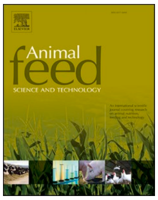
Animal Feed Science and Technology 297 (2023) 115571

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci



Reducing dietary crude protein in broiler diets positively affects litter quality without compromising growth performance whereas a reduction in dietary electrolyte balance further improves litter quality but worsens feed efficiency

William Lambert[a,*], Julio Diaz Berrocoso[b], Bart Swart[b], Marije van Tol[c], Erik Bruininx[d], Els Willems[b]

[a] METEX NØØVISTAGO, 32 rue Guersant, 75017 Paris, France
[b] Agrifirm, Landgoedlaan 20, 7325 AW Apeldoorn, the Netherlands
[c] ORFFA, Minervum 7032, 4817 ZL Breda, the Netherlands
[d] Animal Nutrition Group, Wageningen University & Research, Zodiac building, De Elst 1, 6708 WD, Wageningen, the Netherlands

ARTICLE INFO

Keywords:
Broilers
Low protein diets
Dietary electrolyte balance
Performance
Welfare
Amino acids

ABSTRACT

Reducing crude protein (**CP**) in broiler feed is a nutritional strategy that allows to partially reduce dietary soybean meal (**SBM**) inclusions to reduce feed costs, with proven benefits. In addition, reduction of dietary CP leads to a lower dietary electrolyte balance (**dEB**) due to the concomitant reduction of potassium (**K**). The aim of the present study is therefore two-fold: 1) to evaluate the potential of reduction of dietary CP until performance drops (1, 2 or 3 % points of reduction) and 2) to combine it with a reduction of dEB (by reducing dietary K) to validate the benefit of reduced dEB/K on litter quality without compromising performance. In total, 9100 male Ross 308 chicks were randomly allotted to 35 pens of 260 broilers. They were fed 5 dietary treatments from 10 to 20d and 20–30d.There was a "positive control" diet (CONTROL; 20.7 % CP in grower, 19.5 % CP in finisher) and 3 levels of dietary CP reduction by 1 % (CP-1 %), 2% (CP-2 %) and 3 % (CP-3 %), all diets formulated to respect at least the ideal AA profile for indispensable AA. In addition, an extra diet called "negative control" (NC CP-3 %) was formulated to have the same ingredient composition as the CP-3 % diet but with lower K and dEB level. Reducing dietary CP as low as 3 % points and maintaining AA and dEB levels resulted in similar or better production performance from 10 to 30d for weight gain, feed intake, feed conversion ratio (FCR) and carcass traits, reduced daily water intake and water-to-feed ratio, excreta N and moisture as well as lower litter moisture and footpad lesions (**FPL**). Reducing dEB level at − 3 % CP (negative control) increased feed intake and FCR but reduced water intake, water-to-feed ratio, and FPL. It is therefore possible to feed broilers with lower CP diets without compromising performance, with clear benefit on litter quality. As reducing dietary CP is often associated to reduced dietary K, due to lower SBM inclusion, the extent of reduction of K and the control of dEB by Na, Cl and K need to

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Abbreviations: AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; CP, crude protein; dEB, dietary electrolyte balance; FCR, feed conversion rate; FPS, footpad score; FPL, foot pad lesions; K, potassium; NH3, ammonia; N2O, nitrous dioxide; N, nitrogen; SBM, soybean meal; WFR, water to feed ratio.

[*] Corresponding author.

E-mail address: William.lambert@metex-noovistago.com (W. Lambert).

https://doi.org/10.1016/j.anifeedsci.2023.115571
Received 17 October 2022; Received in revised form 28 December 2022; Accepted 10 January 2023
Available online 13 January 2023

be further explored, especially on litter quality, to facilitate the adoption of low protein diets in practice.

1. Introduction

Implementation of reduced dietary crude protein (**CP**) levels in poultry production offers several advantages in terms of environmental impact and animal welfare. Firstly, by reducing nitrogen (**N**) intake of broilers while maintaining N retention, this strategy supports increased nitrogen retention and redcud N excretion (Bregendahl et al., 2002; Nagaraj et al., 2007; Belloir et al., 2017). According to Belloir et al. (2017) and Méda et al. (2019), there is also potential for reduced CP diets to reduce the volatilization of litter N into gaseous emissions such as ammonia (**NH_3**) and nitrous oxide (**N_2O**). Secondly, excessive levels of CP in the diets of broiler chickens enforce the inclusion of soybean meal (**SBM**), often linked to land use change and therefore global warming potential (Castanheira and Freire, 2013; Da Silva et al., 2010). Reducing dietary CP therefore reduces global warming potential of chicken meat production as demonstrated by Mosnier et al. (2011), Kebreab et al. (2016) and as reviewed by Cappelaere et al. (2021a). Third, it usually results in reduced water intake as demonstrated by Alleman and Leclercq (1997) due to a lower dietary potassium (**K**) and a reduced need to produce uric acid which is a water consuming process. In turn, this reduces litter humidity and when combined with reduced N in the litter, it results in a lower incidence and severity of FPL (Lemme et al., 2019; van Harn et al., 2019).

However, the effect of reduced-CP diets on broiler performance and carcass characteristics is still submitted to controverse as reviewed by Selle et al. (2020) and Hilliar and Swick (2019). Recently, van Harn et al. (2019), demonstrated that reducing dietary CP by 3 %pts from 20.8 % to 17.8 % (11–28d) in grower and from 19.8 % to 16.8 % in finisher (28–35d) phase had no negative effect on growth performance and even a positive effect on feed conversion ratio (**FCR**) was observed. The reduction of dietary CP was formulated to gradually replace SBM and soybean oil by cereals (wheat) and feed-grade Amino Acids (**AA**), down to the use of L-Val, L-Arg, L-Ile, L-Trp and Gly. In a similar study from Maynard et al. (2021), dietary CP could even be reduced further by 4 %pts with the aid of additional L-His, L-Leu and L-Ser. However, Chrystal et al. (2020) found that the reduction of dietary CP beyond 3 %pts led to significant increase in FCR (+9 %), despite the use of a large portfolio of L-AA. On top of a diversity of outcomes, the reduction of dietary CP in the studies did not enable the full removal of SBM from diets with reductions of SBM ranging from 41 % to 72 %, depending on the studies.

In addition, when gradually reducing dietary CP through SBM reduction, a K-rich feedstuff is decreasing, leading to a decrease in dietary electrolyte balance (**dEB**) if not controlled. Borges et al. (2011) tested a reduction of dEB from 240 to 140 mEq/kg which was shown to induce a reduction in body weight of 21d broilers. Therefore, emphasis should be put on the evaluation of optimal dEB requirements as suggested by Kleyn and Ciacciariello (2021) and Mushtaq et al. (2013). To our knowledge, only Chrystal et al. (2020) tested a lower level of dEB (120 vs 230 mEq/kg) in the context of a low CP diet. They found that low levels of dEB did result in a significant reduction of the ileal digestibility of 9 AA out of the 16 tested. In Chrystal et al. (2020), they made the choice to reduce dEB by increasing dietary Cl content whereas in practice, the reduction of dietary CP would mostly be achieved by a reduction of dietary SBM and therefore of dietary K. According to the meta-analysis of Alfonso-Avila et al. (2022), the dietary K content is decreased on average by 0.12 % pts for 1 %pt of dietary CP reduction (as low as 0.5 %), also leading to an overall decrease of dEB when broilers are fed low protein diets and indicating that the careful control of this nutrient is not implemented in the majority of low dietary CP published experiments.

The aim of the present study is therefore two-fold: 1) to evaluate the potential of reduction of dietary CP without compromising broiler performance together with a complete removal of dietary SBM and 2) to combine a reduction of dietary CP with a reduction of dEB by reducing dietary K content.

2. Materials and methods

This experiment was performed in accordance with the Dutch rules and regulations and approved by the Ethics Committee of Agrifirm, the Netherlands.

2.1. Animal procedure

In total, 9100 one-day-old male Ross 308 birds originating from a 44-week-old parent flock were obtained from a commercial hatchery and were housed in a semi-commercial broiler shed in the Netherlands. Birds were randomly allotted in groups of 260 into 35 pens (4.45 m x 3.14 m effective floor surface). The floor was bedded with wood shavings (0.9 – 1.0 kg/m2). During the first two days of age, light was provided 24 h/d). From day 3 to day 30 of age, a day/night schedule of 18 h light and 6 h dark (18 L:6D) was applied. The room temperature was set at approx. 35 °C at the day of arrival and thereafter gradually decreased with 1 degree Celsius to 18–20 °C during the last production days. Feed and water were administered ad libitum. Broilers were vaccinated for Infectious Bronchitis (at hatchery), Newcastle Disease (at 14d) and Infectious Bursal Disease (at 16d).

2.2. Experimental diets

There were 4-feeding phases: starter (0–5d), transition (5–10d), grower (10 to 20d) and finisher (20 to 30d). All broilers received the same starter and transition diets, and from day 10, 5 experimental diets were tested (Tables 1 & 2). The starter and transition phase had the same ingredient composition but were offered as crumble (2,4 mm) and as pellet (2,4 mm) in starter and transition phase, respectively. Among the 5 experimental diets, there was a "positive control" diet (CONTROL) and 3 levels of dietary CP reduction by 1 % (CP-1 %), 2 % (CP-2 %) and 3 % (CP-3 %). In order to reduce dietary CP, SBM was gradually replaced with wheat and potato protein concentrate and feed grade L-AA (L-Lys, L-Thr, DL-Met, L-Val, L-Ile, L-Arg, Gly, L-Trp, L-Leu & L-His) were used to maintain dietary digestible AA at least at the level of the requirement. Potassium carbonate was used to match the K and the dEB at the level of the control. In addition, a fifth diet called "negative control" (NC CP-3 %) was formulated to have the same ingredient composition as the CP-3 % diet but with lower K and dEB level (potassium carbonate was not used in this diet). All diets per feeding phase were formulated according to CVB (2018), to have the same level of digestible Lys (10.5 % and 9.5 % in grower and finisher diets, respectively) and to be fed iso-caloric diets (12.4 MJ/kg and 12.7 MJ/kg in grower and finisher diets, respectively). In addition, the CP-3 % and NC CP-3 % were free of SBM in both feeding phases.

Raw materials (before formulating the diets) and the experimental diets were analyzed for moisture, CP, crude fat, ash, crude fiber, starch, K, Ca, P, Na, K and Cl by Nutricontrol (Veghel, the Netherlands). Dry matter content was gravimetric determined by drying at a constant weight at 103 °C according to ISO 6496 (1998). Following drying, samples were ground to pass a 1 mm screen and kept for further analysis. Kjeldahl nitrogen content was measured using $CuSO_4$ as catalyst according to ISO 5983 (1997) in fresh samples. Crude protein content was calculated as N * 6.25. Crude fat content was determined gravimetric after extraction with light petroleum (boiling point 40–60°C) according to ISO 6492 (1999). Ash was gravimetric determined by incineration at a constant weight at 550 °C according to ISO 5984 (2002). Crude fiber was gravimetric determined as the remaining insoluble organic fraction after acid and alkaline digestion of the sample according to revised ISO 6865 (1988). Starch was analyzed using the polarimetric method according to ISO

Table 1

Ingredient and calculated & analysed nutrient composition of the five experimental grower diets fed from 10–20d of age to broiler chickens.

	CONTROL	CP-1%	CP-2%	CP-3%	NC[a] CP-3%
Ingredient composition, g/kg					
Wheat	328	384	441	497	506
Soybean Meal, 49 %	222	149	73	-	-
Maize	200	200	200	200	200
Oats	75	75	75	75	75
Rapeseed Expeller	50	50	50	50	50
Potato protein concentrate	-	17	34	50	50
Sunflower meal 36 %	30	30	30	30	30
Poultry fat	28	24	21	17	17
Soybean oil	24	19	15	10	10
Monocalcium phosphate	8.0	8.7	9.5	10.2	10.2
Chalk	10.9	11.0	11.1	11.2	11.2
Salt	1.4	0.9	0.5	-	-
Sodium bicarbonate	2.8	3.4	4.1	4.8	4.8
Potassium carbonate	-	2.2	4.4	6.6	-
Premix and enzymes[b]	14.8	14.8	14.8	14.8	14.8
DL-Methionine 98 %	2.22	2.21	2.21	2.20	2.20
L-Threonine 98 %	0.73	1.22	1.71	2.20	2.20
L-Lysine 78 %	2.18	3.40	4.67	5.89	5.89
L-Valine 96 %	-	0.13	0.26	0.38	0.38
L-Tryptophan 98 %	-	0.14	0.28	0.42	0.42
L- Isoleucine 92 %	-	0.59	1.21	1.80	1.80
L-Arginine 98 %	-	1.42	2.87	4.29	4.29
Glycine 98 %	-	1.00	2.03	3.03	3.03
L- Leucine 98 %	-	0.11	0.21	0.32	0.32
L-Histidine 98 %	-	0.21	0.44	0.65	0.65
Formulated nutrient composition, g/kg[c]					
AMEn (MJ/kg)	12.4	12.4	12.4	12.4	12.4
Crude protein	207 (208)	197 (201)	187 (191)	177 (183)	177 (181)
Digestible Lysine	10.5	10.5	10.5	10.5	10.5
Crude Fat	76 (76)	68 (66)	60 (61)	51 (53)	51 (54)
Crude fiber	42 (33)	41 (29)	40 (30)	39 (28)	39 (30)
Starch	379 (408)	412 (440)	445 (458)	478 (487)	478 (462)
dEB[d] (mEq/kg)	226	226	226	226	122
Ca	6.9 (7.9)	6.9 (7.6)	6.9 (7.5)	6.9 (8.3)	6.9 (8.2)
P	6.1 (6.4)	6.0 (6.1)	6.0 (6.1)	5.9 (6.2)	5.9 (6.1)
K	8.5 (8.6)	8.5 (8.6)	8.5 (8.6)	8.5 (8.3)	4.5 (5.4)
Na	1.4 (1.5)	1.4 (1.5)	1.4 (1.5)	1.4 (1.6)	1.4 (1.4)
Cl	1.9 (2.1)	1.9 (2.0)	1.9 (2.0)	1.9 (2.0)	1.9 (2.0)

[a] NC = negative control (low in dEB).

[b] Provided the following (per kilogram of diet): Premix, 5,0 gr; Maxiban 0.5 %, 5.0 gr, EC-Phytase, 3.3 gr; coated calciumbutyrate, 1.0 gr; Choline chloride, 0.3 gr; Belfeed B1100ML, 0.2 gr.

[c] Values into brackets refer to the analyzed nutrient composition.

[d] Dietary Electrolyte Balance.

Table 2
Ingredient and calculated and analysed nutrient composition of the five experimental finisher diets fed from 20–30d of age to broiler chickens.

	CONTROL	CP-1 %	CP-2 %	CP-3 %	NC[a] CP-3 %
Ingredient composition, g/kg					
Wheat	351	404	460	514	520
Soybean Meal, 49 %	198	133	65	-	-
Maize	200	200	200	200	200
Oats	75	75	75	75	75
Rapeseed Expeller	50	50	50	50	50
Potato protein concentrate	-	12	24	36	36
Sunflower meal 36 %	30	30	30	30	30
Poultry fat	29	26	22	19	19
Soybean oil	27	22	17	13	13
Monocalcium phosphate	4.9	5.5	6.2	6.8	6.8
Chalk	12.5	12.4	12.4	12.4	12.4
Salt	1.5	1.0	0.5	-	-
Sodium bicarbonate	2.6	3.4	4.1	4.9	4.9
Potassium carbonate	-	1.9	4.0	5.9	-
Premix and enzymes[b]	14.8	14.8	14.8	14.8	14.8
DL-Methionine 98 %	1.72	1.97	2.23	2.48	2.48
L-Threonine 98 %	0.51	1.05	1.61	2.15	2.15
L-Lysine 78 %	1.83	3.05	4.31	5.53	5.53
L-Valine 96 %	-	0.12	0.25	0.38	0.38
L-Tryptophan 98 %	-	0.12	0.24	0.36	0.36
L- Isoleucine 92 %	-	0.58	1.18	1.76	1.76
L-Arginine 98 %	-	1.19	2.43	3.62	3.62
Glycine 98 %	-	0.80	1.63	2.43	2.43
L- Leucine 98 %	-	0.12	0.24	0.36	0.36
L-Histidine 98 %	-	0.17	0.34	0.50	0.50
Expected nutrient composition, g/kg[c]					
AMEn[d] (MJ/kg)	12.7	12.7	12.7	12.7	12.7
Crude protein	195 (198)	185 (190)	175 (176)	165 (169)	165 (166)
Digestible Lysine	9.5	9.5	9.5	9.5	9.5
Crude Fat	80 (81)	72 (72)	64 (64)	56 (61)	56 (56)
Crude fiber	42 (32)	41 (34)	40 (30)	39 (27)	39 (32)
Starch	396 (388)	426 (416)	457 (457)	487 (491)	487 (477)
dEB[d] (mEq/kg)	211	211	211	211	122
Ca	6.8 (7.6)	6.8 (7.4)	6.8 (7.3)	6.8 (7.9)	6.8 (7.2)
P	5.3 (5.4)	5.2 (5.2)	5.2 (5.2)	5.3 (5.2)	5.4 (5.1)
K	7.9 (8.2)	7.9 (8.1)	7.9 (7.8)	7.9 (7.8)	4.4 (4.5)
Na	1.4 (1.3)	1.4 (1.4)	1.4 (1.4)	1.4 (1.5)	1.4 (1.5)
Cl	1.9 (1.8)	1.9 (1.9)	1.8 (1.9)	1.8 (2.0)	1.8 (1.9)

[a] NC = negative control (low in dEB).

[b] Provided the following (per kilogram of diet): Premix, 5,0 gr; Maxiban 0.5 %, 5.0 gr, EC-Phytase, 3.3 gr; coated calciumbutyrate, 1.0 gr; Choline chloride, 0.3 gr; Belfeed B1100ML, 0.2 gr.

[c] Values into brackets refer to the analyzed nutrient composition.

[d] Dietary Electrolyte Balance.

6493 (2000). Mineral content was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) according to ISO method 11885 (1998).

In addition, the AA and CP content of the raw materials (before formulating the diets) and experimental diets were analyzed by the METEX NØØVISTAGO Customer Laboratory. Total AA and free (added) L-AA analysis followed the EN ISO 13903 procedure except for Trp which followed the Adapted procedure from abrogated standard AFNOR XP V18–114.

2.3. Measurements

Body weight (**BW**) was determined per pen and was measured at d0, d10, d20 and d30 of age. Feed intake per pen was determined at day 10, 20 and 30 days of age. Average daily gain (**ADG**), average daily feed intake (**ADFI**) and FCR were calculated per pen for the following periods: 10–20, 20–30, and 10–30 days of age. Water intake was recorded per pen and water to feed ratio (**WFR**) was calculated at the end of each feeding phase (at days 10, 20, and 30).

At the end of the experiment (d30), 10 birds per pen were selected on BW (with a BW within the range of ± 40 g from the average of the pen) and taken to the commercial slaughterhouse. The experimental unit for carcass measurements was a pooled sample of 10 birds per pen. At the slaughterhouse, the birds were cut up by hand by trained personnel to determine carcass, wing, leg (thigh + drums), and breast meat weight. All yield parameters were expressed as a percentage of carcass weight.

Nitrogen retention was calculated by multiplying broiler body weight gain by a whole-body N content constant of 29 g/kg (Belloir et al., 2017; Equation 2). Thus, N efficiency was calculated per pen according to the following equation:

$$Nitrogenefficiency = \frac{(Nitrogenintake - Nitrogenretention)}{Nitrogenintake} * 100$$

Litter quality was visually scored for all pens at day 10, 13, 16, 20, 23, 27 and 29 d. Scoring is based on the friability and wetness of the litter in each pen on a 1–5 point scale with 1 = dry loose litter, no cakes or wet places; 2 = a single cake in the litter at nipples and feed box, largely dry and loose; 3 = large cakes at nipples and feed box, but loose parts in other places; 4 = most of cage covered with hard cakes and 5 = whole cage consists of soft wet litter, chicks also have dirty legs and feathers.

Litter moisture was determined per pen in a pooled sample collected from 4 different locations in the pen. Litter was collected from the top of the litter all the way until the hard floor. Samples were dried in an oven at 70 °C until constant weight. In addition, at least 6 fresh droppings per pen were collected at day 28. All collected droppings in each pen were pooled into 1 pooled sample. Fecal droppings were analyzed for moisture, CP, N, and uric acid.

The occurrence of foot pad lesions (**FPL**) and their severity (scale 0, 1, or 2,) were determined at 9, 20 and 29 days in 8 birds per pen. FPL were scored per broiler for both feet according to the so-called 'Swedish' classification, i.e., score 0: no lesions or very small discoloration; score 1: discoloration but no deep lesion; score 2: deep lesion with ulcers or scabs, bumble foot (Berg, 1998). The severity of footpad lesions was expressed as footpad score (**FPS**) per pen according to the following equation:

$$FPS = \frac{(100 \times ((0.5 \times numberofbirdsscored1) + (2 \times numberofbirdsscored2)\ )}{totalnumberofscoredbirds}$$

The FPS can therefore range from 0 (all birds having no lesions) to 200 (all birds having score 2).

On day 30, 2 birds per pen were used to collect blood samples. The blood samples were analyzed by Labocea (7 Rue du Sabot, 22440 Ploufragan, France) for total protein, specific proteins (albumin, alpha1, alpha2, beta and gamma), uric acid, and specific energy metabolism indicators (cholesterol and triglycerides). The blood sampling was performed as follows: 4 mL of blood from each animal was collected by venous puncture in the wing and immediately preserved in a 5 mL tube with EDTA-2Na as an anticoagulant. After collection, the tubes were placed on ice and stored at 7°C until further analysis. The blood samples were centrifuged for 15 min at 3000 rpm, 4 ℃, and supernatant as plasma was collected and stored at − 20 ℃.

Table 3

Expected and analyzed amino acid ratio of the five experimental diets in the grower and finisher phase.

	Grower phase (10–20 d of age)					Finisher phase (20–30 d of age)				
	CONTROL	CP-1 %	CP-2 %	CP-3 %	NC[a] CP-3 %	CONTROL	CP-1 %	CP-2 %	CP-3 %	NC[a] CP-3 %
Expected and analyzed FD[b] Lys (g/kg)[c]										
Dig Lys (E)[d]	10.5	10.5	10.5	10.5	10.5	9.5	9.5	9.5	9.5	9.5
Dig Lys (A)[e]	10.1	10.3	10.3	10.5	10.6	9.3	9.5	9.4	9.7	9.5
Expected and analyzed AA ratios to FD Lys (%)										
Thr /Lys (E)	66	66	67	67	67	66	66	67	67	67
Thr /Lys (A)	70	69	69	68	66	67	67	66	66	66
Met /Lys (E)	48	49	50	52	52	48	49	50	52	52
Met /Lys (A)	46	47	47	49	48	42	42	43	44	44
Cys /Lys (E)	26	25	24	22	22	26	25	24	22	22
Cys /Lys (A)	29	27	26	24	26	30	28	27	25	25
Met+Cys/Lys (E)	74	74	74	74	74	74	74	74	74	74
Met+Cys/Lys (A)	74	74	73	73	74	72	71	70	68	68
Trp /Lys (E)	20	19	19	18	18	20	19	19	18	18
Trp /Lys (A)	21	20	20	19	19	22	21	20	19	18
Ile /Lys (E)	69	68	67	67	67	69	68	67	67	67
Ile /Lys (A)	68	69	67	66	67	72	71	68	67	66
Val /Lys (E)	80	80	80	80	80	80	80	80	80	80
Val /Lys (A)	83	81	82	81	80	81	80	78	78	78
Leu /Lys (E)	128	120	112	105	105	128	120	112	105	105
Leu /Lys (A)	127	122	114	108	108	135	125	116	107	107
His /Lys (E)	44	42	39	36	36	46	44	42	36	36
His /Lys (A)	41	39	36	34	35	43	41	38	35	35
Arg /Lys (E)	111	110	109	105	105	114	112	110	105	105
Arg /Lys (A)	103	103	102	99	100	111	106	103	99	99
Gly+Ser/Lys (E)	147	144	142	140	140	153	148	143	140	140
Gly+Ser/Lys (A)	149	149	144	141	143	160	153	147	141	141
Phe+Tyr/Lys (E)	137	127	117	105	105	141	130	118	105	105
Phe+Tyr/Lys (A)	135	130	119	111	112	144	133	120	110	110

[a] NC = negative control (low in dEB).

[b] FD = fecal digestible.

[c] Arg = arginine, His = histidine, Ile = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Thr = threonine, Trp = tryptophan, Val = valine, Cys = cysteine, Gly = glycine, Ser = serine, Tyr = tyrosine.

[d] E = expected ratio.

[e] A = analyzed ratio based on chemically analyzed total amino acid associated to the corresponding AA digestibility coefficient of the feed.

2.4. Statistics

Data were subjected to mixed model analysis, using the lme4 (Bates et al., 2015) package in R (R Core Team, 2020) with the following statistical model:

$$Y_{ij} = \mu + \alpha_i + b_j + \varepsilon_{ij}$$

Where Y_{ij} = dependent variable, μ = overall mean, α_i = fixed treatment effect (i = I, II,., V), b_j = random block effect (j = I, II, III, …, VII), and ε_{ij} = residual error term. Preplanned contrasts were used to determine significant relationships for linear and quadratic effects of CP. In addition, a preplanned contrast was used to evaluate effect of dEB level. Data were analyzed using a group of animals in one pen as the experimental unit. For carcass, wing, leg and breast meat yield, the experimental unit was the pen (with a pooled sample of 10 birds). Litter score data were analyzed as ordinal distributed data, using the same statistical model. For all outcomes, a P-value below 0.05 were considered statistically significant, whereas if P-value is between 0.05 and 0.1, was considered as a trend.

3. Results

3.1. Analytical results of the experimental feeds

As reported in Table 3, analyzed dietary content of indispensable AA (plus Gly and Ser) as ratios to Lysine were in line with the expectations.Results of proximate analysis (CP, fat, starch, K, Na and Cl) were also in line with the formulated levels (Tables 1 and 2).

3.2. Effects on growth performance and water intake

Mortality was low (<2 %) in the experiment and not affected by treatment (data not shown). Overall, the performance of the broilers was good with an average BW of 1864 g (± 11 g) at 30d and a FCR of 1.41 during 0–30 days (± 0.01), close to Aviagen (2019) performance objectives for Ross 308 male broilers.

The impact of reducing dietary CP and dEB on broiler growth performance from 10 to 20d, 20–30d and for the total experimental period (10–30d) is described in Table 4. In the grower phase, reducing dietary CP linearly reduced body weight (*P* = 0.02) and ADG

Table 4

Growth performance, water intake, nitrogen efficiency and European production index of broilers fed experimental diets differing in dietary crude protein and dietary electrolyte balance.

	CONTROL	CP-1 %	CP-2 %	CP-3 %	NC[a] CP-3 %	SE[b]	Linear[c]	Quad[d]	dEB[e]
Dietary CP (%, grower, finisher)	20.7 19.5	19.7, 18.5	18.7, 17.5	17.7, 16.5	17.7, 16.5				
Dietary EB (mEq/kg, grower, finisher)	226, 211	226, 211	226, 211	226, 211	122, 122				
Grower phase (10–20d)[f]									
Body weight D10 (g/bird)	338	337	339	341	342	1.72	0.16	0.26	0.70
Body weight D20 (g/bird)	994	982	974	978	990	5.95	0.02	0.14	0.10
ADG (g/bird/day)	65.0	64.5	63.4	63.5^{b}	64.8^{a}	0.52	0.01	0.49	0.05
ADFI (g/bird/day)	89.1	88.9	90.5	90.5^{b}	92.0^{a}	0.53	<0.001	0.85	0.01
FCR	1.38	1.37	1.43	1.42	1.42	0.01	<0.001	0.95	0.74
Water intake (mL/bird/day)	164.8	160.4	153.7	147.4^{a}	135.3^{b}	1.05	<0.001	0.28	<0.001
Water to Feed Ratio	1.85	1.79	1.7	1.63^{a}	1.47^{b}	0.01	<0.001	0.35	<0.001
Finisher phase (20–30d)									
Body weight D30 (g/bird)	1848	1887	1867	1862	1856	11	0.68	0.05	0.70
ADG (g/bird/day)	85.2	90.2	89.3	88.3	86.4	1.1	0.10	0.01	0.25
ADFI (g/bird/day)	136.0	138.4	137.4	133.2^{b}	137.7^{a}	0.91	0.02	<0.001	<0.001
Feed conversion ratio	1.60	1.54	1.54	1.51^{b}	1.59^{a}	0.02	<0.001	0.26	<0.001
Water intake (mL/bird/day)	260.0	257.3	246.0	230.2^{a}	204.1^{b}	2.12	<0.001	<0.001	<0.001
Water to Feed Ratio	1.91	1.86	1.79	1.73^{a}	1.48^{b}	0.01	<0.001	0.56	<0.001
Whole experimental period (10–30d)									
ADG (g/bird/day)	75.5	77.5	76.4	76.0	75.7	0.53	0.83	0.03	0.65
ADFI (g/bird/day)	112.5	113.9	113.9	111.8^{b}	114.8^{a}	0.69	0.45	0.01	<0.01
FCR	1.49	1.47	1.49	1.47^{b}	1.52^{a}	0.01	0.10	0.98	<0.01
Water intake (mL/bird/day)	212.6	209.2	200.1	189.1^{a}	170.1^{b}	1.31	<0.01	<0.001	<0.01
Water to Feed Ratio	1.89	1.84	1.76	1.69^{a}	1.48^{b}	0.01	<0.01	0.33	<0.01
Nitrogen efficiency %	58	62	65	70^{a}	67^{b}	0	<0.01	0.09	<0.01
European Production Index (0–30d)	424	445	430	436^{a}	426^{b}	3.68	0.18	0.03	0.04

^{ab}In a row, means assigned different lowercase letters are significantly different, P < 0.05 (Tukey's procedure).

[a] NC = negative control (low in dEB).

[b] SE = Standard error (7 replicates of 260 broilers per pen).

[c] Linear = statistical linear effect of reducing dietary crude protein.

[d] Quad = statistical quadratic effect of reducing dietary crude protein.

[e] dEB = statistical t-test effect comparing CP-3 % and NC CP-3 % to test the effect of reduction of dEB in a low crude protein diet.

[f] ADG = Average daily gain, ADFI = Average daily feed intake, FCR = Feed conversion ratio,

($P = 0.01$) and increased ADFI and FCR ($P < 0.001$). Reducing dEB had a limited effect in the grower period with a trend for a higher ADG ($P = 0.05$) due to a significantly higher ADFI ($P = 0.01$) as compared to CP-3 %. Both water intake and WFR were strongly reduced by the reduction of dietary CP (linear effect; $P < 0.001$) and were further reduced by dEB reduction ($P < 0.001$), as compared to CP-3 %.

In the finisher phase, the impact of dietary CP on growth performance was opposite to the grower phase with a significant linear decrease of ADFI ($P = 0.02$) and FCR (from 1.60 to 1.51; $P < 0.001$) when reducing dietary CP and a quadratic effect on ADG ($P = 0.01$), increasing from CONTROL to CP-1 % and then decreasing again with reduction of dietary CP. The reduction of dEB increased ADFI ($P < 0.001$) and therefore FCR ($P < 0.001$). Water intake and WFR were again strongly reduced by the reduction of dietary CP ($P < 0.001$) and further decreased by dEB reduction ($P < 0.001$).

For the whole experimental period (10–30d), the reduction of dietary CP had limited effect on growth performance and only a quadratic effect was observed on ADG ($P = 0.03$) and ADFI ($P = 0.01$). No effect on FCR was observed ($P = 0.10$). However, the reduction of dEB in the CP-3 % resulted in an increase of ADFI (+3 g/d; $P < 0.01$) and a subsequent increase of FCR (+0.05; $P < 0.01$). Water intake was linearly decreased by dietary CP reduction ($P < 0.01$) from 212.6 in the CONTROL to 189.1 mL/day (−11 % reduction) in the CP-3 % and further reduced by dEB reduction (from 189.1 to 170.1 mL/day, a further drop of 10 %; $P < 0.01$). Water to feed ratio followed the same pattern.

Interestingly, N efficiency was strongly improved by dietary CP reduction from 58 % to 70 % ($P < 0.01$) and compromised when reducing dEB, due to the increase in feed (N) intake ($P < 0.01$).

3.3. *Effects on carcass characteristics*

Besides a trend for a linear effect of reducing dietary CP on carcass yield ($P = 0.07$) and leg yield ($P = 0.06$), neither CP nor dEB had any effect on carcass characteristics ($P > 0.10$), including breast meat yield (Table 5). The reduction of dietary CP tended to increase carcass yield from 59.8 % to 60.7 % and to reduce leg yield from 40.2 % to 39.2 %.

3.4. *Effects on excreta, litter, and welfare indicators*

The influence of dietary CP and dEB on excreta, litter and FPS is displayed in Tables 6 and 7, completed by Fig. 1 for graphical visualization. Reducing dietary CP and dEB had a strong and significant effect on moisture content of both excreta and litter ($P < 0.001$; Table 6). For example, litter moisture was linearly reduced by the reduction of dietary CP from 51.3 % to 36.8 %pts (−28 % reduction) and further reduced by the reduction of dEB from 36.8 % to 22.9 %pts (−38 % reduction). Both N ($P = 0.04$) and uric acid ($P < 0.001$) content in the excreta were linearly decreased by the reduction of dietary CP and the reduction of dEB only influenced the K content, a reduction from 75.9 to 40.9 g/kg dry matter ($P < 0.001$).

Consequently, litter score at d20, d23, d27 and d29 was linearly improved by the reduction of dietary CP ($P < 0.001$) but not by the reduction of dEB (Table 7 and Fig. 1). Regarding FPS, there were almost no FPL at d9 (data not shown), and treatment had no effect. At d20, FPS was numerically reduced when reducing CP ($P = 0.06$; Table 7), whereas at d29, there was a linear effect of reducing dietary CP on FPS ($P < 0.01$). However, no effect of dEB was found on FPS either at d20 or d29.

3.5. *Effects on blood parameters*

Reducing dEB had no effect on any of the blood parameters measured in this experiment ($P > 0.10$; Table 8). Crude protein, albumin, interpheron alpha1, alpha2, beta and gamma were not affected by the reduction of dietary CP ($P > 0.10$). However, uric acid in blood was linearly decreased by the reduction of dietary CP ($P = 0.04$). A trend and a significant effect were observed for the two parameters related to energy metabolism: cholesterol ($P = 0.08$) and triglycerides ($P = 0.02$). For example, reducing CP increased cholesterol and triglycerides content in blood by 8.9 % and 29.6 %, respectively in treatment CP-3 % compared to CONTROL.

Table 5
Carcass and cut-up yields of broilers fed diets differing in dietary crude protein and dietary electrolyte balance.

	CONTROL	CP-1 %	CP-2 %	CP-3 %	NC[a] CP-3 %	SE[b]	Linear[c]	Quad[d]	dEB[e]
Dietary CP (%, grower, finisher)	20.7, 19.5	19.7, 18.5	18.7, 17.5	17.7, 16.5	17.7, 16.5				
Dietary EB (mEq/kg, grower finisher)	226, 211	226, 211	226, 211	226, 211	122, 122				
Carcass weight, kg	1.14	1.16	1.15	1.15	1.13	0.01	0.82	0.55	0.34
Carcass yield, %	59.77	60.54	60.94	60.73	60.50	0.38	0.07	0.21	0.69
Leg yield, %	40.21	39.51	39.20	39.23	40.11	0.38	0.06	0.33	0.11
Breast meat yield, %	31.52	31.68	30.99	31.54	31.34	0.37	0.72	0.60	0.71
Wing yield, %	11.17	10.97	11.29	11.01	11.24	0.12	0.75	0.70	0.15

[a] NC = negative control (low in dEB).
[b] SE = standard error (7 replicates of 10 broilers per pen, hand cut-up at d30).
[c] Linear = statistical linear effect of reducing dietary crude protein.
[d] Quad = statistical quadratic effect of reducing dietary crude protein.
[e] dEB = statistical t-test effect comparing CP-3 % and NC CP-3 % to test the effect of reduction of dEB in a low crude protein diet.

Table 6

Excreta characteristics of broilers fed diets differing in dietary crude protein and dietary electrolyte balance.

	CONTROL	CP-1 %	CP-2 %	CP-3 %	NC[a] CP-3 %	SE[b]	Linear[c]	Quad[d]	dEB[e]
Dietary CP (%, grower, finisher)	20.7, 19.5	19.7, 18.5	18.7, 17.5	17.7, 16.5	17.7, 16.5				
Dietary EB (mEq/kg, grower, finisher)	226, 211	226, 211	226, 211	226, 211	122, 122				
Moisture, %	77.0	76.9	76.3	75.2^{a}	73.0^{b}	0.48	0.01	0.26	<0.001
Dry matter, %	23.0	23.1	23.7	24.8^{b}	27.0^{a}	0.48	0.01	0.26	<0.001
Nitrogen, g/kg dry matter	50.4	52.6	46.5	42.0	37.6	3.30	0.04	0.31	0.35
Uric Acid, g/kg dry matter	50.5	50.5	42.3	38.9	30.6	3.06	<0.001	0.56	0.06
Potassium, g/kg dry matter				75.9^{a}	40.9^{b}	3.43			<0.001

[ab] In a row, means assigned different lowercase letters are significantly different, P < 0.05 (Tukey's procedure).

[a] NC= negative control (low in dEB).

[b] SE=Standard error (7 replicates of at least 6 droppings per pen, pooled in 1 sample, collected at d28 (excreta)).

[c] Linear=statistical linear effect of reducing dietary crude protein.

[d] Quad=statistical quadratic effect of reducing dietary crude protein.

[e] dEB=statistical t-test effect comparing CP-3 % and NC CP-3 % to test the effect of reduction of dEB in a low crude protein diet.

Table 7

Litter score, litter moisture and footpad score of broilers fed diets differing in dietary crude protein and dietary electrolyte balance.

	CONTROL	CP-1 %	CP-2 %	CP-3 %	NC[a] CP-3 %	SE[b]	Linear[c]	Quad[d]	dEB[e]
Dietary CP (%, grower, finisher)	20.7, 19.5	19.7, 18.5	18.7, 17.5	17.7, 16.5	17.7, 16.5				
Dietary EB (mEq/kg, grower, finisher)	226, 211	226, 211	226, 211	226, 211	122, 122				
Litter score D9	2.14	2.29	2.14	2.14	2.29	0.16	0.84	0.66	0.53
Litter score D13	1.86	1.57	1.57	1.57	1.43	0.26	0.33	0.46	0.60
Litter score D16	2.29	2.29	2.00	2.14	2.00	0.13	0.24	0.60	0.45
Litter score D20	2.86	2.57	2.14	2.00	2.00	0.13	<0.001	0.58	1.00
Litter score D23	2.71	2.71	1.86	1.57	1.14	0.22	<0.001	0.52	0.18
Litter score D27	3.57	3.14	1.86	1.29	1.00	0.23	<0.001	0.76	0.38
Litter score D29	3.86	3.14	1.86	1.29	1.14	0.18	<0.001	0.70	0.58
Litter moisture D29, %	51.3	46.7	38.0	36.8^{a}	22.9^{b}	1.49	<0.001	0.15	<0.001
FPS[f] D20	10.7	6.3	3.6	2.7	0.0	3.01	0.06	0.56	0.54
FPS D28	22.3	7.1	5.4	0.0	0.0	4.71	<0.01	0.26	1.00

[ab] In a row, means assigned different lowercase letters are significantly different, P < 0.05 (Tukey's procedure).

[a] NC = negative control (low in dEB).

[b] SE = Standard error (7 replicates of 260 broilers per pen (litter score); or 7 replicates of pooled sample collected at 4 different locations per pen (litter moisture) and 7 replicates of 8 birds scored both feet per pen).

[c] Linear = statistical linear effect of reducing dietary crude protein.

[d] Quad = statistical quadratic effect of reducing dietary crude protein.

[e] dEB = statistical t-test effect comparing CP-3 % and NC CP-3 % to test the effect of reduction of dEB in a low crude protein diet.

[f] FPS = Footpad score.

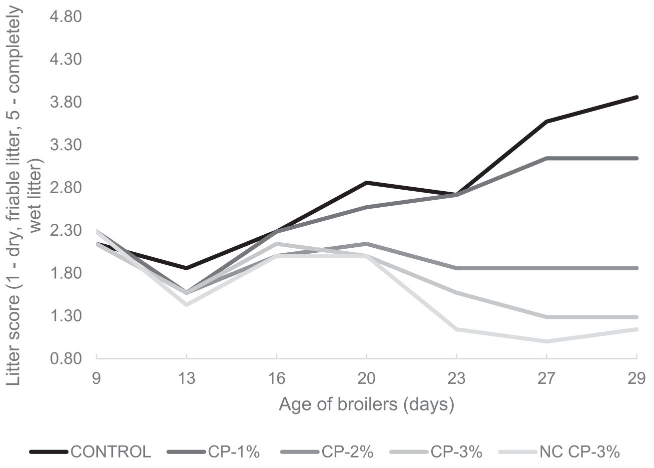

Fig. 1. Litter score of broilers fed diets differing in dietary crude protein and dietary electrolyte balance (7 replicates of 260 broilers per pen at d9, d13, d16, d20, d23, d25 & d29).

Table 8
Blood characteristics of broilers fed diets differing in dietary crude protein and dietary electrolyte balance.

	CONTROL	CP-1 %	CP-2 %	CP-3 %	NC[a] CP-3 %	SE[b]	Linear[c]	Quad[d]	dEB[e]
Dietary CP (%, grower, finisher)	20.7, 19.5	19.7, 18.5	18.7, 17.5	17.7, 16.5	17.7, 16.5				
Dietary EB (mEq/kg, grower, finisher)	226, 211	226, 211	226, 211	226, 211	122, 122				
Crude protein, g/L	36.2	36.1	35.1	36.1	37.3	1.04	0.77	0.62	0.40
Albumin, g/L	10.2	9.8	10.0	10.5	10.1	0.36	0.40	0.17	0.36
Alpha1, g/L	2.1	2.2	2.0	2.2	2.3	0.14	0.78	0.73	0.40
Alpha2, g/L	6.8	6.7	6.6	6.8	7.1	0.25	0.76	0.56	0.36
Beta, g/L	5.7	6.2	5.7	5.7	6.2	0.33	0.59	0.35	0.19
Gamma, g/L	11.1	11.2	10.8	11.0	11.6	0.61	0.87	0.91	0.45
Cholesterol, g/L	1.58	1.66	1.72	1.72	1.73	0.06	0.08	0.49	0.87
Triglycerides, g/L	0.54	0.57	0.66	0.7	0.66	0.06	0.02	0.98	0.61
Uric acid, g/L	58.1	59.9	54.3	44.6	47.6	4.89	0.04	0.23	0.65

[a] NC = negative control (low in dEB).
[b] SE = standard error (7 replicates of 2 broilers per pen at d30).
[c] Linear = statistical linear effect of reducing dietary crude protein.
[d] Quad = statistical quadratic effect of reducing dietary crude protein.
[e] dEB = statistical t-test effect comparing CP-3 % and NC CP-3 % to test the effect of reduction of dEB in a low crude protein diet.

4. Discussion

4.1. Reduction of dietary crude protein combined with a suppression of soybean meal

The first objective of the present study was to elucidate the potential impact of CP reduction in the diets for fast-growing broilers and the effects on performance, carcass traits, litter quality and blood parameters. Recent publications on reduction of dietary CP levels, showed inconsistent results on performance (Van Harn et al., 2018, 2019; Chrystal et al., 2020; Maynard et al., 2021). In these publications, they used the same formulation strategy to reduce dietay CP than in the present experiment. They indeed reduced dietary CP by gradually replacing SBM by cereals and feed-grade while the diets were formulated on minimal amounts of indispensable AA ratios to Lys. Among all the aforementioned low protein diet experiments, none has been testing a complete removal of SBM, totally replaced by wheat and the full set of feed-grade L-AA (down to Gly). Table 9 indeed provides a summary of the Material & Methods of the 4 studies, compared to the present experiment. It can be noticed that all selected experiments formulated low protein diets down to L-Ile, L-Arg, L-Val and L-Trp and few of them including either Gly and/or L-His and L-Leu. Another way to look at the extent of tested protein reduction is the ratio between dLys, and CP. Studies ranged between 5.9 % (present study) and 7.1 % (Chrystal et al., 2020). In the studies where performance was deteriorated (Chrystal et al., 2020; Maynard et al., 2021), the FCR was significantly increased only when the dietary ratio of dLys to CP exceeded 6.4 %. This was confirmed by the studies from van Harn,) et al., (2018, 2019) and the present study, where the reduction of dietary CP was less severe (up to 6.0 % dLys/CP) and no negative effect on performance was observed. It seems therefore achievable to reduce dietary crude protein by 3–4 %pts from synonym to an average dLys/CP of around 6.3–6.4 % and an associated N efficiency up to 70 % without compromising performance if broilers are fed adequate levels of indispensable AA.

The case of Gly is particular in the sense that in one out of the 5 studies, Gly was not added, and this did not result in a decrease of performance. In the current study, the use of feed-grade Gly was preferred as a safety for optimal performance. However, recent findings suggest that although Gly might be a conditionally indispensable AA in fast-growing broilers, dietary Thr has the potential to serve as a direct precursor of Gly and to spare the need to supplement it in low protein diets (Corzo et al., 2009; Ospina-Rojas et al., 2012, 2013; Star et al., 2021). In addition, as one molecule of uric acid requires one molecule of glycine (Siegert and Rodehutscord, 2019) a reduction of dietary CP results in a lower uric acid excretion (as shown in this trial) and therefore reduces the need for dietary glycine or its precursors.

In the present study, reducing dietary CP did not negatively affect overall performance. However, in the grower phase (10–20d), the performance was slightly decreased whereas in the finisher phase (20–30d), performance was slightly improved when feeding broilers with reduced protein levels. This is in line with the findings of van Harn et al. (2018) who found a reduced FCR with low protein diets in the finisher phase. The authors highlighted that the improved feed efficiency could come from a positive stimulation of gut health as indicated by the positive findings on FPL but also by previous research investigating the benefits of low CP diets on gut health (Qaisrani et al., 2015; Liu et al., 2017).

In addition, to our knowledge, this is one of first experiments testing the full replacement of SBM by combining a reduction of dietary CP with the use of alternative feedstuffs without compromising growth performance. Méda et al. (2019) tested the full replacement of SBM by reducing dietary CP combined with the use of fababeans, sunflower meal and rapeseed meal in 21–35d broilers. Performance was maintained except for a slight but significant negative effect on FCR (+0.03). The partial (50 %) or full (100 %) replacement of SBM by insect meal was tested by Velten et al. (2018) and Murawska et al. (2021), respectively. Important reductions in growth performance in both studies were reported. In our experiment, SBM was gradually replaced by cereals (wheat), feed-grade AA (see Table 9) and potato protein concentrate. This nutritional strategy seems to maintain performance of Ross 308 broilers fed with diets free of SBM.

Reducing dietary CP did not influence carcass traits or muscle deposition in this experiment. In van Harn et al. (2019), breast meat

Table 9

Material and methods of five recent low dietary crude protein trials for fast-growing broilers, conducted within similar conditions.

Reference	Country	Genetics	Number of experimental phases	Period of test (d)	Housing system	Replicates per treatment	Broilers per pen	Maximum of CP reduction	Highest dLys[a]/ CP reached (%)	Highest N efficiency reached (%)	Feed-gradeL-AA used[b]
Maynard et al. (2021)	USA	Cobb 500	3	1–41	Pens	8	34	-4 %pt	6.58	74	Val, Arg, Ile, Leu, Trp, His, Gly, Ser
Chrystal et al. (2020)	Australia	Ross 308	1	14–35	Cages	7	6	-4.4 %pt	7.05	77	Val, Arg, Ile, Leu, Trp, His, Gly
van Harn et al. (2018)	The Netherlands	Ross 308	2	10–35	Pens	10	13	-3.4 %pt	6.0	69	Val, Arg, Ile, Trp
Van Harn et al., 2019	The Netherlands	Ross 308	2	11–35	Pens	17	13	-3 %pt	5.9	71	Val, Arg, Ile, Trp, Gly
Present study	The Netherlands	Ross 308	2	10–30	Semi-commercial	7	260	-3 %pt	5.9	70	Val, Arg, Ile, Leu, Trp, His, Gly

[a] dLys/CP = ratio between digestible Lysine and crude protein.

[b] Beyond L-Lys, L-Thr & DL-Met, AA = Amino Acids.

yield was significantly lowered in the lowest dietary CP level (17.8 % and 16.8 %, respectively, in grower and finisher diets) whereas in van Harn et al. (2018) and Ospina-Rojas et al. (2014), breast meat yield (and other carcass traits) was not affected by the reduction of dietary CP. As carcass parameters were not evaluated in Maynard et al. (2021) and Chrystal et al. (2020) it is therefore difficult to conclude on a consensus on the impact of very low protein diets on muscle deposition and meat yield.

4.2. Reduction of dietary electrolyte balance by reducing dietary potassium

According to Kleyn and Ciacciariello (2021), ensuring adequate electrolyte and mineral nutrition to broilers is a prerequisite for optimal performance but the requirements of Na, K and Cl need to be revisited considering the modern broiler genetics. Quiniou and Narcy (2019) attempted to provide an optimal dEB for broilers but no more precise than between 200 and 300 mEq/kg. In this experiment, the decrease of dEB from 230 to 120mEq/kg resulted in a significant increase of ADFI (+4 g/d) and FCR (+0.05), indicating that dietary supply of dEB for 10–30d broilers was not sufficient to allow optimal performance. This is in line with the trial from Borges et al. (2005) with a decrease of dEB from 230 to 140 mEq/kg and with the suggested requirements from Vieites et al. (2005). Vieites et al. (2005) tested levels of dEB ranging from 0 to 350 meg/kg were tested by steps of 50 mEq/kg at two levels of dietary CP. In short, the level of CP did not influence the dEB requirement and the average requirement was suggested to be between 160 and 190 mEq/kg. The only other study testing the reduction of dEB in a low protein diet situation is the work from Chrystal et al. (2020) where a reduction of dEB (from 230 to 120 mEq/kg) was tested in the context of a very low CP diet (15.6 % CP). It did not result in a decrease of performance, but significantly lowered the jejunal digestibility of 9 AA with a 3.69 % reduction in the mean digestibility coefficient of the 16 analyzed AA, probably due to an increase of AA endogenous flows as indicated by Adedokun et al. (2017). These results on AA digestibility also confirm the previous findings from Ravindran et al. (2008). The main difference between the work of Chrystal et al. (2020) and the present experiment is that, in their case, the decrease of dEB was achieved with a slight decrease in K and a strong increase in Cl (from 0.16 % to 0.42 %), whereas the reduction of dEB in the present study was achieved by maintaining the same levels of Na (0.23 %) and Cl (0.19 %) as the control and decreasing dietary K (from 0.85 % to 0.45 %) resulting in a decrease of dEB of 110 pts (from 230 to 120mEq/kg). This way of reducing dEB would be more likely to occur in practice as SBM is a K-rich feedstuff being gradually replaced by cereals in a low dietary CP context. It is therefore likely that K was more limiting in the present experiment that dEB. Although K requirement is not well described as indicated by the range of Aviagen recommendation: between 0.4 % and 0.9 %, in 8–42 days broilers, K requirement was estimated to be between 0.63 and 0.76 depending on the performance indicator being evaluated (Oliveira et al., 2005), therefore higher than the lowest level tested in this experiment. In addition, we observed that the effect was more predominant in the finisher phase than in the grower phase, probably as K requirement increases with age (Oliveira et al., 2005). Further investigation on the effect of dEB reduction on performance under different combinations of Na, K or Cl is required to better advice practical nutritionists.

4.3. Combining low dietary crude protein and low dietary potassium for optimal litter quality

Despite the intrinsic complexity of the condition, the direct causes of FPL unanimously recognized to be linked to litter quality and especially litter moisture (Youssef et al., 2011; Shepherd and Fairchild, 2010). Firstly, litter moisture softens the footpad tissue, making it more susceptible to physical damages (Dunlop et al., 2016) and second, litter N has the capacity to irritate the skin and have deleterious effects on the FPL (Ekstrand et al., 1997). Among other nutritional strategies, the reduction of dietary CP is therefore recognized as a solution to reduce FPL as it plays on both the humidity of the litter and the litter N content (Nagaraj et al., 2007; van Harn et al., 2018, 2019). In Alfonso-Avila et al. (2022), based on a meta-analysis of low dietary CP experiments, they predicted that the reduction of 1 %pt of dietary CP (e.g., from 19 % to 18 %) could lead to a reduction of water intake by 4.0 % (based on 19 trials) and a reduction of litter moisture by 6.4 % (based on 13 trials). Interestingly, in most of the papers analyzed in this meta-analysis, dietary CP was reduced by replacing SBM by cereals therefore leading to reduced dietary K and often, reduced dEB. It thus cannot be concluded if the effect of the reduction of dietary CP comes from a reduction of dietary N intake or a reduction in K (or dEB). In this experiment, one of the objectives was to disentangle the reduction of dietary CP (PC, CP-1 %, CP-2 % & CP-3 % at a constant K and dEB level) from the effect of a reduction of dEB and K (CP-3 % vs NC CP-3 % at a constant level of dietary CP). The reduction of water intake is on average − 3.8 % per %point of CP reduction, in line with Alfonso-Avila et al. (2022) whereas K was kept constant. Litter moisture was reduced by 10 % on average, much more than the predictions from Alfonso-Avila et al. (2022). The reduction of dEB from 230 to 120 mEq/kg (together with K from 0.85 to 0.45) further reduced water intake, despite an increase in feed intake, and litter moisture, demonstrating the effect of K on the thirst of animals. Interestingly, when comparing to the CONTROL, the positive contribution of reducing CP on the reduction in litter moisture was equal to the contribution of reducing K (50 % each).

The reduction of litter moisture explained by both the reduction of dietary CP and the reduction of dietary dEB led to an improved visual appearance of the litter quality and to the decrease of the FPL. As only reducing dietary CP directly reduces litter N content, it seems logical that the effect on FPL is mostly associated to the decrease in dietary CP. Overall the effect on FPL is in line with the findings of Lemme et al. (2019) and van Harn,) et al., (2018, 2019).

4.4. Low dietary crude protein influences energy metabolism

Broilers fed low protein diets tend to show a higher abdominal fat deposition (Bregendahl et al., 2002; Belloir et al., 2017; Chrystal et al., 2020) and confirmed by meta-analysis (Cappelaere et al., 2021b). In this experiment, it was unfortunately not possible to measure abdominal fat content, but cholesterol and triglycerides were measured in the blood and were linearly increased when reducing CP, indicating a change in energy metabolism with a probable increase in energy availability to the animal when fed low protein diets. The present increase in blood cholesterol and triglycerides when low protein diets could suggest an increase in energy availability to the animal when reducing dietary CP. The underlying mechanisms are not fully clarified but there are three possible explanations: 1) broilers often slightly increase their feed intake when fed low protein diets and therefore have a higher dietary energy intake (Belloir et al., 2017); 2) low CP diets have the ability to exert an energy sparing effect (Chrystal et al., 2020; Maynard et al., 2021;) and 3) broilers are fed with higher starch, lower CP and lower fat levels in low CP diets and this switch in energy source is not taken into account in the current AMEn system. The choice in energy systems would need to be further investigated to provide a more accurate system for broilers (Noblet et al. (2022)) and could help to avoid the increase in abdominal fat or the energy storage under the form of cholesterol and triglycerides. There is no consensus over the effect of low CP diets on blood energy indicators. Ndazigaruye et al. (2019) reported that reducing dietary CP by 2.5 %pt increased significantly serum total cholesterol in line with the present study. However, the tested reduction of dietary CP was done via a reduction of digestible AA supply, below the recommendations for optimal growth. On the other hand, with a more similar experimental protocol, Attia et al. (2017) found a significant increase in blood cholesterol in low protein diets, in line with the present study.

5. Conclusion

In the present experiment, it was confirmed that broilers fed with low CP diets formulated with adequate dietary levels of AA and dEB exert similar performance, feed efficiency and meat traits. On the contrary, reducing dEB by reducing dietary K leads to detrimental effects on feed intake and feed efficiency. Although reducing dietary CP linearly improved litter quality and foot pad lesions, the treatment that exhibited the best litter quality was the combination of low CP diets and low dEB/K level. Further research should be conducted on the specific influence of K but also other electrolytes (Na, Cl) in low dietary CP situations.

CRediT authorship contribution statement

William Lambert: Conceptualization, Methodology, Writing – original draft. **Els Willems:** Conceptualization, Methodology, Supervision, Project administration, Writing – review & editing. **Julio Diaz Berrocoso:** Formal analysis, Writing – review & editing. **Bart Swart:** Writing – review & editing. **Marije van Tol:** Writing – review & editing. **Erik Bruininx**: Writing – review & editing.

Conflict of interest

The authors were employees of AGRIFIRM, an animal compound feed producer, of ORFFA and of METEX NOOVISTAGO, two feed additive companies, at the time of the experiment.

Acknowledgements

The authors would like to thank the staff of Laverdonk Research Farm, Laverdonk, the Nertherlands for their support. Thanks to Nutricontrol, Veghel, the Netherlands, for analysis of all the excreta and feed samples. In addition, we would like to thank the trained personnel of the slaughterhouse for manual cutting of the broilers. Special thanks to Sonia Mauclair and the Customer Laboratory of METEX NØØVISTAGO, Amiens, France for analyzing the feeds for crude protein, total and free AA.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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