Animal Nutrition 7 (2021) 472–480

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Production performance and plasma metabolite concentrations of broiler chickens fed low crude protein diets differing in Thr and Gly

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ARTICLE INFO

Article history:
Received 8 May 2020
Received in revised form
26 July 2020
Accepted 8 September 2020
Available online 22 January 2021

Keywords:
Broiler
Threonine
Glycine
Low protein
Performance
Plasma metabolite

ABSTRACT

The aim of the study was to test the interaction between Thr and Gly in low crude protein (CP) diets in 7 to 28 d broilers on production performance and plasma metabolites. A total of 2,040 broilers were allocated to 17 treatments. A positive control (PC) diet (20.5% CP) was formulated to be adequate in dietary Thr and Gly. A negative control (NC) diet (18.5% CP, deficient in Thr and Gly) was supplemented with crystalline L-Thr and Gly to obtain a 4 Thr × 4 Gly design. Dietary Thr was tested at an apparent faecal digestibility (AFD) Thr-to-Lys ratio, which was 55%, 58%, 61% or 64%, and dietary Gly was tested at an AFD (Gly + Ser)-to-Lys ratio, which was 135%, 142%, 149% or 156%. Plasma samples were collected at 28 d. The low CP diet, formulated at 64% Thr and 156% Gly, resulted in a higher body weight gain (BWG) ($P < 0.01$) and similar feed conversion ratio (FCR) as the high CP treatment (PC). FCR was improved ($P < 0.001$) by L-Thr supplementation. Quadratic response to dietary Thr was significant for feed intake (FI), BWG and FCR ($P < 0.01$). A near-significant interaction for Thr × Gly was observed for FI and BWG ($P_{\text{linear}} = 0.091$ and $P = 0.074$, respectively). Gly did not affect production performance. An interaction between Thr × Gly on plasma free AA level was observed ($P < 0.05$). Free AA concentration in plasma linearly decreased with increase in AFD Thr-to-Lys ratio, and increased with increase in AFD (Gly + Ser)-to-Lys ratio. Plasma uric acid concentration was higher in PC than in all of the other diets, and plasma triglyceride concentration was decreased by L-Thr supplementation, but not by Gly. In conclusion, Gly was not limiting for growth at low dietary CP level unless Thr was deficient, showing that adequate amounts of Thr in broiler diets can overcome marginal supply of Gly and Ser and allow reduction of dietary CP from 20.5% to 18.5% for broilers from 7 to 28 d of age.

1. Introduction

Lowering dietary crude protein (CP) levels in broiler feed is important to improve intestinal health, and to reduce foot pad lesions and N excretion in the environment (Jianlin et al., 2004; Widyaratne et al., 2008). When CP content is reduced, it is critical to consider the limiting effect of indispensable amino acids (IAA) on production performance (Lemme et al., 2019), and the need to supplement them in the diets at the required levels. Among them, Thr is considered the third-limiting amino acid (AA) in commercial corn-soybean meal based broiler diets (Fernandez et al., 1994). This IAA is needed for tissue protein synthesis, in particular in skeletal muscles, and it is the first AA incorporated in mucus and

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2020.09.003

immunoglobulins (Kidd, 2000). Threonine has been extensively investigated and reviewed since feed grade L-Thr has been available worldwide. Researchers have recently updated the Thr requirements and recommended higher dietary levels than previously suggested for modern broilers, especially in the starter phase (Dozier et al., 2015; Mehri et al., 2014; Ospina-Rojas et al., 2014).

Furthermore, when reducing CP by 4% points or more, production performance of broilers is depressed in starter-grower broilers, even though all IAA requirements are covered (Aftab et al., 2006), indicating the importance of some key dispensable amino acids (DAA) on performance. Among all DAA that have been tested, Gly could partially restore performance (Corzo et al., 2009; Dean et al., 2006). Gly is involved in many metabolic pathways and functions in broilers, as biosynthesis of different proteins like collagen, heme, creatine, glutathione, as well as the primary end product of AA catabolism, uric acid (Li and Wu, 2018). Gly is likely the next limiting AA after Arg, Val, and Ile especially in plant-based diets (Lemme et al., 2019). Gly has also many precursors and has proven interactions with other AA such as Thr and Cys (Siegert et al., 2015ab). Therefore, Gly requirement is difficult to estimate and is subjected to a wide variability. The official recommendation from the US (NRC, 1994) is 114% total (Gly + Ser)-to-Lys ratio, whereas from Brazil (Rostagno et al., 2017) it is 147% digestible (Gly + Ser)-to-Lys ratio, and Wu (2014) even estimated an optimal true digestible (Gly + Ser)-to-Lys ratio of 245% for broilers from 0 to 21 d of age based on AA accretion method. Some of this variability can be explained by the age of the animals, as it is known that young birds have higher Gly requirements compared to older birds (Rostagno et al., 2017). Better knowledge on Gly requirements in broilers is needed to reduce CP content in broiler diets without impairing performance.

Threonine is a source of Gly synthesis in poultry. As reviewed by Wang et al. (2013), Thr can be degraded by 2 enzymes: Thr aldolase and Thr dehydrogenase. Both enzymes have Gly-dependent pathways, because they yield Gly as a result of Thr degradation. In poultry, Thr dehydrogenase is the main enzyme involved in Thr degradation as a potential source of Gly. The quantitative importance of Gly synthesis from Thr may depend on species, but it has been reported that in poultry, rats, pigs, and cats Thr dehydrogenase is the major enzyme for initiating approximately 80% Thr degradation (Wang et al., 2013).

It has been shown that when broilers in the starter and grower phase were fed adequate or excessive dietary Thr levels, requirement for Gly was lower than when the broilers were fed deficient dietary Thr levels (Ospina-Rojas et al., 2013a,b). Nevertheless, only limited published information is available on the interaction between Thr and Gly (Siegert and Rodehutscord, 2019), and further investigation is required, especially in practically formulated diets.

In the interest of lowering CP levels in broiler feed, an experiment was performed to study and quantify the interaction between Thr and Gly with 2% points of reduced dietary CP levels and supplementation of crystalline L-Thr and Gly to broilers from 7 to 28 d of age. Effects on production performance and plasma metabolites were studied.

2. Materials and methods

2.1. Birds and housing

A total of 2,040 male Ross 708 broilers were housed in pens in the broiler facilities of Schothorst Feed Research (Lelystad, the Netherlands). Wood shavings were used as litter material. Birds arrived at 0 d, and they were fed a commercial starter diet from 0 to 7 d, an experimental grower diet from 7 to 28 d, and a commercial finisher diet from 28 to 35 d. The trial was finished at 35 d. The ambient temperature was gradually decreased from 34.5 °C at 0 d to 20.0 °C at 35 d. Room temperature and relative humidity were recorded daily. The light was continuously on for the first 24 h to give birds the opportunity to readily find feed and water. After that, the light schedule was 2D:22L during 1 d, and then changed to 4D:10L:2D:8L during the remaining experimental period. Feed and water were supplied ad libitum throughout the entire experiment. The birds were vaccinated against infectious bronchitis, Newcastle disease, and infectious bursal disease at 0, 10 and 20 d, respectively. The Institutional Animal Care and Use Committee approved each protocol (DEC 2014–17, version 2).

2.2. Treatments

The experiment was performed with 17 treatments and 6 replicates each, with 20 broilers per replicate. The design of the experiment is described in Table 1. Treatment 1 (T1) was the positive control (PC) diet with a CP level of 20%, an AFD Thr-to-Lys ratio of 64% and an AFD (Gly + Ser)-to-Lys ratio of 156%. Treatment 2 (T2) was the negative control (NC) diet, with a CP level of 18.5%, an AFD Thr-to-Lys ratio of 55% and an AFD (Gly + Ser)-to-Lys ratio of 135%. Ingredients and composition of the PC and NC diets are given in Table 2. On top of the NC diet, the addition of 3 supplemental levels of feed grade L-Thr and Gly in 4-step dose levels resulted in the treatments 3 to 17 (T3 to T17). The birds were fed a constant N-corrected apparent metabolizable energy level of 12.55 MJ/kg, a digestible Lys level of 1.0 g/kg and adequate IAA levels (INRA tables; Sauvant et al., 2004) in all treatments. From 7 to 28 d of age, the birds were fed the experimental diets in pelleted form. The broilers were supplied a crumbled commercial starter maize-soya-wheat diet (energy, 12.26 MJ/kg; CP, 21.2%; AFD Lys, 1.2%) from day of arrival to 7 d of age, and a pelleted commercial finisher wheat-soya-maize diet (energy, 12.75 MJ/kg; CP, 19.4%; AFD Lys, 1.0%) from 28 to 35 d of age.

2.3. Analysis of feedstuffs and feed

Prior to feed manufacturing, the most critical feedstuffs (wheat, maize, soybean meal, sunflower seed meal and peas) were analysed for complete AA profile. Based on the analyses, experimental PC and NC diets were formulated (Table 2), and experimental diets were optimised according to the experimental design (Table 1). Thereafter, the complete AA profile was analysed in each of the experimental diets (Table 3). Amino acid analyses of feedstuffs and experimental diets were determined by a JLC-500/V AminoTac AA analyser (Jeol, Croissy-sur-Seine, France) as performed by Ajinomoto Animal Nutrition Europe Customers Laboratory (Amiens, France). Total and free AA were analysed by EN ISO 13903 method, except for total and free Trp which was analysed by a method adopted from abrogated standard AFNOR XP V18-114. Furthermore, the PC and NC diet were analysed for Weende nutrients (moisture [ISO 6496], ash [NEN 3329], crude protein [ISO/CD 15670], and crude fat [ISO DIS 6492]) by Schothorst Feed Research.

The recalculated AFD Thr-to-Lys ratios and AFD (Gly + Ser)-to-Lys ratios (based on the analysed AA values) were in line with the expected values (Table 3). The PC diet showed slightly lower AFD Thr-to-Lys ratio and AFD (Gly + Ser)-to-Lys ratio than expected.

2.4. Response variables

2.4.1. Production performance

Body weight of birds per pen was measured at 0, 7 (to standardise mean BW per pen), 28, and 35 d of the experiment. Feed intake (FI) per pen was measured from 0 to 7, 7 to 28, 28 to 35 d. Body weight gain (BWG) and feed conversion ratio (FCR) were

Table 1
Experimental design.[1]

Treatment		CP, %	AFD Thr-to-Lys ratio, %	AFD (Gly + Ser)-to-Lys ratio, %
T1	PC	20.5	64	156
T2	NC	18.5	55	135
T3	as T2 + 0.03 L-Thr	18.5	58	135
T4	as T2 + 0.06 L-Thr	18.5	61	135
T5	as T2 + 0.09 L-Thr	18.5	64	135
T6	as T2 + 0.07 Gly	18.5	55	142
T7	as T6 + 0.03 L-Thr	18.5	58	142
T8	as T6 + 0.06 L-Thr	18.5	61	142
T9	as T6 + 0.09 L-Thr	18.5	64	142
T10	as T2 + 0.14 Gly	18.5	55	149
T11	as T10 + 0.03 L-Thr	18.5	58	149
T12	as T10 + 0.06 L-Thr	18.5	61	149
T13	as T10 + 0.09 L-Thr	18.5	64	149
T14	as T2 + 0.21 Gly	18.5	55	156
T15	as T14 + 0.03 L-Thr	18.5	58	156
T16	as T14 + 0.06 L-Thr	18.5	61	156
T17	as T14 + 0.09 L-Thr	18.5	64	156

CP = crude protein; AFD = apparent faecal digestible; PC = positive control; NC = negative control.

[1] Experimental diets were fed from 7 to 28 d. L-Thr and Gly provided by Ajinomoto Animal Nutrition Europe.

calculated for 0 to 7, 7 to 28, 28 to 35 d. Mortality was registered from 0 until 35 d.

2.4.2. Plasma analysis

Blood sampling was performed in fed birds at 28 d. Samples were taken from 10 birds per treatment (170 birds in total). Plasma was stored at −20 °C until further analysis. The plasma samples were analysed for uric acid (UA), glucose, triglycerides (TG) and total AA circulating levels by INRA (Nouzilly, France). Plasma α-amino-non-protein N concentrations (providing an estimate of total free AA) were measured after extraction with 10% (vol/vol) sulphosalicylic acid, using 2% ninhydrin reagent (Sigma Chemicals) and L-Ser as the standard (Dupont et al., 2008). Plasma glucose, TG and UA concentrations were quantified by colorimetric enzymatic methods using kits obtained from bioMérieux according to the methods of Trinder (1969), Fossati and Prencipe (1982), and Artiss and Entwistle (1981), respectively.

2.5. Statistical analysis

Observations were marked as outlier and excluded from the dataset prior to statistical analyses if the residual (fitted − observed value) > 2.5 × standard error on the residuals of the data set (6 pens for performance in T8, T11, T15, T16 [1 pen] and T2 [2 pens]; 2 birds for UA analyses [T8, T16], 2 birds for glucose [T8, T14], 5 birds for TG [T2, T6, T7, T8, T13]). For performance results, pen was the experimental unit, whereas for plasma analyses individual bird was the experimental unit. The experimental data were analysed by linear and quadratic analysis using GenStat statistical software (19.1st edition). The interaction between Thr × Gly was analysed for treatments 2 to 17 according to the following equation:

$$Y_{ijk} = \mu + \text{Block}_i + \text{Thr}_j + \text{Gly}_k + \text{Thr} \times \text{Gly}_{jk} + e_{ijk}, \tag{1}$$

where Y_{ijk} is the response parameter, μ is the overall mean, Block_i is the random effect of replicated block ($i = 1$ to 6), Thr_j is the effect of AFD Thr-to-Lys ratio ($j = 1$ to 4), Gly_k is the effect of AFD (Gly + Ser)-to-Lys ratio ($k = 1$ to 4), Thr × Gly is the interaction effect, and e_{ijk} is the random error term.

To test the differences in performance of the PC (T1) compared to NC (T2) and T17 with the highest Thr and Gly addition (similar level as PC), these 3 treatments were analysed according to the following equation:

$$Y_{ij} = \mu + \text{Block}_i + \text{Treatment}_j + e_{ij}, \tag{2}$$

where Y_{ij} is the response parameter, μ is the overall mean, Block_i is the random effect of replicated block ($i = 1$ to 6), Treatment_j is the effect of AA and CP level ($j = 1$ to 3), and e_{ij} is the random error term.

For plasma analyses, a similar statistical equation was used as for testing interaction between Thr × Gly on performance for T2 to 17 (Eq. (1)). Besides, an equation was used in which all treatments were compared for plasma analysis results (Eq. (2)).

Treatment means were compared by least significant differences (LSD). $P < 0.05$ was considered to be statistically significant, whereas $0.05 \le P < 0.10$ was considered to be a near-significant trend.

3. Results

3.1. Production performance

Comparing PC (T1), NC (T2) and T17 (similar level as PC) showed that BWG was significantly higher for T17 compared to PC and NC treatments ($P = 0.002$; Table 4). FCR was similar between PC and T17, and both had a significantly lower FCR compared to NC ($P = 0.028$). No effect on mortality rate was observed ($P > 0.05$).

Results on effects of Thr and Gly, and the interaction between Thr × Gly on FI, BWG and FCR from 7 to 28 d are given in Table 5. There was a trend to an interaction between Thr and Gly for BWG ($P = 0.074$) and FI ($P_{\text{linear}} = 0.091$), where Gly affected BWG and FI at 55% AFD Thr-to-Lys ratio.

FCR was linearly and quadratically affected ($P < 0.001$ and $P = 0.013$, respectively) by L-Thr supplementation, whereas BWG and FI were quadratically affected ($P < 0.001$ and $P = 0.002$, respectively) by L-Thr supplementation. Overall, AFD (Gly + Ser)-to-Lys ratio did not affect performance neither linearly nor quadratically ($P > 0.05$).

No differences were observed among all treatments for BWG, FI and FCR from 0 to 7 d and 28 to 35 d in which all broilers were fed a common starter and finisher diet, respectively (data not shown). No differences were observed for mortality rate from 0 to 7, 7 to 28, and 28 to 35 d (average mortality 2.8% from 0 to 35 d (range 0.8% for T5, T13, T17 to 5.0% for T11), data not shown). Besides, BW at 35 d was not significantly different among treatments (average BW 2,344 g, range 2,255 to 2,418 g), as well as FCR from 0 to 35 d (average FCR 1.500 g/g, range 1.485 to 1.519 g/g).

Table 2
Ingredients and nutritional composition of the PC and NC experimental diets in the grower phase (7 to 28 d).[1]

Item	PC	NC
Ingredients, %		
Maize	28.00	32.00
Wheat	22.03	25.72
Soybean meal (>48% CP)	25.94	18.00
Peas (<22% CP)	10.00	9.05
Sunflower seed meal (38% CP)	4.00	6.00
Animal blended fat	6.44	5.27
Limestone	1.08	1.12
Mono calcium phosphate	0.42	0.49
Salt	0.29	0.28
Sodium bicarbonate	0.10	0.10
L-Lys-HCl (79%)	0.04	0.27
DL-Met (99%)	0.26	0.30
L-Thr (98%)	0.02	0.02
L-Trp (98%)	0.00	0.01
L-Val (99%)	0.00	0.05
Phytase[2]	0.13	0.13
Glu-Xyl[3]	0.25	0.25
Vitamin-mineral premix[4]	0.50	0.50
Anticoccidiostat[5]	0.50	0.50
Composition, %		
AMEn, MJ/kg	12.6	12.6
Moisture[6]	11.4 (11.4)	11.5 (11.5)
Crude ash[6]	4.7 (4.5)	4.6 (4.3)
CP[6]	20.7 (20.4)	18.4 (18.6)
Crude fibre	3.0	3.2
Crude fat[6]	8.5 (90.3)	7.4 (91.1)
Starch	34.6	38.9
Ca	0.6	0.6
P	0.5	0.5
K	0.9	0.8
Na	0.2	0.2
Cl	0.2	0.3
AFD AA-to-Lys ratio, %		
AFD Lys-to-Lys ratio	100	100
AFD Met-to-Lys ratio	51	53
AFD (Met + Cys)-to-Lys ratio	76	76
AFD Thr-to-Lys ratio	64	55
AFD Trp-to-Lys ratio	22	19
AFD Val-to-Lys ratio	82	75
AFD Ile-to-Lys ratio	75	64
AFD Arg-to-Lys ratio	124	106
AFD Leu-to-Lys ratio	137	119
AFD His-to-Lys ratio	44	38
AFD Ala-to-Lys ratio	76	66
AFD Asp-to-Lys ratio	176	144
AFD Glu-to-Lys ratio	346	308
AFD Phe-to-Lys ratio	88	75
AFD Pro-to-Lys ratio	101	91
AFD Tyr-to-Lys ratio	60	50
AFD (Gly + Ser)-to-Lys ratio	156	135

PC = positive control; NC = negative control; AFD = apparent faecal digestible; AMEn = nitrogen-corrected apparent metabolizable energy.

[1] Diets were formulated upon chemical analysis of DM, CP, and AA content of the main feedstuffs (maize, soybean meal, peas, sunflower seed meal). Apparent faecal digestible AA composition was calculated according to tables of Schothorst Feed Research, the Netherlands.

[2] Phyzyme (200 FTU/kg diet; Dupont, United Kingdom).

[3] Rovabio Excel (beta-glucanase at 1,500 U/kg diet and beta-xylanase at 1,100 U/kg diet, Adisseo, France).

[4] Vitamin-mineral concentrate supplied per kilogram of diet: vitamin A 12,000 IU; vitamin D_3 2,400 IU; vitamin E 50 mg; thiamine 2.0 mg; riboflavin 7.5 mg; pantothenic acid 12 mg; niacin 35 mg; folic acid 1.0 mg; vitamin B_6 3.5 mg; vitamin B_{12} 25 μg; biotin 200 μg; vitamin K_3 1.5 mg; choline chloride 460 mg; Cu ($CuSO_4.5H_2O$) 12 mg; Fe ($FeSO_4$. H_2O) 80 mg; Mn (MnO) 85 mg; Zn ($ZnSO_4.H_2O$) 60 mg; I (KI) 0.8 mg; Se (Na_2SeO_3) 0.15 mg.

[5] Monteban (Elanco, the Netherlands).

[6] Analysed moisture, crude ash, CP, and crude fat level given between parentheses.

3.2. Plasma analysis

Comparing PC (T1), NC (T2) and T17 with the highest AFD Thr-to-Lys ratio and AFD (Gly + Ser)-to-Lys ratio (comparable level as PC) showed that plasma UA was significantly higher for PC treatment compared to NC and T17 (P = 0.003; Table 6). However, plasma AA, TG and glucose were similar among PC, NC and T17 ($P > 0.05$).

Results on the effects of Thr and Gly, and the interaction between Thr × Gly on plasma analysis are given in Table 7. A significant interaction between Thr × Gly on plasma free AA level was observed ($P = 0.014$), with a linear decrease of AFD Thr-to-Lys ratio and linear increase of AFD (Gly + Ser)-to-Lys ratio levels for plasma AA concentration. A linear and quadratic decrease in plasma TG concentration was observed ($P < 0.001$ and $P = 0.005$, respectively) in response to L-Thr supplementation. Plasma TG concentration was not affected by Gly supplementation ($P > 0.05$).

Plasma glucose concentration was linearly increased ($P = 0.016$) by L-Thr supplementation, whereas a trend in the quadratic response was observed (P = 0.087). Plasma glucose concentration was not affected by Gly supplementation ($P > 0.05$).

Plasma UA concentration was not affected by AFD Thr-to-Lys ratio, AFD (Gly + Ser)-to-Lys ratio, or by the interaction between Thr × Gly ($P > 0.05$).

4. Discussion

Reducing dietary protein in poultry diets is of interest to improve poultry industry sustainability (Hilliar et al., 2019). Supplementing low CP diets (CP level of 16% to 18%) with both IAA and DAA seems to be necessary to maintain adequate performance levels (Corzo et al., 2005; Dean et al., 2006; Ospina-Rojas et al., 2013a). The current study indicates that CP can be reduced by 2.3% points in the grower phase without loss of performance when adequate levels of AA are applied. FCR of the treatment with low CP diet, formulated at 64% Thr and 156% Gly (T17), and PC treatment were similar, having a similar AFD Lys, Thr and Gly level and 2.3% points difference in CP, whereas the NC diet with a lower AFD Thr and Gly level showed a higher FCR. However, low CP diet with 64% Thr and 156% Gly (T17) showed a significantly higher BWG and a numerically higher FI than PC treatment. In the present study, reducing CP and supplementing with a slightly higher IAA and Gly resulted in similar FI and FCR, improved BWG, reduced N excretion and plasma UA compared to the PC treatment. In studies testing decreasing dietary CP levels, the FCR is often impaired whereas the FI is not affected (Belloir et al., 2015). However, when the dietary CP is low and IAA needs are not met, broilers can regulate their protein intake via hyperphagia based on the energy to protein ratio (Smith and Pesti, 1998; Swennen et al., 2007). Besides, by reducing CP some AA will be adequately supplied while others, if not supplemented in the diet, are reduced causing an imbalance in AA. It is known that piglets can sense AA imbalance and adapt their feed intake accordingly (Jansman et al., 2019), and this might also be true for broilers.

Plasma UA concentration was decreased in low CP diets. Uric acid is the end product of AA catabolism, and, when dietary CP is reduced, the excess of AA to catabolize is also reduced, resulting in lower need for UA synthesis and lower N excretion. These results therefore confirm that low CP diets result in lower UA synthesis, and thereby will contribute to lower N excretion, as widely reported in literature (Belloir et al., 2017; Alfonso-Avila et al., 2019;

Table 3
Formulated and analysed (in parentheses) total and AFD AA composition of the experimental diets (% of the diet).[1]

Item	Treatments																
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17
Total AA, %																	
Lys	1.14 (1.15)	1.12 (1.15)	1.12 (1.12)	1.12 (1.12)	1.12 (1.12)	1.12 (1.13)	1.12 (1.12)	1.12 (1.11)	1.12 (1.10)	1.12 (1.13)	1.12 (1.11)	1.12 (1.12)	1.12 (1.11)	1.12 (1.12)	1.12 (1.11)	1.12 (1.11)	1.12 (1.11)
Thr	0.76 (0.74)	0.66 (0.66)	0.71 (0.70)	0.74 (0.73)	0.77 (0.76)	0.66 (0.69)	0.71 (0.70)	0.74 (0.72)	0.77 (0.75)	0.66 (0.68)	0.71 (0.70)	0.74 (0.73)	0.77 (0.75)	0.66 (0.68)	0.71 (0.69)	0.74 (0.73)	0.77 (0.76)
Met	0.54 (0.54)	0.56 (0.54)	0.56 (0.52)	0.56 (0.53)	0.56 (0.53)	0.56 (0.53)	0.56 (0.53)	0.56 (0.54)	0.56 (0.53)	0.56 (0.52)	0.56 (0.53)	0.56 (0.53)	0.56 (0.53)	0.56 (0.52)	0.56 (0.53)	0.56 (0.53)	0.56 (0.52)
Met + Cys	0.84 (0.84)	0.84 (0.83)	0.84 (0.80)	0.84 (0.82)	0.84 (0.81)	0.84 (0.82)	0.84 (0.81)	0.84 (0.82)	0.84 (0.82)	0.28 (0.29)	0.28 (0.29)	0.28 (0.28)	0.28 (0.29)	0.28 (0.29)	0.28 (0.29)	0.28 (0.29)	0.28 (0.28)
Trp	0.25 (0.26)	0.21 (0.23)	0.21 (0.23)	0.21 (0.22)	0.21 (0.22)	0.21 (0.22)	0.21 (0.22)	0.21 (0.22)	0.21 (0.22)	0.84 (0.81)	0.84 (0.81)	0.84 (0.81)	0.84 (0.82)	0.84 (0.81)	0.84 (0.82)	0.84 (0.82)	0.84 (0.80)
Val	0.94 (0.91)	0.86 (0.86)	0.86 (0.87)	0.86 (0.86)	0.86 (0.85)	0.86 (0.86)	0.86 (0.86)	0.86 (0.86)	0.86 (0.85)	0.21 (0.22)	0.21 (0.22)	0.21 (0.22)	0.21 (0.23)	0.21 (0.22)	0.21 (0.22)	0.21 (0.22)	0.21 (0.22)
Ile	0.85 (0.82)	0.72 (0.72)	0.72 (0.73)	0.72 (0.72)	0.72 (0.73)	0.72 (0.72)	0.72 (0.73)	0.72 (0.72)	0.72 (0.72)	0.86 (0.87)	0.86 (0.84)	0.86 (0.86)	0.86 (0.86)	0.86 (0.86)	0.86 (0.85)	0.86 (0.84)	0.86 (0.85)
Leu	1.55 (1.54)	1.35 (1.36)	1.35 (1.36)	1.35 (1.36)	1.35 (1.37)	1.35 (1.37)	1.35 (1.35)	1.35 (1.35)	1.35 (1.35)	0.72 (0.74)	0.72 (0.72)	0.72 (0.72)	0.72 (0.73)	0.72 (0.74)	0.72 (0.72)	0.72 (0.71)	0.72 (0.73)
Arg	1.38 (1.34)	1.17 (1.19)	1.17 (1.19)	1.17 (1.17)	1.17 (1.17)	1.17 (1.19)	1.17 (1.17)	1.17 (1.16)	1.17 (1.16)	1.35 (1.37)	1.35 (1.34)	1.35 (1.36)	1.35 (1.36)	1.35 (1.37)	1.35 (1.35)	1.35 (1.36)	1.35 (1.37)
Phe	0.98 (0.98)	0.84 (0.85)	0.84 (0.87)	0.84 (0.87)	0.84 (0.87)	0.84 (0.87)	0.84 (0.87)	0.84 (0.85)	0.84 (0.86)	1.17 (1.19)	1.17 (1.16)	1.17 (1.17)	1.17 (1.16)	1.17 (1.19)	1.17 (1.16)	1.17 (1.16)	1.17 (1.18)
Tyr	0.69 (0.69)	0.58 (0.59)	0.58 (0.60)	0.58 (0.60)	0.58 (0.59)	0.58 (0.60)	0.58 (0.59)	0.58 (0.59)	0.58 (0.59)	0.84 (0.87)	0.84 (0.86)	0.84 (0.86)	0.84 (0.86)	0.84 (0.87)	0.84 (0.86)	0.84 (0.86)	0.84 (0.87)
His	0.50 (0.50)	0.44 (0.45)	0.44 (0.45)	0.44 (0.45)	0.44 (0.45)	0.44 (0.45)	0.44 (0.45)	0.44 (0.44)	0.44 (0.44)	0.58 (0.60)	0.58 (0.59)	0.58 (0.59)	0.58 (0.60)	0.58 (0.60)	0.58 (0.59)	0.58 (0.59)	0.58 (0.60)
Ser	0.98 (0.97)	0.84 (0.84)	0.84 (0.85)	0.84 (0.85)	0.84 (0.85)	0.84 (0.87)	0.84 (0.85)	0.84 (0.84)	0.84 (0.84)	0.44 (0.45)	0.44 (0.44)	0.44 (0.45)	0.44 (0.45)	0.44 (0.45)	0.44 (0.45)	0.44 (0.44)	0.44 (0.45)
Ala	0.91 (0.89)	0.80 (0.81)	0.80 (0.81)	0.80 (0.81)	0.80 (0.82)	0.80 (0.82)	0.80 (0.81)	0.80 (0.81)	0.80 (0.81)	0.84 (0.85)	0.84 (0.85)	0.84 (0.85)	0.84 (0.84)	0.84 (0.85)	0.84 (0.84)	0.84 (0.86)	0.84 (0.86)
Asp	2.01 (1.95)	1.66 (1.66)	1.66 (1.66)	1.66 (1.66)	1.66 (1.67)	1.66 (1.68)	1.66 (1.65)	1.66 (1.63)	1.66 (1.64)	0.80 (0.82)	0.80 (0.81)	0.80 (0.81)	0.80 (0.81)	0.80 (0.82)	0.80 (0.81)	0.80 (0.81)	0.80 (0.82)
Glu	3.78 (3.69)	3.35 (3.37)	3.35 (3.25)	3.35 (3.25)	3.35 (3.26)	3.35 (3.29)	3.35 (3.25)	3.35 (3.21)	3.35 (3.22)	1.66 (1.67)	1.66 (1.64)	1.66 (1.66)	1.66 (1.65)	1.66 (1.67)	1.66 (1.64)	1.66 (1.65)	1.66 (1.67)
Gly	0.87 (0.85)	0.76 (0.78)	0.76 (0.78)	0.76 (0.78)	0.76 (0.79)	0.83 (0.85)	0.83 (0.84)	0.83 (0.84)	0.83 (0.84)	3.35 (3.28)	3.35 (3.22)	3.35 (3.24)	3.35 (3.24)	3.35 (3.27)	3.35 (3.22)	3.35 (3.24)	3.35 (3.26)
Pro	1.14 (1.11)	1.03 (1.03)	1.03 (1.03)	1.03 (1.04)	1.03 (1.02)	1.03 (1.03)	1.03 (1.00)	1.03 (1.03)	1.03 (1.02)	0.90 (0.92)	0.90 (0.90)	0.90 (0.92)	0.90 (0.90)	0.97 (0.98)	0.97 (0.99)	0.97 (0.97)	0.97 (0.98)
Gly + Ser	1.85 (1.82)	1.60 (1.62)	1.60 (1.63)	1.60 (1.63)	1.60 (1.64)	1.67 (1.72)	1.67 (1.69)	1.67 (1.68)	1.67 (1.68)	1.74 (1.77)	1.74 (1.75)	1.74 (1.77)	1.74 (1.74)	1.81 (1.83)	1.81 (1.83)	1.81 (1.83)	1.81 (1.84)
AFD AA-to-Lys ratio[1], %																	
Lys	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)
Thr	64 (62)	55 (54)	58 (57)	61 (60)	64 (63)	55 (57)	58 (57)	61 (60)	64 (63)	55 (56)	58 (58)	61 (60)	64 (63)	55 (57)	58 (57)	61 (61)	64 (64)
Met	51 (51)	53 (50)	53 (49)	53 (50)	53 (50)	53 (50)	53 (50)	53 (52)	53 (51)	53 (49)	53 (51)	53 (50)	53 (51)	53 (49)	53 (51)	53 (51)	53 (50)
Met + Cys	76 (75)	76 (73)	76 (72)	76 (74)	76 (73)	76 (74)	76 (73)	76 (75)	76 (76)	76 (73)	76 (74)	76 (74)	76 (76)	76 (76)	76 (76)	76 (76)	76 (73)
Trp	22 (23)	19 (20)	19 (21)	19 (20)	19 (20)	19 (20)	19 (20)	19 (20)	19 (20)	19 (20)	19 (20)	19 (20)	19 (21)	19 (20)	19 (20)	19 (20)	19 (20)
Val	82 (78)	75 (73)	75 (76)	75 (75)	75 (74)	75 (74)	75 (75)	75 (76)	75 (75)	75 (75)	75 (74)	75 (75)	75 (76)	75 (75)	75 (75)	75 (74)	75 (75)
Ile	75 (75)	64 (62)	64 (65)	64 (64)	64 (65)	64 (63)	64 (65)	64 (65)	64 (65)	64 (65)	64 (65)	64 (64)	64 (65)	64 (66)	64 (65)	64 (64)	64 (65)
Leu	137 (135)	119 (117)	119 (120)	119 (120)	119 (121)	119 (120)	119 (119)	119 (120)	119 (121)	119 (120)	119 (119)	119 (120)	119 (121)	119 (121)	119 (120)	119 (121)	119 (122)
Arg	124 (120)	106 (105)	106 (108)	106 (106)	106 (106)	106 (107)	106 (106)	106 (106)	106 (107)	106 (107)	106 (106)	106 (106)	106 (106)	106 (106)	106 (106)	106 (106)	106 (108)
Phe	88 (87)	75 (74)	75 (78)	75 (78)	75 (78)	75 (77)	75 (78)	75 (77)	75 (78)	75 (77)	75 (77)	75 (77)	75 (77)	75 (78)	75 (77)	75 (77)	75 (78)
Tyr	60 (59)	50 (50)	50 (52)	50 (52)	50 (51)	50 (51)	50 (51)	50 (51)	50 (52)	50 (51)	50 (51)	50 (51)	50 (52)	50 (52)	50 (51)	50 (51)	50 (52)
His	44 (44)	38 (38)	38 (39)	38 (39)	38 (39)	38 (39)	38 (39)	38 (38)	38 (39)	38 (39)	38 (38)	38 (39)	38 (39)	38 (39)	38 (39)	38 (38)	38 (39)
Ser	86 (84)	73 (71)	73 (74)	73 (74)	73 (73)	73 (75)	73 (74)	73 (74)	73 (74)	73 (73)	73 (75)	73 (74)	73 (74)	73 (74)	73 (74)	73 (75)	73 (75)
Ala	76 (73)	66 (65)	66 (67)	66 (67)	66 (68)	66 (67)	66 (67)	66 (67)	66 (68)	66 (67)	66 (67)	66 (67)	66 (67)	66 (68)	66 (67)	66 (67)	66 (68)
Asp	176 (169)	144 (140)	144 (144)	144 (144)	144 (145)	144 (144)	144 (143)	144 (143)	144 (145)	144 (144)	144 (144)	144 (144)	144 (144)	144 (145)	144 (144)	144 (144)	144 (146)
Glu	346 (335)	308 (302)	308 (299)	308 (299)	308 (300)	308 (300)	308 (299)	308 (298)	308 (301)	308 (299)	308 (299)	308 (298)	308 (301)	308 (301)	308 (299)	308 (301)	308 (302)
Gly	69 (67)	62 (62)	62 (64)	62 (64)	62 (64)	69 (70)	69 (70)	69 (70)	69 (71)	76 (77)	76 (77)	76 (78)	76 (77)	83 (84)	83 (85)	83 (84)	83 (85)
Pro	101 (97)	91 (89)	91 (91)	91 (92)	91 (90)	91 (90)	91 (88)	91 (92)	91 (92)	91 (90)	91 (90)	91 (90)	91 (89)	91 (90)	91 (91)	91 (92)	91 (92)
Gly + Ser	155 (151)	135 (133)	135 (138)	135 (138)	135 (138)	142 (145)	142 (144)	142 (144)	142 (145)	149 (150)	149 (151)	149 (152)	149 (150)	156 (158)	156 (159)	156 (159)	156 (160)

[1] Apparent faecal digestible (AFD) as formulated and as re-calculated based on AA analysis (between parentheses). AFD AA composition was calculated according to tables of Schothorst Feed Research, the Netherlands.

Table 4
Effect of dietary AFD Thr-to-Lys ratio and AFD (Gly + Ser)-to-Lys ratio in treatments 1, 2 and 17 on production performance from 7 to 28 d.[1]

Item	AFD Thr-to-Lys ratio, %	AFD (Gly + Ser)-to-Lys ratio, %	CP, %	FI, g	BWG, g	FCR, g/g
T1 (PC)	64	156	20.5	2,069	1,482^{a}	1.397^{a}
T2 (NC)	55	135	18.5	2,100	1,473^{a}	1.427^{b}
T17	64	156	18.5	2,154	1,538^{b}	1.400^{a}
P-value (LSD)				0.165 (91.3)	0.002 (30.1)	0.028 (0.0226)

AFD = apparent faecal digestible; PC = positive control; NC = negative control; FI = feed intake, BWG = body weight gain, FCR = feed conversion ratio; LSD = least significant differences.

[a, b] Means within the same column with no common superscript differ significantly ($P \leq 0.05$).

[1] Data presented as means ($n = 6$ pens per treatment).

Table 5
Effect of dietary AFD Thr-to-Lys ratio and AFD (Gly + Ser)-to-Lys ratio on production performance from 7 to 28 d.[1]

Item	AFD Thr-to-Lys ratio, %	AFD (Gly + Ser)-to-Lys ratio, %	FI, g	BWG, g	FCR, g/g
Treatments					
	55	135	2,102	1,465	1.427
	58	135	2,233	1,588	1.406
	61	135	2,203	1,573	1.400
	64	135	2,187	1,561	1.401
	55	142	2,194	1,530	1.434
	58	142	2,207	1,561	1.414
	61	142	2,180	1,543	1.403
	64	142	2,118	1,523	1.391
	55	149	2,184	1,532	1.425
	58	149	2,227	1,585	1.395
	61	149	2,177	1,546	1.408
	64	149	2,182	1,548	1.410
	55	156	2,177	1,526	1.426
	58	156	2,210	1,563	1.421
	61	156	2,175	1,562	1.399
	64	156	2,146	1,534	1.400
AFD Thr-to-Lys ratio					
	55		2,164^{a}	1,513^{a}	1.428^{b}
	58		2,219^{b}	1,574^{c}	1.409^{a}
	61		2,184^{ab}	1,556^{bc}	1.403^{a}
	64		2,158^{a}	1,541^{b}	1.400^{a}
AFD (Gly + Ser)-to-Lys ratio					
		135	2,181	1,547	1.409
		142	2,175	1,539	1.410
		149	2,192	1,553	1.410
		156	2,177	1,546	1.412
P-value (LSD)					
AFD Thr-to-Lys ratio			0.004 (34.9)	<0.001 (24.0)	<0.001 (0.0093)
Linear			0.335	0.092	<0.001
Quadratic			0.002	<0.001	0.013
AFD (Gly + Ser)-to-Lys ratio			0.761 (35.0)	0.759 (24.1)	0.948 (0.0092)
Linear			0.895	0.732	0.600
Quadratic			0.714	0.978	0.958
Interaction			0.140 (70.0)	0.074 (48.2)	0.116 (0.0185)
Lin.Lin			0.091	0.041	0.688
Lin.Qua			0.069	0.036	0.787
Qua.Lin			0.178	0.161	0.663
Qua.Qua			0.342	0.152	0.661

AFD = apparent faecal digestible; FI = feed intake, BWG = body weight gain, FCR = feed conversion ratio; LSD = least significant differences.

[a–c] Means within the same column with no common superscript differ significantly ($P \leq 0.05$).

[1] Data presented as means ($n = 6$ pens per treatment).

Table 6
Effect of dietary AFD Thr-to-Lys ratio and AFD (Gly + Ser)-to-Lys ratio in positive control (T1), negative control (T2) and treatment 17 (T17, comparable ratios as PC) on plasma metabolite concentrations at 28 d.[1]

Treatment	AFD Thr-to-Lys ratio, %	AFD (Gly + Ser)-to-Lys ratio, %	CP, %	PFAA, mg/mL	TG, g/L	Glucose, g/L	UA, mg/L
T1 (PC)	64	156	20.5	0.47	0.88	2.38	65.3^{b}
T2 (NC)	55	135	18.5	0.47	1.00	2.30	47.1^{a}
T17	64	156	18.5	0.50	0.92	2.36	54.3^{a}
P-value (LSD)				0.466 (0.072)	0.290 (0.155)	0.750 (0.223)	0.003 (9.98)

AFD = apparent faecal digestible; PFAA = plasma free amino acids; TG = triglyceride; UA = uric acid; LSD = least significant differences.

[a, b] Means within the same column with no common superscript differ significantly ($P \leq 0.05$).

[1] Data presented as means ($n = 10$ birds per treatment).

Table 7
Effect of dietary AFD Thr-to-Lys ratio and AFD (Gly + Ser)-to-Lys ratio on plasma metabolite concentrations at 28 d.[1]

Item	AFD Thr-to-Lys ratio, %	AFD (Gly + Ser)-to-Lys ratio, %	PFAA, mg/mL	TG, g/L	Glucose, g/L	UA, mg/L
Treatments						
	55	135	0.47^{abc}	1.00	2.30	46.7
	58	135	0.53^{cde}	1.19	2.13	53.1
	61	135	0.47^{abcd}	1.09	2.26	45.7
	64	135	0.43^{a}	0.96	2.42	53.5
	55	142	0.49^{abcde}	1.23	2.19	48.3
	58	142	0.46^{abc}	1.09	2.23	47.5
	61	142	0.46^{abc}	1.19	2.35	47.3
	64	142	0.51^{bcde}	0.90	2.39	51.1
	55	149	0.61^{f}	1.15	2.35	51.2
	58	149	0.54^{ef}	1.04	2.17	48.5
	61	149	0.54^{de}	1.09	2.28	54.7
	64	149	0.46^{ab}	0.94	2.32	49.3
	55	156	0.55^{ef}	1.10	2.18	47.8
	58	156	0.52^{bcde}	1.02	2.22	51.9
	61	156	0.52^{bcde}	1.16	2.30	56.4
	64	156	0.50^{bcde}	0.92	2.36	54.3
AFD Thr-to-Lys ratio						
	55		0.53^{b}	1.12^{b}	2.26^{ab}	48.5
	58		0.51^{b}	1.08^{b}	2.19^{a}	50.3
	61		0.50^{ab}	1.13^{b}	2.30^{ab}	51.0
	64		0.48^{a}	0.93^{a}	2.37^{b}	52.0
AFD (Gly + Ser)-to-Lys ratio						
		135	0.47^{a}	1.06	2.28	49.8
		142	0.48^{a}	1.10	2.29	48.6
		149	0.54^{b}	1.06	2.28	50.9
		156	0.52^{b}	1.05	2.26	52.6
P-value (LSD)						
AFD Thr-to-Lys ratio			0.014 (0.033)	<0.001 (0.082)	0.020 (0.118)	0.434 (4.34)
Linear			0.001	<0.001	0.016	0.105
Quadratic			0.679	0.005	0.087	0.821
AFD (Gly + Ser)-to-Lys ratio			<0.001 (0.033)	0.590 (0.082)	0.977 (0.118)	0.297 (4.34)
Linear			<0.001	0.529	0.791	0.117
Quadratic			0.390	0.428	0.723	0.347
Interaction			0.014 (0.066)	0.132 (0.163)	0.844 (0.236)	0.351 (8.69)
Lin.Lin			0.384	0.930	0.873	0.683
Lin.Qua			0.555	0.116	0.636	0.308
Qua.Lin			0.248	0.265	0.354	0.271
Qua.Qua			0.115	0.198	0.704	0.594

AFD = apparent faecal digestible; PFAA = plasma free amino acids; TG = triglyceride; UA = uric acid; LSD = least significant differences.
[a-f] Means within the same column with no common superscript differ significantly ($P \leq 0.05$).
[1] Data presented as means ($n = 10$ birds per treatment).

Lemme et al., 2019). Lemme et al. (2019), for example, measured an improved N utilisation and related effects on N excretion, litter quality and volume, and foot pad lesions when reducing CP level in the broiler diets from starter to finisher phase (1 to 40 d). The meta-analysis on the impact of reducing CP in diets conducted by Alfonso-Avila et al. (2019) showed that N efficiency can be increased by 2.3% for each percent in CP reduction and that litter moisture can be reduced by 4% for each percent in CP reduction in broiler diets. Low CP diets therefore improve the sustainability of broiler production by efficiently reducing environmental burden associated with N excretion (Belloir et al., 2017; Hilliar et al., 2019).

During the last few years, research focussed on Gly requirement and its indispensability for broiler chickens. Researchers often came to the conclusion that high levels of dietary Gly (>176% [Gly + Ser]-to-Lys ratio) were required for optimal growth and health (Corzo et al., 2004; Schutte et al., 1997; Waguespack et al., 2009). However, feed grade Gly is not commercially available in the European Union (EU). Glycine is known to be converted from Thr metabolism, suggesting that supplementation of this AA may satisfy Gly requirements. This concept was proven when using an experimental design in which the basal mixture for all diets consisted of corn and casein, and not when using practically formulated diets (Siegert et al., 2015a). According to these findings, diet compositions that consider the sparing effect of Thr on Gly can contribute to the beneficial reduction of dietary CP, even though these findings appear to be related to the overall level of IAA.

In the current study, Gly supplementation did not affect production performance, with exception of BWG at the lowest dietary AFD Thr-to-Lys ratio level. This is in contrast with some studies, in which FCR was improved with Gly supplementation (Corzo et al., 2004; Waguespack et al., 2009). It is possible that the dietary CP content (and thereby Gly level) in the current study was not low enough to observe a response to Gly, as Gly requirement is increased at low dietary CP levels (Dean et al., 2006; Waterhouse and Scott, 1961). Another hypothesis is that the AA profile of the low CP diet was close to the actual AA requirements of the broilers for optimal growth, sparing the Gly needed for UA synthesis and therefore lowering Gly requirements. This seems to be confirmed by the significantly lower plasma UA concentration of treatments with 20.5% CP compared to the control with 18.5% CP measured at 28 d. In addition, it could also be argued that birds from this experiment were older (7 to 28 d) than birds usually followed in Gly dose–response studies (0 to 18 d), and that Gly requirement of starter broilers is higher than that of growing-finishing broilers (Rostagno et al., 2017). Moreover, there was no significant effect of Gly level on most circulating metabolites, and in particular UA. In literature, there is usually no reported effect of Gly supplementation on serum or plasma UA concentration (Ngo et al., 1977;

Table 8
Diet composition and age of broilers from studies investigating Thr and Gly interactions.[1]

Item	Waldroup et al. (2005)	Ospina-Rojas et al. (2013a)	Corzo et al. (2009)	Ospina-Rojas et al. (2013b)	Siegert et al. (2015b) [2]
Age, d	1 to 21	1 to 21	21 to 42	21 to 35	1 to 21
CP, %	15.0	19.1	17.1	16.6	17.4
AFD Lys, %	1.07	1.23	0.94	1.05	1.34
AFD Thr-to-Lys ratio, %	60 to 94	62 to 73	56 to 67	61 to 68	43 to 72
AFD (Met + Cys)-to-Lys ratio, %	60	70	77	72	66
AFD Cys-to-Lys ratio, %	17	18	24	19	25
AFD Val-to-Lys ratio, %	64	73	72	75	96
AFD (Gly + Ser)-to-Lys ratio, %	96 to 133	127 to 162	139 to 149	112 to 140	99 to 178
Thr × Gly interaction	No	Yes, for FCR	Yes, for BWG	Yes, for FCR	Yes, for BWG and FCR

AFD = Apparent faecal digestibility; BWG = body weight gain.
[1] Values are recalculated using tables of Schothorst Feed Research, except for Siegert et al. (2015b).
[2] Expressed in analysed total Thr-to-Lys ratio and total Gly_{equi}-to-Lys ratio.

Hofmann et al., 2019), except in the case of concomitant supplementation of Gly + Glu (Namroud et al., 2008) and addition of Gly for the lowest levels of dietary CP and Gly + Ser (Yuan et al., 2012). In the current experiment it is concluded that CP level has an impact on UA synthesis, which might influences Gly requirement, but the Gly effect on UA in plasma was not demonstrated.

The FCR decreased with increased AFD Thr-to-Lys ratio, indicating that Thr requirement for FCR was at least 64% AFD Thr-to-Lys ratio in broilers from 7 to 28 d. However, FI and BWG showed a quadratic response with a peak in the response at 58% AFD Thr-to-Lys ratio. It is common that FCR responds more to Thr than BWG in Thr dose–response studies (Leclercq, 1998; Mack et al., 1999). In recent trials, researchers have found increased requirement for Thr in modern broilers up to 68% digestible Thr-to-Lys ratio, indicating that today's broilers require higher levels of dietary Thr, as this AA is necessary for optimal growth, gut health, and immune response (Dozier et al., 2015; Jiang et al., 2014; Star et al., 2012). In addition, Thr may also regulate lipid metabolism, by reducing plasma triglyceride level when supplemented at 64% ADF Thr-to-Lys ratio compared to the other Thr levels in the present study. Jiang et al. (2017, 2019) suggested that dietary Thr supplementation improved hepatic lipid metabolism of Pekin ducks by regulating lipid synthesis, transport and oxidation. Similar observations were found in mammals (Yoshida et al., 1961; Xiao et al., 2016).

Interaction between Thr and Gly was first observed by Baker et al. (1972). Since then, only 5 groups of researchers have published conclusions on the interaction between Thr and Gly (Corzo et al., 2009; Ospina-Rojas et al., 2013a,b; Siegert et al., 2015b; Waldroup et al., 2005). In Table 8, dietary nutrient composition and age of broilers used in the aforementioned studies are presented. Interestingly, only Waldroup et al. (2005) did not observe any interaction between Thr and Gly in broilers, while the other studies showed an interaction for either FCR or BWG. In the current study, a tendency to interaction between Thr and Gly on production performance was also observed. However, BWG of broilers only responded when both dietary Gly and Thr levels were very low. This indicates that in the case of a severe Thr deficiency, less Thr is converted to Gly, which therefore needs to be supplemented to the diet. Similarly, in broilers from 21 to 35 d, Ospina-Rojas et al. (2013b) observed that FCR showed a stronger response to Gly when broilers were fed 65% digestible Thr-to-Lys ratio compared to 72% digestible Thr-to-Lys ratio. Response of FCR to dietary Thr level was also stronger when birds were fed 135% AFD (Gly + Ser)-to-Lys ratio level compared to 149%. This is also supported by the results of Corzo et al. (2009), who observed that BWG of broilers from 21 to 42 d responded more to Thr when they were fed 143 compared to 153% digestible (Gly + Ser)-to-Lys ratio level. The extent of the replacement effect of dietary Thr to Gly depends on the respective concentrations and appears to be related to the overall level of IAA (Siegert et al., 2015), and more specifically to Met + Cys, choline, Arg, and metabolites from Gly, guanidino acetic acid and creatine, as reviewed by Siegert and Rodehutscord (2019).

5. Conclusion

In the current trial, Gly was not limiting for growth at low CP levels unless Thr was deficient. Besides, low CP diets resulted in lower plasma UA concentrations, indicating the contribution to lower N excretion. This shows that adequate amounts of Thr (64% AFD Thr-to-Lys ratio) in broiler diets can overcome the marginal supply of Gly and Ser and allow reduction of dietary CP from 20.5% to 18.5% for broilers from 7 to 28 d of age while maintaining similar growth performance.

Author contributions

Laura Star: conceptualization, supervision, investigation, methodology, formal analysis, writing- original draft preparation; **Sophie Tesseraud**: methodology, writing – review and editing; **Marije van Tol**: writing – review and editing; **Ilaria Minussi**: writing – review and editing; **Etienne Corrent**: conceptualization, methodology; **William Lambert**: investigation, writing – review and editing.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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