BW (42 d) was linearly decreased ($P < 0.05$) when the diets had lower AME and the Lys:AME was maintained. The final BW in treatments with the increased Lys:AME was not significantly different compared with the control ($P > 0.10$) but followed a positive quadratic response relative to dietary AME (Figure 1; $P < 0.05$). Similarly, there were no differences in ADG at the end of the starter or grower-1 phases ($P > 0.10$; Table 4). At the end of the grower-2 and finisher phases, treatment 5 was significantly lower compared with the control ($P < 0.05$). Moreover, treatments with the constant Lys:AME linearly decreased ADG in the grower-2 and finisher phases relative to AME ($P < 0.05$), but no differences were determined in treatments with increased Lys:AME.

There were no differences in ADFI during the starter or grower-1 phases ($P > 0.10$). During the grower-2 phase, ADFI increased linearly ($P < 0.05$) relative to the dietary AME and independently of the Lys:AME. In the finisher period, ADFI increased linearly ($P < 0.05$) when the Lys:AME was kept constant but quadratically ($P < 0.05$) when the Lys:AME was increased. During the starter phase, FCR was similar in all the treatments ($P > 0.10$). In the grower-1, grower-2, and finisher phases, FCR significantly and linearly ($P < 0.05$) increased with reduced the dietary AME. The linear FCR slopes in the constant Lys:AME group were 10 and 54% greater than the slopes of the group with the increased Lys:AME in the grower-2 and finisher phases, respectively.

On the overall performance (0−42 d), lowering the dietary AME with a constant Lys:AME linearly decreased BW and ADG, and increased ADFI and FCR (Table 5). When the Lys:AME augmented, the final BW, ADG, and ADFI presented a positive quadratic response ($P < 0.05$), and FCR increased linearly ($P < 0.05$). The FCR slope when the Lys:AME was kept constant was 38% higher than when the Lys:AME increased. Overall mortality was not different across treatments ($P > 0.10$). The calculated total AME intake during the entire study decreased linearly ($P < 0.05$) when lowering the AME content, but there were no differences between both Lys:AME groups (Table 5).

Organ and Carcass Weights

There were no differences in the absolute or relative weight of the proventriculus at the end of each feeding phase ($P > 0.10$; Table 6). There was a tendency for a greater gizzard weight (g) in treatment 3 compared to 1 ($P = 0.073$) at the end of the grower-1 phase (20 d); other treatments were not different ($P > 0.10$). At the end of the grower-2 phase (31 d), the gizzard weight was greater ($P < 0.05$) in treatment 3 compared with 7, all other treatments were intermediate ($P > 0.10$). Similar results were obtained when gizzard was expressed relative to BW.

The random chickens selected for carcass yield did not have different BW ($P > 0.10$; Table 7). Carcass and breast meat yield, expressed as absolute or relative values, were not significantly different among the treatments ($P > 0.10$). The protein content in the carcass composition was significantly ($P < 0.05$) higher in treatment 9 than in any other treatment; conversely, the fat

Table 4. Average BW, ADG, ADFI, and FCR per phase of chickens fed diets with reduced AME content and with constant or increased Lys:AME ratio.

	Treatment											Regression analysis[1]	
	Control	Reduced AME, constant Lys:AME				Reduced AME, increased Lys:AME						Constant Lys: AME	Increased Lys: AME
	1	2	3	4	5	6	7	8	9	SEM	*P*-value		
BW, g/bird													
09 d	223	223	222	222	221	218	221	228	220	2.9	0.402	-	-
20 d	894	886	884	888	875	884	885	904	879	9.6	0.503	NS	NS
31 d	1,982	1,950	1,933	1,953	1,904	1,956	1,947	1,977	1,931	19	0.063	L	NS
42 d	3,307^{a}	3,264^{a}	3,241^{a}	3,232^{ab}	3,140^{b}	3,248^{a}	3,251^{a}	3,258^{a}	3,287^{a}	25	<0.001	L	Q
ADG, g/d													
Starter,	19.9	20.0	19.9	19.8	19.7	19.4	19.7	20.5	19.6	0.32	0.188	-	-
Grower-1,	61.0	60.4	60.7	60.5	59.5	60.5	60.4	61.7	60.1	0.67	0.518	NS	NS
Grower-2,	99^{a}	96^{ab}	95^{ab}	95^{ab}	94^{b}	97^{ab}	96^{ab}	98^{ab}	97^{ab}	1.1	0.015	L	NS
Finisher,	120^{a}	119^{ab}	119^{ab}	116^{ab}	112^{b}	118^{ab}	119^{ab}	116^{ab}	119^{a}	1.5	0.017	L	NS
ADFI, g/d													
Starter,	21.6	21.4	21.2	21.5	21.1	20.9	21.2	21.8	21.0	0.30	0.190	-	-
Grower-1,	76.7	76.4	77.0	78.0	77.3	76.0	76.4	78.2	77.2	0.80	0.573	NS	NS
Grower-2,	148^{c}	147^{c}	150^{abc}	151^{abc}	150^{abc}	149^{bc}	149^{abc}	154^{ab}	154^{a}	1.4	<0.001	L	L
Finisher,	200^{c}	204^{bc}	204^{bc}	208^{abc}	211^{ab}	200^{c}	203^{bc}	206^{bc}	215^{a}	2.2	<0.001	L	L,Q
FCR, g/g													
Starter,	1.084	1.078	1.066	1.082	1.074	1.08	1.073	1.078	1.079	0.0071	0.669	-	-
Grower-1,	1.258^{d}	1.266^{cd}	1.280^{cb}	1.279^{cb}	1.300^{a}	1.256^{d}	1.265^{cd}	1.269^{cbd}	1.284^{ab}	0.0041	<0.001	L	L
Grower-2,	1.497^{g}	1.529^{fg}	1.570^{cde}	1.587^{cbd}	1.631^{a}	1.535^{ef}	1.551^{def}	1.591^{bc}	1.616^{ab}	0.0089	<0.001	L	L
Finisher,	1.681^{e}	1.720^{cde}	1.716^{de}	1.815^{ab}	1.862^{a}	1.705^{de}	1.712^{de}	1.779^{bcd}	1.793^{abc}	0.018	<0.001	L	L

Abbreviations: ADFI: average daily feed intake; ADG: average daily gain; AME: apparent metabolizable energy; BW: body weight; FCR: feed conversion ratio.

Values are least-square means ± SEM; n = 10.

a,b,c,d,e,f,g Different superscripts in the same row denote ($P < 0.05$) difference.

[1]Linear and quadratic regression analysis of the different variables relative to the AME content in the diets. L or Q denote significant linear or quadratic regression ($P < 0.05$), respectively; NS = not significant ($P > 0.05$).

content was the lowest in treatment 9 ($P < 0.05$). There were no differences in protein or fat contents among other treatments ($P > 0.10$).

DISCUSSION

Among other factors, formulating commercial diets to achieve a high AME content, indirectly reduces the use of alternative, more fibrous, and usually cheaper, feed ingredients (e.g., barley, oats, sunflower meal, and rapeseed meal). The present study evaluated progressive AME reductions with greater inclusion of alternative feed ingredients on the growth performance, organ weight, and carcass characteristics of broiler chickens. Additionally, the effect of maintaining a constant or increasing the Lys:AME was tested.

During diet formulation, the minimum oil content was set to maintain acceptable pellet quality, and this minimum amount was kept equal between both Lys:AME groups. With a reduction of ~30% of the oil content in the finisher diets in treatments 5 and 9, the pellet hardness was the highest (2.6 and 2.3 kPa, respectively) and about double the pellet hardness of the control treatment. Studies on the relation between added oil/fat content in the diet and pellet quality have reported that 3 to 6% added oil is ideal for optimizing pellet durability

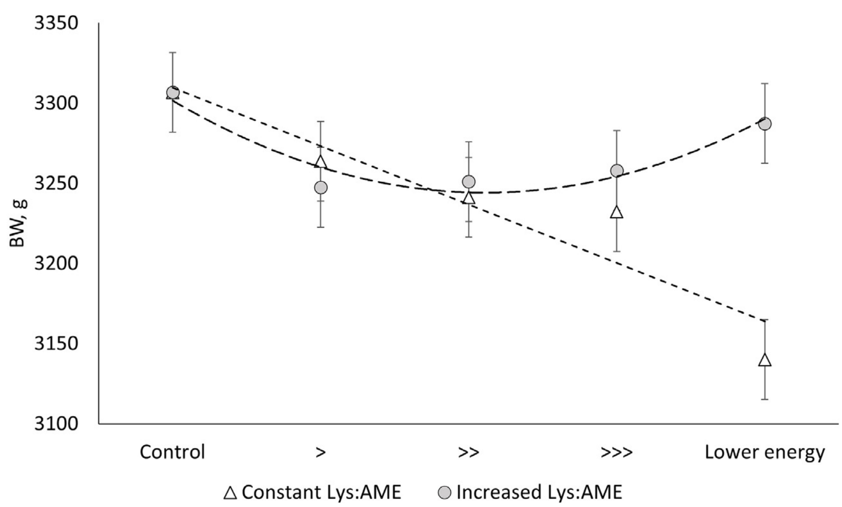

Figure 1. Average BW at 42 d of chickens fed diets with reduced AME content with constant or increased Lys:AME ratio. Data points are least-square means ± SE (n = 10).

Table 5. Overall performance (ADG, ADFI, FCR, and mortality) and total AME intake of chickens fed diets with reduced AME content and with constant or increased Lys:AME ratio.

	Treatment											Regression analysis[1]	
	Control	Reduced AME, constant Lys:AME				Reduced AME, increased Lys:AME							
	1	2	3	4	5	6	7	8	9	SEM	*P*-value	Constant Lys:AME	Increased Lys:AME
ADG, g/d	77.7^{a}	76.7^{a}	76.1^{a}	75.9^{ab}	73.7^{b}	76.3^{a}	76.4^{a}	76.5^{a}	77.2^{a}	0.60	<0.001	L	Q
ADFI, g/d	116^{bc}	116^{bc}	116^{bc}	119^{ab}	118^{abc}	115^{c}	116^{bc}	119^{abc}	121^{a}	0.90	<0.001	L	Q
FCR, g/g	1.487^{d}	1.515^{c}	1.528^{c}	1.571^{b}	1.602^{a}	1.513^{c}	1.521^{c}	1.555^{b}	1.569^{b}	0.0055	<0.001	L	L
Mortality, %	5.4	5.4	5.8	6.7	6.1	3.8	3.5	6.1	5.8	1.3	0.868	-	-
AME intake, Mcal	14.34^{a}	14.18^{ab}	13.94^{abc}	13.88^{bc}	13.58^{c}	14.09^{ab}	13.89^{bc}	13.87^{bc}	13.80^{bc}	0.11	<0.001	L	L
Δ relative to Control, %		−1.09	−2.82	−3.22	−5.33	−1.78	−3.17	−3.26	−3.77				

Abbreviations: ADFI: average daily feed intake; ADG: average daily gain; AME: apparent metabolizable energy; FCR: feed conversion ratio.

Values are least-square means ± SEM; n = 10.

[a,b,c,d]Different superscripts in the same row denote significant differences ($P < 0.05$).

[1]Linear and quadratic regression analysis of the different variables relative to the AME content in the diets. L or Q denote significant linear or quadratic regression ($P < 0.05$), respectively; NS = not significant ($P > 0.05$).

without excessive pellet hardness (Briggs et al., 1999; Moritz et al., 2002; Mohammadi et al., 2019); pellet integrity is significantly compromised when the oil content surpasses 7.5% (Briggs et al., 1999). Thus, the reduction in oil content in the low AME diets resulted in acceptable pellet quality. It should also be noted that the highest pellet hardness was observed in the finisher phase when the bird is bigger and the grinding capacity of the gizzard is further developed (Engberg et al., 2002; Abdollahi and Ravindran, 2013). Therefore, the reported pellet quality in the low AME treatments does not appear to represent a problem that hindered feed intake.

The increment of feed intake when reducing the dietary AME content to maintain energy homeostasis is documented, and dietary AME is considered among the main factors when predicting feed intake in different animal models (Taylor and Kyriazakis, 2021). However, Classen (2017) proposed that the modern broiler chickens have lost the capacity for adjusting feed intake as a result of the intensive genetic selection for fast growth. This does not appear entirely correct as per results reported by Taylor et al. (2021) and herein. The capacity for quantitatively increasing feed intake depends, among many other factors, on the dietary energy content and gut capacity, and therefore the bird size (energy-dependent feed intake; Gouz, 2007; Taylor et al., 2021). The present study aimed to push the feed intake capacity of the bird over time (Sahraei and Shariatmadari, 2007), while avoiding sudden changes in feed bulkiness, as reported elsewhere (Nascimento et al., 2020; Taylor et al., 2021). To avoid long-term negative effects of the starter phase on the overall performance, all birds were given a similar starter phase diet ensuring gastrointestinal tract development and aiming to improve the digestive capacity of alternative ingredients in the following phases. Moreover, the feed intake in the starter phase represented only 4.0% of the total feed intake, making the inclusion of alternative feed ingredients in the grower and finisher phases more relevant.

Despite the significant AME reductions in the grower-2 and finisher phases (−8 and −12% at the lowest AME contents, respectively), which together represented about 80% of the total feed intake, the calculated total AME intake was only reduced by 5.33 and 3.77% in the lowest AME treatments (treatments 5 and 9, respectively), and it decreased linearly relative to the AME content. This calculated AME intake, however, does not account for the reduced viscosity of the digesta and the potentially improved digestibility of amino acids because of the non-starch polysaccharide (**NSP**)-degrading enzymes (**NSPases**) added (Castañón et al., 1997; Kocher et al., 2000; Meng et al., 2005). Therefore, there is higher unaccounted AME intake as the AME of the diet was lowered. Indeed, as the NSP content increased with the addition of more fibrous ingredients (barley, rapeseed meal, and sunflower meal), it was also decided to increase the amount of NSPases in the treatments with the lowest AME. Adding NSPases has been

Table 6. Proventriculus and gizzard weights at the end of the grower-1, grower-2, and finisher phases of chickens fed diets with reduced AME content and with constant or increased Lys:AME ratio.

	Treatment						
	control	Reduced AME constant Lys:AME		Reduced AME increased Lys:AME			
	1	3	5	7	9	SEM	*P*-value
Proventriculus, g							
20d	4.5	4.3	4.6	4.4	4.7	0.25	0.639
31d	6.9	6.7	7.5	7.2	6.8	0.43	0.368
42d	8.5	8.2	8.7	9	9.4	0.61	0.598
Proventriculus, %BW							
20d	0.511	0.515	0.538	0.495	0.542	0.021	0.295
31d	0.377	0.348	0.403	0.354	0.364	0.022	0.412
42d	0.267	0.272	0.282	0.302	0.316	0.020	0.264
Gizzard, g							
20d	10.4	12.8	11.0	12.0	12.1	0.60	0.073
31d	19.4[ab]	23.8[a]	22.2[ab]	17.3[b]	19.5[ab]	1.3	0.015
42d	25.4	23.8	27.9	26.1	26.8	1.6	0.536
Gizzard, %BW							
20d	1.21[b]	1.57[a]	1.30[ab]	1.35[ab]	1.41[ab]	0.082	0.041
31d	1.06[ab]	1.25[a]	1.21[a]	0.85[b]	1.04[ab]	0.083	0.025
42d	0.81	0.79	0.92	0.89	0.91	0.062	0.510

Values are least-square means ± SEM; n = 10.
[a,b]Different superscripts in the same row denote significant differences ($P < 0.05$).

reported to differently improve dietary energy digestibility, depending on the NSP content, the ingredients, and the amount of NSPases added (Marquardt et al., 1994; Castañón et al., 1997; Austin et al., 1999; Cho and Kim, 2013; Williams et al., 2014). It is reasonable to conclude that the potential energy provision from NSPase-ingredient interaction was higher as the AME was reduced and the fiber content increased (additive effect). The actual total AME intake may be greater than the reported, especially in the low AME diets.

The dietary amino acid balance or protein content may also alter feed intake in broiler chickens. Within similar AME contents, chickens may consume more of a diet that provides a better balance of amino acids and energy (i.e., Lys:AME; Barekatain et al., 2021). Herein, feed intake was not different when the Lys:AME increased, implying that the increment in BW within the same AME content was related to better nutrient utilization rather than higher nutrient intake. The latter explanation is in agreement with the low body fat content found in the birds fed the lowest AME with the increased Lys:AME. Then, it is possible that the energy released from the NSPases in the high-fiber diets was better utilized with the increment in SID-Lys. Furthermore, this interaction between the increment in SID-Lys, NSPases, and the greater amount of substrate may explain the quadratic response in the final BW seen in treatments with the increased Lys:AME (Figure 1).

The higher SID-Lys in treatment 9 was accompanied by a proportional increment on all other essential amino acids. The only amino acid ratio that was intentionally increased was the Thr to Lys ratio to support changes in Thr requirements driven by increased mucin production (Bortoluzzi et al., 2018; Montagne et al., 2003). It is reported that visceral organs may further develop and represent a higher proportion of BW when fed high-fiber diets (Jimenez-Moreno et al., 2013; Taylor et al., 2021). However, our results failed to detect differences between treatments. A possible explanation may lay in the amount of days chickens were eating the highest-fiber

Table 7. Carcass and breast meat yield, and carcass composition of chickens fed diets with reduced AME content and with constant or increased Lys:AME ratio.

	Treatment						
	Control	Reduced AME constant Lys:AME		Reduced AME increased Lys:AME			
	1	3	5	7	9	SEM	*P*-value
BW 43 d, g/bird	3,509	3,575	3,474	3,387	3,480	70	0.441
Carcass, g/bird	2,403	2,406	2,351	2,277	2,335	55	0.462
Carcass, %BW	67.2	67.3	67.1	67.1	67.0	0.45	0.993
Breast, g/bird	750	776	731	728	737	24	0.626
Breast, %BW	21.5	21.6	20.9	21.4	21.1	0.39	0.649
Breast, %carcass	32.0	32.1	31.0	31.9	31.5	0.45	0.409
Carcass composition							
Protein, %DM	47.9[b]	49.4[b]	49.3[b]	48.5[b]	52.0[a]	0.66	<0.001
Fat, %DM	34.0[a]	33.5[a]	33.3[a]	33.9[a]	30.5[b]	0.70	0.003

Values are least-square means ± SEM; n = 20 for carcass yield; n = 10 for carcass composition.
[a,b]Different superscripts in the same row denote significant differences ($P < 0.05$).

diets (20−42 d), while from 0 to 20 d diets were rather the same or similar to the control treatment. It is also possible that the inclusion of alternative ingredients was not as high as reported in other studies (15−60% fibrous ingredients; Jimenez-Moreno et al., 2013; Taylor et al., 2021). Conversely, the carcass yield relative to BW remained unchanged, and the total carcass weight depended solely on the bird BW. The proventriculus weight remained unchanged across the study, but the gizzard weight increased as the AME content was reduced at the end of the grower-1 and grower-2 phases. The higher gizzard weight is probably related to the ingredients used, pellet hardness, and the function the gizzard plays on feed grinding (Svihus, 2011).

In conclusion, in this study, we explored the progressive reduction of the CVB (2018) recommended AME contents (up to 12%) with a constant or increased Lys:AME. The final BW was significantly and linearly decreased when birds were fed lower AME diets, but it showed a positive quadratic response when the Lys:AME was increased. The feed intake increased linearly when lowering the AME, but the calculated AME intake was slightly but significantly reduced. The carcass and breast meat yields relative to BW were not different. Progressive reduction of the AME content and proper adjustment of the Lys:AME may be an interesting nutritional strategy that can facilitate the inclusion of alternative feed ingredients. The effect of adding NSPases when increasing the inclusion of fibrous ingredients warrants further research.

DISCLOSURES

WDM, SS, and AIG currently work at Trouw Nutrition. JMR worked at Trouw Nutrition and FSQ was an intern during the execution of this trial.

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