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## EDITED BY

Michael D. Flythe,  
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## REVIEWED BY

Sarah C. Pearce,  
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United States  
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Konkuk University, Republic of Korea  
Samiru Sudharaka Wickramasuriya,  
Agricultural Research Service (USDA),  
United States

## \*CORRESPONDENCE

Marie-Pierre Létourneau-Montminy  
✉ marie-pierre.letourneau-montminy@  
ulaval.ca

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# Meta-analysis of the effect of low-protein diets on the growth performance, nitrogen excretion, and fat deposition in broilers

Théophile de Rauglaudre<sup>1,2,3</sup>, Bertrand Méda<sup>2</sup>,  
Simon Fontaine<sup>4</sup>, William Lambert<sup>4</sup>, Sébastien Fournel<sup>3</sup>  
and Marie-Pierre Létourneau-Montminy<sup>1\*</sup>

<sup>1</sup>Département des Sciences animales, Université Laval, Québec, QC, Canada, <sup>2</sup>Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), Université de Tours, Biologie des Oiseaux et Aviculture (BOA), Nouzilly, France, <sup>3</sup>Département des sols et de génie agroalimentaire, Université Laval, Québec, QC, Canada, <sup>4</sup>METEX Animal Nutrition, Paris, France

In broilers, the effects of crude protein (CP) reduction on animal performance are heterogeneous. This could limit the use of this strategy in commercial farms despite its potential to improve the sustainability of production. The objective of this meta-analysis was to study the effect of lowering dietary CP in fast-growing broilers with a focus on growth performance. A database was built from 29 papers published after 2016, for a total of 106 trials and 268 treatments. To be included in the database, trials had to be iso-energy and iso-lysine. Trials in which the growth rate for the control treatment was below 90% of the genetic potential of the birds were not included. The effect of the CP level was analyzed by multiple linear regression, with the trial as a random effect. A subsample of 33 trials (AACON) met the recommended amino acid (AA)-to-lysine ratios for indispensable AAs. In this subdatabase, average daily gain and average daily feed intake were maintained when dietary CP was reduced. The feed conversion ratio increased by 1.3% when CP was reduced by a one percentage point. The same CP reduction decreased daily nitrogen (N) excretion by 10.4%, whereas N retention was not affected by CP. In conclusion, respecting the ideal protein concept with the use of feed-grade AA allows for a decrease in dietary CP and N excretion with a limited impact on growth performance.

## KEYWORDS

amino acid, sustainability, chicken, crude protein, nitrogen balance, abdominal fat pad, intake, efficiency

## 1 Introduction

The world population is growing, with a projected 9.5 billion inhabitants by 2050 (UN, 2019). The demand for chicken meat, which is the most consumed meat in the world (FAO, 2020), is expected to increase by 13% by 2031 (OCDE, 2020). At the same time, humanity is facing an unprecedented climatic and environmental crisis. For example, the Earth's surface temperature has risen faster in the last 50 years than in the previous 2,000 years,

mainly as a result of the increased production of anthropogenic greenhouse gases (GHGs; IPCC, 2021). Although poultry production accounts for only 8.7% of GHGs produced by the livestock sector, which in turn accounts for 14.5% of global human-induced GHG emissions, the poultry sector must implement new strategies to continue to meet the demand for animal protein without exacerbating the environmental crisis (Gerber et al., 2013). Environmental impacts of the broiler production are mainly related to the feed production stage (67%–78% of climate change, 55%–69% of energy use, 52%–70% of eutrophication, and 25%–55% of acidification, (Leinonen et al., 2012; da Silva et al., 2014; Méda et al., 2021). Nutrient losses to the environment from manure management also cause significant environmental impacts (Gerber et al., 2013). Broiler chickens excrete approximately 40%–60% of the nitrogen (N) they ingest, and up to 50% of this excreted N can be lost through volatilization (Guiziou and Béline, 2005; Wiedemann et al., 2016; Belloir et al., 2017).

Therefore, lowering the dietary crude protein (CP) content could be a potential lever to reduce environmental impacts (Méda et al., 2011; Gerber et al., 2013; Greenhalgh et al., 2020). In many countries, lowering the dietary CP content results in reduced use of soybean meal (SBM) from South America, which is associated with high environmental impacts (Dalgaard et al., 2008). Decreasing CP also reduces N losses through volatilization, reducing the impacts associated with ammonia (NH<sub>3</sub>) and nitrous oxide (N<sub>2</sub>O) emissions (Méda et al., 2017; Van Emous et al., 2019). A decrease in NH<sub>3</sub> emissions also improves the respiratory health of birds and workers (Naseem and King, 2018). Finally, reducing dietary CP content can also improve gut health (Qaisrani et al., 2015; Luise et al., 2021) and decrease the occurrence of pododermatitis (Minussi et al., 2021). However, growth performance in response to CP is heterogeneous: there is no unequivocal decrease or increase (Alfonso-Avila et al., 2022; Frayssé et al., 2015). This is likely a result of the amino acids (AAs) that are not always controlled when reducing CP and become limiting when CP content decreases. These include AAs that are less studied such as glycine, histidine, or phenylalanine (Kidd et al., 2021). A meta-analysis was carried out, based on recent studies in fast-growing broilers, to test the low-CP strategy while controlling essential AAs with the addition of free (crystalline or synthetic) AAs.

## 2 Materials and methods

### 2.1 Data collection and coding

A literature search was conducted on the public databases Web of Science™ (Clarivate™, Philadelphia, PA, USA) and PubMed® (National Library of Medicine, Bethesda, MD, USA). The following keywords were used to search titles and abstracts: chicken(s), broiler (s), Ross, Cobb, reduce(d) protein diets, reduce(d) CP, low protein, low CP, levels of CP, and protein dilution. To be included in the data set, the trials had to use diets formulated with the same levels of digestible lysine (digLys) and apparent metabolizable energy (AME) or nitrogen-corrected AME (AMEn) within each trial. Trials were

removed if the daily Lys intake of the control broilers deviated by more than 20% from the recommended Lys intake, which is derived from Hauschild et al. (2015). Only trials using fast-growing strains (Ross 308 or Cobb 500) were considered, and the bird performance in the control treatment was required to be at least 90% of the genetic potential of the breeder's guidelines. In addition, feed composition, experimental conditions (bird variety, sex, age, ambient temperature, etc.), and growth performance had to be detailed. Using these selection criteria, a general database (GEN-database) was built with 29 publications from 2016 to 2021, regrouping 106 trials and 268 treatments (see Supplementary Material). The database contained qualitative data such as strain and sex, and qualitative data such as CP content and average daily gain (ADG). The nutrient composition of each diet was recalculated using INRAE feedstuff tables (INRAE-CIRAD-AFZ, 2018) to limit biases related to the use of different formulation matrices in the papers. A general description of the GEN database is provided in Table 1. A subdatabase of treatment where AAs were controlled (AACON-database) was also created, including 33 trials (79 treatments) of the original database (31%) where the levels of Met + Cys/Lys, Thr/Lys, Trp/Lys, Arg/Lys, Val/Lys, Ile/Lys, His/Lys, Gly + Ser/Lys, and Phe + Tyr/Lys were at 95% or above the reference requirements (Table 1). These requirements were calculated by considering digAA-to-digLys ratios taken from Rostagno et al. (2017). To recalculate the N balance (i.e., N intake, N retention, and N excretion), an average value of 29 g N retained/kg body weight gained (BWG) was considered (Belloir et al., 2017). As abdominal fat deposition was measured with different units (e.g., percentage of the live weight, percentage of the carcass), a fat deposition variable was created by aggregating the different fat deposition data expressed as a ratio of the control.

### 2.2 Statistical analyses

The effect of CP on the variables of interest was studied in both databases using Minitab® (2021) to conduct a multivariate analysis with the trial as a random effect:

$$Y_{ij} = \mu + a_i + b_{1ij}CP + b_{2ij}CP^2 + E_{ij}, \quad (1)$$

where  $Y_{ij}$  is the value of the studied variable in trial  $i$  for diet  $j$ ,  $\mu$  is the global intercept,  $a_i$  is the trial effect,  $b_1$  and  $b_2$  are the linear and quadratic coefficients of the CP effect, respectively, and  $E_{ij}$  is the residual error. The linear model was used when the quadratic effect was not significant. The effect of CP was studied for several datasets. Finally, to ensure the quality of the CP response models, the normality of residuals, the independence of the error, and the homogeneity of the variance were tested. Effects were considered significant at a  $P$ -value < 0.05 and trends at a  $P$ -value < 0.10.

As stated previously, a fixed coefficient for N retention was used to calculate N balance, except in the 30 trials (81 treatments) that measured N retention. To ensure the accuracy of using a fixed coefficient, these 30 trials were used to compare the measured versus calculated N retention based on the intercept, the slope, and the  $R^2$

TABLE 1 Description of the GEN-database and the AACON-databases.

	Mean	SD	Min	Median	Max
<b>GEN-database (108 trials)</b>					
Initial age (d)	13.30	8.21	1.00	10.00	29.00
Final age (d)	28.55	8.20	11.00	28.00	42.00
Average age (d)	20.93	7.79	6.00	19.50	34.50
Trial duration (d)	15.25	5.15	6.00	14.00	28.00
CP <sup>1</sup> in control (%)	20.12	1.84	16.61	20.10	24.14
CP <sup>1</sup> reduction (% point)	2.52	1.01	0.13	2.53	4.95
AMEn <sup>2</sup> (kcal/kg)	2940	138	2552	2951	3292
SID <sup>3</sup> lysine (g/kg)	10.51	0.89	8.10	10.54	12.70
<b>AACON-database (33 trials)</b>					
Initial age (d)	14.44	9.99	1.00	13.00	29.00
Final age (d)	28.03	10.28	11.00	35.00	42.00
Average age (d)	21.23	9.81	6.00	21.00	34.50
Trial duration (d)	13.80	5.25	6.00	13.00	28.00
CP <sup>1</sup> in control (%)	21.05	1.93	18.42	20.66	24.14
CP <sup>1</sup> reduction (% point)	1.62	0.69	0.13	1.56	3.22
AMEn <sup>2</sup> (kcal/kg)	2894	140	2552	2868	3132
SID <sup>3</sup> lysine (g/kg)	10.56	1.28	8.10	10.09	12.70

<sup>1</sup>CP, dietary crude protein.

<sup>2</sup>AMEn, apparent metabolizable energy corrected for zero nitrogen retention.

<sup>3</sup> SID, standardized ileal digestibility.

obtained between observed and predicted values. The mean square prediction error (MSPE) was broken down into error in central tendency (ECT), error due to regression (ER), and error due to disturbance (ED), as suggested by Pomar and Marcoux (2005); Theil (1966), and Bibby and Toutenburg (1977). The ECT indicates the extent to which the average of the predicted dependent variables deviates from the average of the observed values. The ER measures the deviation of the least squares regression coefficient from 1, which is the value obtained if the observed and simulated results were in complete agreement. The ED is the variation in the observed values that are not accounted for by least squares regression of the observed on predicted values. This error is the unexplained variance, and it represents the portion of MSPE that cannot be eliminated by linear transformation (Theil, 1966). In addition, relative MSPE (rMSPE), expressed as a percentage of the observed mean, was used as a measure of the prediction error.

## 3 Results

### 3.1 Databases description

All the birds in the selected publications were male. The origin of the articles in order of importance is Australia (34%, 42%), Europe (24%, 17%), Asia (21%, 17%), North America (14%, 17%),

South America (3%, 0%), and the Middle East (3%, 8%) in the GEN-database and AACON-database, respectively. The differences between the two databases were less than 5% for average age, final age, CP content of control feed, AMEn content, and standardized ileal digestible (SID) Lys level (Table 1). The maximum CP reduction reached was 4.9% in the GEN-database and 3.2% in the AACON-database. The levels of AA : Lys were in average 5% lower in the GEN-database than in the AACON-database, with the largest differences being 9% for Gly + Ser : Lys, His : Lys, and Phe + Tyr : Lys (Supplementary Table 1).

### 3.2 Effects of lowering crude protein on diet composition

In the GEN-database, the decrease in CP content was systematically achieved by a decrease in dietary SBM content (g/kg;  $SBM = -487.0 + 38.5 \times CP$ ;  $P < 0.001$ ;  $R^2 = 95\%$ ;  $rMSE = 23.9$ ). Decreasing dietary CP content by one percentage point led to a decrease in SBM content of 39 g/kg (i.e., a decrease of 19%). As a consequence, because SBM was approximately 6.6 times richer in potassium (K) than corn (INRAE-CIRAD-AFZ, 2018), the decrease in SBM content led to a decrease in dietary K content ( $K = 4.53 + 0.013 \times SBM$ ;  $P < 0.001$ ;  $R^2 = 87\%$ ;  $rMSE = 0.587$ ; i.e., a 7% decrease for a one percentage point CP reduction in feed). This

resulted in the decrease of dietary electrolyte balance (DEB' calculated using  $K^+$ ,  $Na^+$ , and  $Cl^-$  contents; Mongin, 1981) by 19.5 mEq/kg for the same CP reduction. Simultaneously, the cereal content increased by 32 g/kg in response to the reduction of SBM incorporation. Dietary starch content (g/kg) was correlated with cereal content ( $R^2 = 63\%$ ;  $P < 0.001$ ). Therefore, starch content increased by 5% for a dietary CP decrease of one percentage point (starch =  $81.49 - 2.09 \times CP$ ;  $P < 0.001$ ;  $R^2 = 93\%$ ; rMSE = 1.67). Corn was the most used cereal, but 24 trials were conducted on a wheat basis without corn. For a one percentage point CP decrease, the synthetic AA content of the feed was increased by 5.2 g/kg (i.e., an increase of 28%).

### 3.3 Effect of lowering crude protein on animal performance

In the GEN-database, the decrease in dietary CP content led to a linear ( $P < 0.001$ ) and quadratic ( $P < 0.001$ ) decrease in average daily feed intake (ADFI) and ADG (Figure 1; Table 2). In contrast, in the AACON-database, the dietary CP content did not affect ADFI and ADG.

These changes in animal performance in the GEN-database led to a linear increase ( $P < 0.001$ ) in the feed conversion ratio (FCR) (g/g) by 1.9% for a CP decrease of one percentage point. However, when the levels of digestible AA were controlled (AACON-database), this increase in FCR was lower (+1.2%). Lowering dietary CP content led to a linear increase in fat deposition in the form of abdominal deposition (Table 2) of 8.8% for a CP decrease of one percentage point in the GEN-database; in the AACON-database, based on 12 trials, lowering the CP content did not affect fat deposition ( $P = 0.152$ ).

### 3.4 Validation of daily nitrogen retention recalculation

The daily N retained was predicted with good accuracy with the rMSPE (4%), mostly because of the disturbance (86%), followed by a central tendency error of 12% and a regression error of 2% (Figure 2). For a one percentage point reduction of dietary CP, the daily recalculated values for N intake, N retention, and N excretion were decreased by 4%, 2%, and 11%, respectively, in the GEN-database ( $P < 0.001$ ; Table 2). In the AACON-database, a similar decrease in CP content decreased daily N intake by 6% ( $P < 0.001$ ; Table 2), did not affect N retention, and decreased daily N excretion by 14% ( $P < 0.001$ ). In both databases, N retention efficiency was increased by approximately 4% ( $P < 0.001$ ).

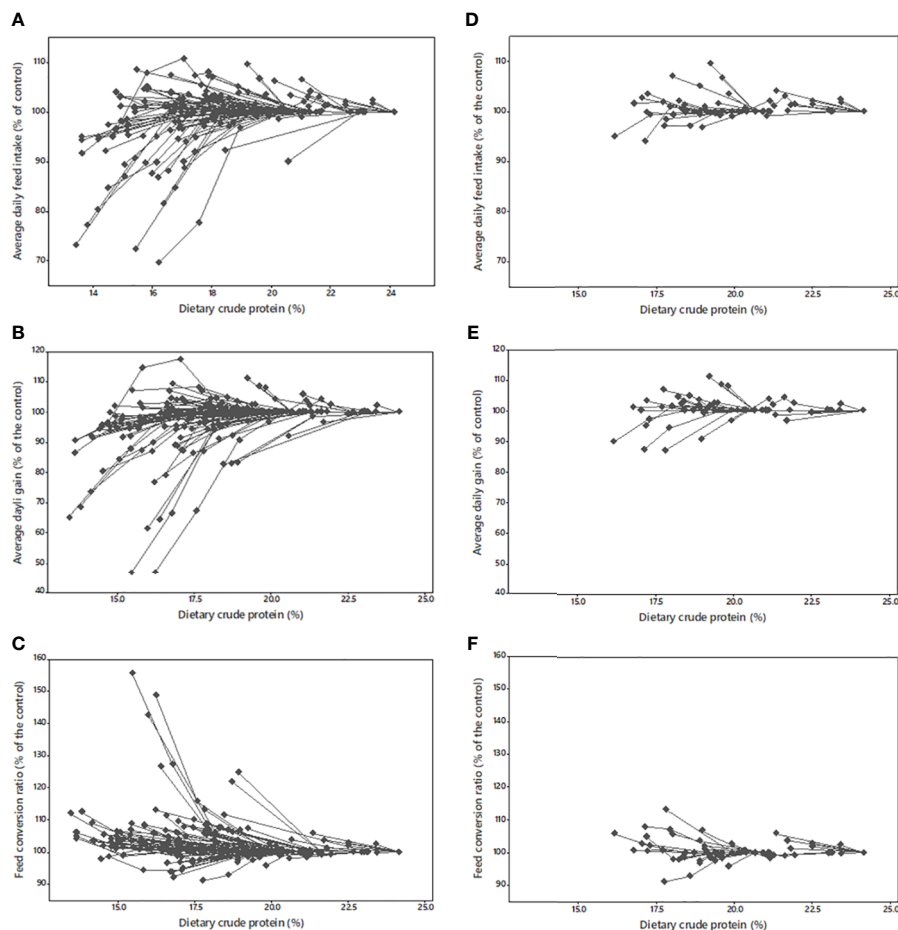
## 4 Discussion

The effect of reducing CP on diet composition agreed with the results of the meta-analysis by Alfonso-Avila et al. (2022) focus on the N utilization, litter quality, and water consumption, which also showed that a CP reduction of 1% reduced SBM by 25 g/kg, dietary

K content by 0.47 g/kg, and the DEB by 19.3 mEq/kg. In comparison, in the GEN-database, the SBM, dietary K, and DEB were reduced by 39 g/kg, 0.50 g/kg, and 19.5 mEq/kg, respectively. The higher decrease in SBM could be explained by a higher usage of free AAs. Despite a more rapid SBM reduction, the equivalent decrease in K and DEB can be explained by the use of K carbonate in some trials. The lowest K value was 3.3 g/kg, while requirements range from 6.3 g/kg to 7.1 g/kg (Oliveira et al., 2005). Very few trials have tested the impact of restoring K content in low-CP diets, except Lambert et al. (2023), who showed that K supplementation to restore DEB in low-CP diets improved growth performance. The fact that decreasing K decreases water consumption is an opportunity to improve litter quality. However, little research is available on the K requirements of chickens. It will therefore be important to determine the level of K to adopt in the LCP strategy, which will maximize litter quality without degrading growth performance.

In the GEN-database, decreasing CP induced a slight increase in ADFI and ADG until they reached a maximum (CP = 20.4% and 21.0%, respectively), after which performance decreased with decreasing CP. For example, reducing CP from 24% to 23% increased ADFI by 1.4%, whereas reducing CP from 19% to 18% reduced ADFI by 0.8%. This quadratic response is consistent with Alfonso-Avila et al. (2022), which did not use selection rules based on the AA level. It is interesting to note that approximately 56% of the trials showed an increase in ADFI, while 26% of the trials showed a concomitant increase in ADG, and 14% showed a positive effect on all growth parameters. In the AACON-database, ADFI and ADG were not impacted by CP levels, indicating that the effect observed in the GEN-database can be linked to a deficiency in AAs. According to the most accepted hypotheses on feed intake regulation, chickens consume to meet their most limiting nutrient (Emmans, 1987) but, in practice, multiple factors may limit the animal from reaching their desired feed intake, such as the bulkiness of the feed (Taylor and Kyriazakis, 2021). The decrease in CP caused a reduction in crude fiber content (Alfonso-Avila et al., 2022), and, therefore, in water-holding capacity, so bulk capacity does not seem to explain the decrease in feed intake in this case. The energy balance, in particular fat deposition, is also reported to drive feed intake (Gous et al., 1990). Trials were all formulated to be iso-AMEn; however, recent results have shown that energy digestibility increased with decreasing CP (Chrystal et al., 2020a; Maynard et al., 2021). It is therefore possible that this increase in AMEn coupled with the increase in fat deposition caused the decreased ADFI. Moreover, intake is regulated both in the short term (e.g., hormonal regulation) and long term (e.g., energetic balance; Bouvarel et al., 2010). For example, as a long-term regulation, the decreased intake observed in the GEN-database could be partly linked to the reduced growth performance, because feed intake is lower in smaller birds.

The current results show that maintaining the levels of Met + Cys, Thr, Trp, Arg, Val, Ile, His, Gly + Ser, and Phe + Tyr in the right ratio to Lys allowed a decrease in CP content without impairing animal performance. Therefore, in the AACON-database, dietary CP could be decreased to 16% without degrading growth performance. In addition, in some trials not



**FIGURE 1** Broiler within experiment responses of average daily feed intake, average daily gain, and feed conversion ratio using the general database (A–C) and the AACON-database where amino acid levels are controlled (D–F).

**TABLE 2** Effect of crude protein (CP, in %) on animal performance, fat deposition, and nitrogen balance in the general database (GEN-database) and in the AACON-database where amino acid levels are controlled.

	Regression equation	P-value			R <sup>2</sup>	RMSE
		Intercept	CP	CP <sup>2</sup>		
<b>GEN-database</b>						
ADFI (g/d)	1.20 + 12.34 × CP – 0.312 CP <sup>2</sup>	NS	< 0.001	< 0.001	99.3	5.37
ADG (g/d)	–49.30 + 12.53 × CP – 0.30 CP <sup>2</sup>	< 0.05	< 0.001	< 0.001	97.46	5.27
FCR (g/g)	2.05 – 0.03 × CP	< 0.001	< 0.001	NS	90.33	0.09
Abdominal fat pad (% of control)	303.70 – 10.33 × CP	< 0.001	< 0.001		77.38	13.46
N intake (g/d)	–6.06 + 0.84 × CP – 0.02 × CP <sup>2</sup>	< 0.001	< 0.001	< 0.001	99.05	0.18
N retention (g/d)	–1.43 + 0.36 × CP – 0.01 × CP <sup>2</sup>	< 0.05	< 0.001	< 0.001	97.42	0.15
N excretion (g/d)	–5.32 + 0.56 × CP – 0.01 × CP <sup>2</sup>	< 0.001	< 0.001	< 0.001	97.5	0.16
N retention efficiency (%)	172.90 – 9.04 × CP + 0.18 × CP <sup>2</sup>	< 0.001	< 0.001	< 0.001	95.03	3.04
<b>AACON-database</b>						
ADFI (g/d)		< 0.05	NS	NS	99.9	2.81

(Continued)

TABLE 2 Continued

	Regression equation	P-value			R <sup>2</sup>	RMSE
		Intercept	CP	CP <sup>2</sup>		
ADG (g/d)		NS	NS	NS	99.5	3.00
FCR (g/g)	$1.91 - 0.02 \times \text{CP}$	< 0.001	< 0.001		98.2	0.04
Abdominal fat pad (% of control)		NS	NS	NS	11.7	14.3
N intake (g/d)	$-8.04 + 1.04 \times \text{CP} - 0.02 \times \text{CP}^2$	< 0.001	< 0.001	< 0.001	99.85	0.091
N retention (g/d)		NS	NS	NS	99.45	0.09
N excretion (g/d)	$-9.05 + 0.92 \times \text{CP} - 0.02 \times \text{CP}^2$	< 0.001	< 0.001	< 0.001	99.31	0.10
N retention efficiency (%)	$107.89 - 2.36 \times \text{CP}$	< 0.001	< 0.001		97.3	1.38

ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio; N, nitrogen; NS, not significant.

included in the AACON-database because they did not control all of the essential AAs, a decrease in CP did not decrease growth performance (Figure 1). This shows that there is still work to be done to fully understand the success factors of CP reduction. The decrease in CP content also leads to the emergence of interest in Gly, His, and Phe + Tyr requirements that are usually not considered in feed formulation for broilers. For example, out of the 166 low-protein diets in the GEN-database, supplementation of Gly was done in 81 treatments, while 16 were supplemented with L-His and 34 with L-Phe. An increase in the FCR was observed in both databases when the CP was decreased. Degradation of the FCR was 37% lower in the CON-AA than in the GEN-database. Therefore, controlling AAs is crucial for successful results with decreased CP.

The data in this meta-analysis confirmed that abdominal fat is increased when CP content is decreased, as previously observed (Belloir et al., 2017; Chrystal et al., 2020b; Cowieson et al., 2020). Abdominal fat represents approximately 20% of total fat (Tůmová and Teimouri, 2010) and is correlated with carcass fat (Hood, 1982; Namroud et al., 2008). Because chicken fat has a low economic value, this fat deposition should be avoided (Fouad and El-Senousey, 2014). Our data indicate that fat deposition is lower

when AAs are controlled (Supplementary Figure 1). Therefore, using AA-to-Lys ratios corresponding to the ideal protein profile limits fat deposition; however, this phenomenon still occurs, indicating that the effect of CP on fat deposition is not solely related to AA limitations. This is consistent with the results from Chendrimada et al. (2007), which showed the inhibitory effect of protein with non-essential AA (i.e., Ala and Glu) and mineral N, on the malic enzyme involved in fat deposition.

Our results confirmed that an average of 29 g N/kg BW gain can be used to recalculate the N balance. A single value for the N content of the gain can be used because the water and fat contents are negatively correlated (Liu et al., 2017; Caldas et al., 2019). The decrease in the N content of the gain that should have been caused by the increase in fat is compensated by a decrease in water content. When all essential AAs are controlled, lowering dietary CP content reduces daily N excretion by 14% and increases N retention efficiency by four percentage points for a CP decrease of between 190 g/kg and 180 g/kg ( $P < 0.001$ ; Table 2). This decreased excretion is consistent with the 10% value reported by Méda et al. (2011). The combination of reduced N excretion and decreased volatility of the N excreted has been shown to lead to a synergetic decrease in total

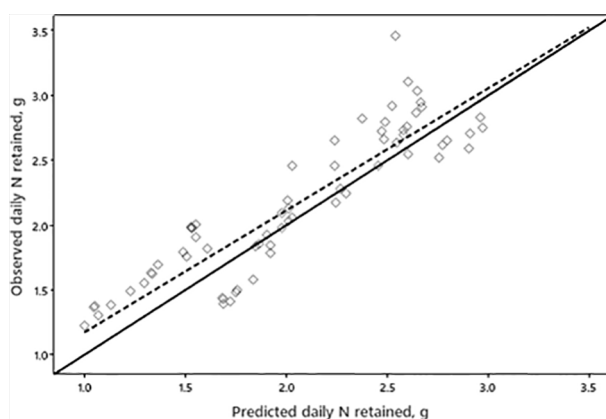


FIGURE 2

The relationship between observed and predicted daily N retained, no. of observations = 81, relative mean square prediction error = 4%, error in central tendency = 12%, error due to regression = 2%, and error due to disturbance = 86%,  $x = y$  line (—) and regression line (---).

N volatilization in a broiler house (Belloir et al., 2017). To further assess the environmental effects, emissions of nitrogenous gases, such as NH<sub>3</sub> and N<sub>2</sub>O, should be studied to determine how they are specifically impacted by the reduction in dietary CP.

In conclusion, a decrease in dietary CP content, without impairing animal performance, is possible when the levels of indispensable AA are precisely controlled. This strategy can also reduce the need for imported SBM and limit N emissions.

## Data availability statement

The raw data supporting the conclusions of this article will be made available, on demand, by the authors, without undue reservation.

## Ethics statement

The animal study was reviewed and approved by the Comité de protection des animaux de l'Université Laval (CPAUL) and the Comité de protection des animaux du Center de recherche en sciences animales de Deschambault (CPA-CRSAD).

## Author contributions

TR built the database, performed the graphical and statistical analysis, and wrote the first draft of the manuscript. M-PL-M was the PI of the project. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

Authors SF and WL were employed by company METEX Animal Nutrition.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fanim.2023.1214076/full#supplementary-material>

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