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Evaluating the impact of reduced-protein diets with varying ingredient combinations on broiler chickens' performance, body composition and nutrient utilization

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ABSTRACT

Ingredient availability is a factor that could impact nutrient utilization and consequently broiler performance, especially when feeding reduced crude protein (**CP**) diet. In this study, 2304 male Ross 308 broiler chicks were allotted into nine treatments in a 3 × 3 factorial arrangement. All birds were offered a common starter diet from 0 to 7d. On d8, they were assigned to treatments in groups of eight replicates of 32 birds each. The experimental diets were formulated based on three cereal combinations – corn; wheat-corn; sorghum-wheat, each of three CP levels: control CP, 20 g/kg CP reduction (LCP20), and 40 g/kg CP reduction (LCP40). The CP levels of 230, 210, and 190 g/kg (grower I); 215, 195, and 175 g/kg CP (grower II); and 205, 185, and 165 g/kg (finisher) were maintained. The feed conversion ratio (**FCR**) was similar and superior in broilers fed the control and LCP20 diets during the early growth phase (8–28 days) but worsened during the finisher phase. Protein and fat intake declined with lower CP level. Fat deposition increased while nitrogen (**N**) excretion reduced with lower CP levels. Corn-based diets had highest carcass weight and breast yield at all levels of CP. Wheat-corn and sorghum-wheat diets gave similar results up to 20 g/kg CP reduction. Amino acid (**AA**) digestibility increased as dietary protein reduced. Broilers fed wheat-corn or sorghum-wheat diets showed lower but comparable body weight gain (**BWG**), and up to 20 g/kg CP reduction, BWG was unaffected regardless of diet composition. However, broilers on corn-based diets demonstrated improved FCR. These findings

Abbreviations: AIDE, Apparent ileal digestible energy; AIDC, Apparent ileal digestibility coefficient; AME, Apparent metabolizable energy; AMEn, Apparent metabolizable energy corrected for zero nitrogen retention; ANe, Apparent nitrogen excretion; ANeC, Apparent nitrogen excretion co-efficient; ANOVA, Analysis of variance; BW, Body weight; BWG, Body weight gain; CO_2, Carbon iv oxide; CP, Crude protein; DCL, Digestible crude lipid; DCP, Digestible crude protein; DM, Dry matter; EE, Effective energy; FCR, Feed conversion ratio; Fd, Fat deposition; FI, Feed intake; FOM, Feed organic matter; GE, Gross energy; Glu, Glutamate; kF, Ratio of fat deposition to intake; kP, Ratio of protein deposition to intake; N, Nitrogen; NE, Net energy; Ni, Nitrogen intake; NR, Nitrogen retained; Pd, protein deposition; SID, Standard ileal digestibility; TiO_2, Titanium dioxide; *wd*, Fecal organic matter; *wu*, Urinary nitrogen; *z*, Proportion of lipid retained.

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suggest that wheat-corn or sorghum-wheat diet may yield similar performance to corn in broilers when dietary CP is reduced up to 20 g/kg.

1. Introduction

The impact of livestock production on the environment has been extensively documented (de Vries and de Boer, 2010; Greenhalgh et al., 2020; Leip et al., 2015; Meier et al., 2015; Steinfeld et al., 2006). The intensification of pig and poultry production, owing to their efficiency, has raised environmental concerns globally (Ferket et al., 2002; Mallin and Cahoon, 2003; OECD/FAO, 2021). Moreover, the escalating global demand for broiler meat (Shahbandeh, 2022; Whitnall and Pitts, 2019) has exerted additional pressure on the already skyrocketed soybean demand (Greenhalgh et al., 2020; OECD, 2018). Consequently, developing strategies that mitigate the nitrogenous waste emission and soybean dependence in poultry production has become imperative (Liu et al., 2021), thus addressing multiple issues simultaneously.

Reducing the CP content of broiler diets and supplementing with non-bound (crystalline) AAs has been proposed as a promising approach to curb increasing N excretion in broiler chickens (Chrystal et al., 2020a; Greenhalgh et al., 2020; Liu and Selle, 2017; Selle et al., 2020; Woyengo et al., 2023). Reduced CP diets are often formulated by decreasing the amount of protein source (usually soybean meal) and increasing the inclusion of cereals with higher inclusions of AAs, to meet the nutritional requirements. Although current economic constraints may limit its implementation, it is bound to become affordable in the future due to increased demand (Liu et al., 2021), which had been partially proven (Kareem et al., 2025). While CP reduction is not a novel concept (Selle et al., 2020), recent years have witnessed an intensified focus on this approach. Nevertheless, its adoption among producers has been slow, partially due to consistent observation that lowering CP adversely affects broiler growth performance and results in increased fat deposition. Chrystal et al. (2021) observed reduced growth performance and increased protein digestibility in broiler chickens fed wheat-based diets with CP levels reduced from 200 to 160 g/kg. Similarly, Bregendahl et al. (2002) found that reducing CP from 210 to 170 g/kg in corn-soybean diets reduced broiler growth rate and feed efficiency. Namroud et al. (2008) observed increased fat deposition and abdominal fat pad as the level of CP in broilers' diets decreased, thereby supporting the assertion that the excess starch from reduced CP diets is metabolized to fat in broiler chickens.

Due to the diversity of feed ingredients utilized for poultry feed formulation across different regions (Ravindran, 2013), there is a pressing need for research on reduced CP that considers these differences. Liu et al. (2014) noted that protein was more digestible in corn-based diets than wheat and sorghum; but sorghum had better digestibility than wheat. This implies that the response of broiler chickens to dietary crude protein reduction may vary depending on the specific feed ingredients used (Heger et al., 1996), underscoring the importance of a deeper understanding of this phenomenon. So, in this study, we formulated diets of varied ingredients so that the responses of broilers due to CP reduction could be evaluated in a broader scenario. We hypothesized that reducing dietary CP of wheat or sorghum-based broiler diets up to 20 g/kg will elicit a similar response to corn-based diets but may become debilitating when reduced up to 40 g/kg. This study therefore aimed to evaluate the response of broiler chickens to different ingredient combinations while reducing dietary CP, focusing on growth performance, carcass yield, nitrogen excretion, body composition, and protein digestibility.

2. Materials and methods

2.1. Ethical statement

This study was conducted at the Poultry Science Laboratory of the School of Agricultural and Veterinary Sciences, São Paulo State University (**UNESP**). All procedures were approved by the Animal Care and Use Committee of UNESP on February 20, 2022 (Protocol n° 639/22).

2.2. Experimental birds, housing, and management

A total of 2304 one-day-old male Ross 308 broiler chicks, with average weight of 42 g, were obtained from a commercial hatchery. All birds were randomly allocated to separate pens, with wood shavings as litter, nipple drinkers, and feeders. The poultry house was equipped with an environmental controlled system to maintain temperature and humidity according to breeder guidelines (Aviagen, 2018).

On d 8, the birds were individually weighed and then randomly distributed among nine dietary treatments, each with eight replicates. This arrangement resulted in a total of 72 experimental units (pens), with 32 birds per pen, ensuring uniformity in average bird weight across all pens. Each pen had dimensions of 2.0 × 1.5 m, and a stocking density of approximately 11 birds/m^2 was maintained. The birds had continuous access to both water and feed throughout the experimental period. Lighting was provided for the birds continuously until seven days of age. Subsequently, on d 8, a photoperiod of 18 h was scheduled, till the end of the experiment.

2.3. Experimental diets

The birds were initially provided with a standard starter diet complying with the guideline recommendation by Aviagen (2019) from 0 to 7d post-hatch. At d 8, nine isocaloric experimental diets were offered, consisting of three CP levels: Control CP; 20 g/kg CP reduction (LCP20); 40 g/kg CP reduction (LCP40), and three combinations of cereal as main ingredients of each diets: Corn, wheat and corn, sorghum and wheat. All other nutrients in the experimental diets met or exceeded the nutrient specification guide provided by Aviagen (2019). A three-phase feeding program was employed: grower I (8 – 18 d), grower II (18 – 28 d), and finisher (28 – 38 d)

Table 1
Composition of experimental diet at the grower I phase (8–18 d).

	Corn (g/kg)			Wheat and corn (g/kg)			Sorghum and wheat (g/kg)		
Ingredients (g/kg)	Control[1]	LCP20[2]	LCP40[3]	Control	LCP20	LCP40	Control	LCP20	LCP40
Corn, grain	531.40	596.80	669.70	285.00	285.00	285.00	-	-	-
Sorghum grain, low tannin	-	-	-	-	-	-	377.70	450.60	546.20
Wheat, grain	-	-	-	287.00	368.30	456.40	200.00	200.00	200.00
Soybean oil	39.40	27.40	13.40	46.70	35.90	22.00	47.10	35.10	17.20
Peanut meal	70.00	70.00	70.00	-	-	-	-	-	-
Meat and bone meal	-	-	-	-	-	-	20.00	20.00	20.00
Soybean meal	313.70	253.50	183.80	336.20	257.10	165.70	317.10	246.70	148.10
Dicalcium Phosphate	17.00	17.40	17.80	16.80	17.10	17.60	11.30	11.80	12.40
Limestone	7.80	8.00	8.30	7.70	8.10	8.50	5.50	5.70	6.10
Salt (NaCl)	2.70	2.70	2.60	2.80	2.70	2.70	2.70	2.70	2.70
Premix (Minerals)[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Premix (Vitamins)[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DL-Methionine (990)[5]	3.00	3.50	4.00	2.70	3.20	3.70	3.00	3.60	4.40
L-Lysine HCl (780)	1.90	3.70	5.80	1.80	4.00	6.60	2.20	4.40	7.30
L-Threonine (985)	0.70	1.40	2.30	0.60	1.60	2.70	0.90	1.80	3.10
L-Valine (965)	-	1.00	2.10	-	1.10	2.40	0.10	1.20	2.80
L-Isoleucine (904)	-	0.60	1.70	-	0.50	1.80	-	0.60	2.20
Glycine (985)	-	-	1.70	-	-	2.60	-	0.30	3.70
L-Arginine (900)	-	-	0.40	-	0.70	3.10	-	1.20	4.10
L-Tryptophan (980)	-	-	0.20	-	-	0.20	-	-	0.20
L-Phenylalanine (966)	-	-	-	-	-	1.80	-	-	1.90
L-Histidine (900)	-	-	-	-	-	0.10	-	-	0.70
Choline chloride, 600 g/kg	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Coccidiostat	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Inert	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Potassium carbonate	1.60	3.10	5.00	1.80	3.80	6.20	2.00	3.80	6.40
Sodium bicarbonate	1.30	1.40	1.50	1.40	1.50	1.60	0.80	0.80	0.90
Adsorbent	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated nutritional composition, g/kg unless noted otherwise									
Crude Protein	230	210	190	230	210	194	230	210	190
Digestible Protein	206	187	169	199	178	161	195	177	159
AME (MJ/kg)	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6
Net Energy (MJ/kg)[6]	11.3	11.4	11.5	11.3	11.5	11.6	11.4	11.5	11.7
Ether Extract	72.2	61.4	48.6	74.2	62.9	48.3	75.3	63.8	46.3
Starch	347.5	389.4	436.1	344.4	389.4	438.2	358.6	405.9	468.0
Ash	38.7	35.8	32.3	37.6	33.9	29.6	40.7	37.4	32.6
Calcium	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4
Available Phosphorus	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
Sodium (Na)	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Chlorine (Cl)	2.6	3.0	3.4	2.7	3.0	3.6	2.7	3.1	3.7
Potassium (K)	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2
SID Lysine	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5
SID Methionine (Met)	5.7	5.9	6.2	5.5	5.7	5.9	5.8	6.0	6.4
SID Met.+Cyst	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
SID Threonine	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4
SID Glycine (Gly)	8.1	7.3	8.0	7.7	6.8	8.2	8.0	7.3	9.0
SID Serine (Ser)	10.0	8.9	7.7	10.0	8.9	7.5	9.6	8.4	6.7
SID Gly+Ser	18.1	16.2	15.7	17.8	15.7	15.7	17.6	15.7	15.7
Electrolyte Balance (mEq/kg)	229	219	208	230	218	205	227	215	200

[1]Control CP
[2]LCP20: Control CP – 20 g/kg
[3]LCP40: Control CP – 40 g/kg
[4] Provided per kg of vitamin premix: Folic Acid (min) 1600 mg; Vitamin B5 - Pantothenic acid (min) 24.96 g; Biotin (min) 80 mg; Butyl hydroxide toluene 100 mg; Niacin (min) 67.20 g; Selenium (min) 600 mg; Vitamin A (min) 13440000 UI; Vitamin B1 (min) 3492 mg; Vitamin B12 (min) 19200 mcg; Vitamin B2 (min) 9600 mg; Vitamin B6 (min) 4992 mg; Vitamin D3 (min) 3200000 UI; Vitamin K3 (min) 2880 mg. Provided per kg of mineral premix: Copper (min) 15 g; Iron (min) 90 g; Iodine (min) 1500 mg; Manganese (min) 150 g; Zinc (min) 140 g.
[5] Purities (g/kg) of non-bound amino acids are included in parentheses.
[6] Calculated using the effective energy equation of Emmans et al. (1994)
AME – Apparent Metabolizable Energy; NE – Net energy; SID - Standard ileal digestibility

phases, with similar nutritional profile between treatments. The control, 20 g/kg, and 40 g/kg reduced CP diets were formulated with targeted levels: 230, 210, and 190 g/kg CP (grower I); 215, 195, and 175 g/kg CP (grower II); and 205, 185, and 165 g/kg CP (finisher), respectively.

The detailed composition of the experimental diets formulated for each phase are shown in Tables 1, 2, and 3. An indigestible

Table 2
Composition of experimental diet at the grower II phase (18–28 d).

	Corn (g/kg)			Wheat and corn (g/kg)			Sorghum and wheat (g/kg)		
Ingredients (g/kg)	Control[1]	LCP20[2]	LCP40[3]	Control	LCP20	LCP40	Control	LCP20	LCP40
Corn, grain	564.00	625.70	693.90	250.00	250.00	250.00	-	-	-
Sorghum grain, low tannin	-	-	-	-	-	-	410.20	476.60	567.60
Wheat, grain	-	-	-	364.80	444.40	537.90	200.00	200.00	200.00
Soybean oil	45.40	34.40	21.40	54.80	44.50	29.80	53.90	43.40	26.80
Peanut meal	70.00	70.00	70.00	-	-	-	-	-	-
Meat and bone meal	-	-	-	-	-	-	20.00	20.00	20.00
Soybean meal	278.00	221.60	157.80	287.50	210.40	114.40	280.70	217.50	124.80
Dicalcium Phosphate	15.10	15.40	15.90	14.80	15.20	15.60	9.40	9.80	10.40
Limestone	7.20	7.40	7.70	7.20	7.60	8.00	4.90	5.10	5.50
Salt (NaCl)	2.70	2.70	2.60	2.80	2.70	2.70	2.70	2.70	2.70
Premix (Minerals)[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Premix (Vitamins)[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DL-Methionine (990)[5]	2.40	2.80	3.30	2.10	2.50	3.10	2.40	2.90	3.70
L-Lysine HCl (780)	1.50	3.10	5.00	1.60	3.70	6.50	1.80	3.70	6.50
L-Threonine (985)	0.30	1.10	1.90	0.40	1.30	2.50	0.60	1.40	2.60
L-Valine (965)	-	0.50	1.50	-	0.70	2.10	-	0.70	2.20
L-Isoleucine (904)	-	0.30	1.30	-	0.30	1.70	-	0.30	1.80
Glycine (985)	-	-	0.70	-	-	2.00	-	-	2.60
L-Arginine (900)	-	-	-	-	0.60	3.10	-	0.80	3.50
L-Tryptophan (980)	-	-	0.20	-	-	0.10	-	-	0.10
L-Phenylalanine (966)	-	-	-	-	-	1.90	-	-	1.20
L-Histidine (900)	-	-	-	-	-	0.10	-	-	0.50
Choline chloride, 600 g/kg	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Coccidiostat	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Inert	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Potassium carbonate	2.50	4.00	5.70	3.00	5.00	7.50	3.00	4.60	7.10
Sodium bicarbonate	1.40	1.50	1.60	1.40	1.50	1.60	0.80	0.80	0.90
Adsorbent	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated nutritional composition, g/kg unless noted otherwise									
Crude Protein	215	195	175	215	195	177	215	195	175
Digestible Protein	191	174	155	182	161	143	181	163	145
AME (MJ/kg)	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Net Energy (MJ/kg)[6]	11.7	11.8	11.9	11.8	11.9	12.0	11.8	11.9	12.1
Ether Extract	78.6	68.7	57.0	81.1	70.3	54.9	82.1	72.1	55.9
Starch	368.4	408.0	451.6	365.1	409.2	461.0	379.7	422.8	481.9
Ash	36.2	33.4	30.3	34.6	31.0	26.5	38.2	35.2	30.8
Calcium	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6
Available Phosphorus	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Sodium (Na)	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Chlorine (Cl)	2.6	2.9	3.2	2.6	3.0	3.6	2.6	3.0	3.5
Potassium (K)	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2
SID Lysine	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3
SID Methionine (Met)	5.0	5.2	5.4	4.7	4.9	5.1	5.0	5.3	5.6
SID Met.+Cyst	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6
SID Threonine	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
SID Glycine (Gly)	7.6	6.8	6.6	7.2	6.3	7.1	7.4	6.5	7.6
SID Serine (Ser)	9.3	8.4	7.3	9.4	8.2	6.8	9.0	7.9	6.3
SID Gly+Ser	17.0	15.2	13.9	16.6	14.6	13.9	16.4	14.4	13.9
Electrolyte Balance (mEq/kg)	231	223	213	230	219	205	230	219	206

[1]Control CP
[2]LCP20: Control CP – 20 g/kg
[3]LCP40: Control CP – 40 g/kg
[4] Provided per kg of vitamin premix: Folic Acid (min) 1600 mg; Vitamin B5 - Pantothenic acid (min) 24.96 g; Biotin (min) 80 mg; Butyl hydroxide toluene 100 mg; Niacin (min) 67.20 g; Selenium (min) 600 mg; Vitamin A (min) 13440000 UI; Vitamin B1 (min) 3492 mg; Vitamin B12 (min) 19200 mcg; Vitamin B2 (min) 9600 mg; Vitamin B6 (min) 4992 mg; Vitamin D3 (min) 3200000 UI; Vitamin K3 (min) 2880 mg. Provided per kg of mineral premix: Copper (min) 15 g; Iron (min) 90 g; Iodine (min) 1500 mg; Manganese (min) 150 g; Zinc (min) 140 g.
[5] Purities (g/kg) of non-bound amino acids are included in parentheses.
[6] Calculated using the effective energy equation of Emmans et al. (1994)
AME – Apparent Metabolizable Energy; NE – Net energy; SID - Standard ileal digestibility

marker (titanium dioxide) was added to the finisher diets of the broiler chickens from 33 d to 38 d at 5 g/kg. Dry matter (**DM**), CP, AME, ether extract, and AA analyses of all organic feed ingredients were determined by Near-Infrared Spectroscopy (**NIRS**) by AMINONIR® (Evonik Operations GmbH, Germany) before formulating each diet, and the result of these analyses was employed in the

Table 3
Composition of experimental diet at the finisher phase (28–38 d).

	Corn			Wheat and corn			Sorghum and wheat		
Ingredients (g/kg)	Control[1]	LCP20[2]	LCP40[3]	Control	LCP20	LCP40	Control	LCP20	LCP40
Corn, grain	582.20	644.00	712.00	230.00	230.00	230.00	-	-	-
Sorghum grain, low tannin	-	-	-	-	-	-	428.40	496.20	588.00
Wheat, grain	-	-	-	409.50	491.80	581.30	200.00	200.00	200.00
Soybean oil	50.30	39.30	26.30	60.90	50.10	35.60	59.30	48.40	31.50
Peanut meal	70.00	70.00	70.00	-	-	-	-	-	-
Meat and bone meal	-	-	-	-	-	-	20.00	20.00	20.00
Soybean meal	254.00	197.50	134.00	255.50	175.30	83.30	256.20	191.50	97.90
Dicalcium Phosphate	15.20	15.60	16.10	15.00	15.30	15.80	9.60	10.00	10.60
Limestone	7.30	7.50	7.80	7.40	7.70	8.10	5.00	5.20	5.60
Salt (NaCl)	2.70	2.70	2.60	2.70	2.70	2.70	2.70	2.70	2.70
Premix (Minerals)[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Premix (Vitamins)[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DL-Methionine (990)[5]	2.20	2.70	3.10	1.90	2.30	2.90	2.30	2.80	3.50
L-Lysine HCl (780)	1.60	3.20	5.10	1.80	4.10	6.70	1.90	3.90	6.70
L-Threonine (985)	0.30	1.10	1.90	0.50	1.40	2.50	0.60	1.40	2.60
L-Valine (965)	-	0.50	1.50	-	0.80	2.10	-	0.80	2.30
L-Isoleucine (904)	-	0.30	1.40	-	0.40	1.80	-	0.40	1.90
Glycine (985)	-	-	0.50	-	-	1.80	-	-	2.50
L-Arginine (900)	-	-	0.10	-	1.00	3.40	-	1.00	3.70
L-Tryptophan (980)	-	-	0.20	-	-	0.20	-	-	0.20
L-Phenylalanine (966)	-	-	-	-	-	2.30	-	-	1.50
L-Histidine (900)	-	-	-	-	-	0.20	-	-	0.60
Choline chloride, 600 g/kg	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Coccidiostat	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Titanium dioxide (TiO_2)[6]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Potassium carbonate	3.20	4.70	6.40	3.90	5.90	8.30	3.70	5.30	7.80
Sodium bicarbonate	1.40	1.50	1.60	1.50	1.60	1.70	0.80	0.80	0.90
Adsorbent	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated nutritional composition, g/kg unless noted otherwise									
Crude Protein	205	185	165	205	185	168	205	185	165
Digestible Protein	182	164	146	171	151	133	172	154	136
AME (MJ/kg)	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
Net Energy (MJ/kg)[7]	12.0	12.1	12.2	12.1	12.2	12.3	12.1	12.2	12.4
Ether Extract	83.6	73.7	62.0	86.3	75.0	59.9	87.4	77.0	60.5
Starch	380.0	419.6	463.2	377.0	422.6	472.1	391.5	435.5	495.1
Ash	34.9	32.2	29.0	33.1	29.3	25.0	37.0	33.9	29.4
Calcium	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6
Available Phosphorus	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Sodium (Na)	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Chlorine (Cl)	2.6	2.9	3.3	2.6	3.1	3.6	2.6	3.0	3.6
Potassium (K)	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2
SID Lysine	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8
SID Methionine (Met)	4.7	4.9	5.1	4.4	4.6	4.8	4.8	5.0	5.4
SID Met.+Cyst	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
SID Threonine	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
SID Glycine (Gly)	7.3	6.5	6.1	6.8	5.9	6.5	7.1	6.1	7.1
SID Serine (Ser)	8.9	7.9	6.8	8.9	7.7	6.4	8.5	7.4	5.8
SID Gly+Ser	16.2	14.4	12.9	15.8	13.7	12.9	15.6	13.5	12.9
Electrolyte Balance (mEq/kg)	231	222	213	231	218	204	229	217	204

[1]Control CP
[2]LCP20: Control CP – 20 g/kg
[3]LCP40: Control CP – 40 g/kg
[4] Provided per kg of vitamin premix: Folic Acid (min) 1600 mg; Vitamin B5 - Pantothenic acid (min) 24.96 g; Biotin (min) 80 mg; Butyl hydroxide toluene 100 mg; Niacin (min) 67.20 g; Selenium (min) 600 mg; Vitamin A (min) 13440000 UI; Vitamin B1 (min) 3492 mg; Vitamin B12 (min) 19200 mcg; Vitamin B2 (min) 9600 mg; Vitamin B6 (min) 4992 mg; Vitamin D3 (min) 3200000 UI; Vitamin K3 (min) 2880 mg. Provided per kg of mineral premix: Copper (min) 15 g; Iron (min) 90 g; Iodine (min) 1500 mg; Manganese (min) 150 g; Zinc (min) 140 g.
[5] Purities (g/kg) of non-bound amino acids are included in parentheses.
[6] Inert was added to the diet at the inclusion level of 5 g/kg from d 29–32 and replaced with titanium dioxide in the diet from d 33–38.
[7] Calculated using the effective energy equation of Emmans et al. (1994)
AME – Apparent Metabolizable Energy; NE – Net energy; SID - Standard ileal digestibility

diet formulations. After feed production at each phase, 200 g feed samples of each experimental diet were collected and stored, for further analysis (AMINOLab®, Evonik Operations GmbH, Germany) of DM, CP, gross energy (**GE**), and AAs (methionine, cysteine, lysine, threonine, tryptophan, arginine, isoleucine, leucine, valine, histidine, phenylalanine, glycine, and serine). The AAs were determined using the High-Performance Liquid Chromatography (**HPLC**), while CP was determined by Dumas' method. The analyzed CP, ether extract, apparent metabolizable energy (**AME**), and total AA contents of the diets at grower I, grower II, and finisher phases are presented in Table 4. The net (effective) energy of the diets was calculated following the equation of Emmans (1994) as shown below.

$$NE(MJ/kg) = AMEn - (wd * FOM) – (0.16 * wu * DCP) + (12 * z * DCL)$$

Where, NE (MJ/kg)) = Net energy; AME_n (MJ/kg) = Apparent metabolizable energy corrected to zero nitrogen retention; $wd = 3.80$; FOM (g) = Feed organic matter; $wu = 29.2$; DCP (g/g) = Digestible crude protein; z = 0.3; DCL (g/g) = Digestible crude lipid

2.4. Growth performance

The body weight (**BW**), BWG, and feed intake (**FI**) were recorded on days 8, 18, 28, and 38. The FCR was calculated from the BWG and FI. The FCR was corrected daily for mortality.

2.5. Body composition

Body composition analysis was determined by comparative slaughter technique (Sakomura and Rostagno, 2016). At d 7 post-hatch, 12 birds were randomly selected to establish the baseline body composition. At 40 d post-hatch, one bird per replicate with representative BW of the group was selected. The birds were fasted for 24 h, weighed, and then euthanized via CO2 asphyxiation.

After feather sampling, the birds were defeathered to record the free-feather BW, and the carcasses were stored at −20ºC. Subsequently, frozen carcasses were cut (Delta Grill DG-1003, Brazil), minced (CAF 22 DSM, Brazil) and samples homogenized. Carcass samples were stored at −80°C (ColdLab CL374–86V, USA), for subsequent lyophilization (Edwards Supermudulyo-220 freeze dryer, USA). Partial dried samples were ground using a ball grinder (Marconi MA 350, Brazil) and then analyzed for DM, ether extract, and protein content.

The feathers were dried in an oven for 72 h for DM determination, and subsequent CP analysis. Carcasses samples underwent ether

Table 4
Analyzed nutrients and total amino acid composition of the diets at grower I, grower II, and finisher phases.

	Corn			Wheat and Corn			Sorghum and wheat		
	Control[1]	LCP20[2]	LCP40[3]	Control	LCP20	LCP40	Control	LCP20	LCP40
Phases	Analyzed nutrients and total amino acid composition, g/kg unless noted otherwise								
Grower I (8 – 18d)									
CP	237	219	199	237	213	199	234	213	196
Ether extract	73	57	44	73	57	49	72	66	44
AME (MJ/kg)	12.3	12.1	12.0	12.5	12.4	12.2	12.6	12.4	12.4
Methionine	6.3	6.5	6.5	6.1	6.0	6.8	6.0	7.0	6.8
Lysine	13.3	13.3	12.3	13.3	12.8	13.3	12.9	13.1	12.6
Threonine	8.8	8.5	8.0	8.9	8.5	8.6	8.7	8.5	8.2
Cysteine	3.4	3.2	3.0	3.8	3.5	3.2	3.4	3.1	2.7
Met+Cys	9.7	9.7	9.5	9.9	9.5	10.1	9.4	10.1	9.4
Grower II (18 – 28 d)									
CP	226	204	184	219	200	185	217	201	179
Ether extract	68	61	49	73	64	55	69	70	50
AME (MJ/kg)	12.3	12.4	12.4	12.8	12.6	12.7	12.8	12.9	13.0
Methionine	6.0	5.7	6.0	5.3	5.7	5.8	5.8	5.8	6.6
Lysine	12.7	11.6	11.5	11.3	11.7	11.1	11.7	11.5	11.9
Threonine	8.3	7.8	7.8	7.9	7.9	7.7	8.1	7.9	8.1
Cysteine	3.5	3.1	2.8	3.6	3.4	3.0	3.2	3.0	2.5
Met+Cys	9.5	8.8	8.8	8.9	9.1	8.9	9.1	8.8	9.1
Finisher (28–38 d)									
CP	220	188	185	206	187	167	211	184	168
Ether extract	81	71	63	69	79	56	96	70	61
AME (MJ/kg)	12.6	12.6	12.6	12.9	13.1	12.8	13.2	12.8	12.7
Methionine	5.7	5.1	6.4	4.7	5.1	5.0	5.6	5.2	6.2
Lysine	12.0	10.7	12.6	10.4	10.6	9.9	11.3	10.1	11.2
Threonine	8.2	7.2	8.2	7.6	7.4	6.7	8.0	7.3	7.4
Cysteine	3.3	2.9	2.8	3.5	3.2	3.0	3.2	2.8	2.3
Met+Cys	9.0	8.0	9.3	8.2	8.4	7.9	8.7	7.9	8.5

[1]Control CP
[2]LCP20: Control CP – 20 g/kg
[3]LCP40: Control CP – 40 g/kg

extract (AOAC, 2005: method 920.39) for defatting (AnkomXT15 Extractor, USA) prior to CP analysis. Both feather and carcass were weighed (Ohaus Adventurer AR2140, USA) and dried at 105°C for 16 h for DM quantification. Protein was determined by Kjeldahl procedure (AOAC, 2005: method 2001.11) and the gross energy by adiabatic bomb calorimeter (IKA C2000 basic, Germany).

Protein deposition (**Pd**) was calculated for carcass and feather, while fat deposition (**Fd**) was calculated from carcass. Deposition rates (g/day) were derived from the difference between final (d 40) and reference (d 8) body composition.

$$Pd = \frac{BW * \%CP_{DM} in\ carcass\ and\ feather}{number\ of\ days}; Fd = \frac{BW * \%fat_{DM} in\ carcass}{number\ of\ days}$$

The protein and fat intake were calculated from the FI and the analyzed feed composition:

$$Protein\ intake = \%CP_{DM} in\ diet * FI;\ Fat\ intake = \%fat_{DM} in\ diet * FI$$

Utilization rates of protein (**kP**) and fat (**kF**) were determined as:

$$kP = \frac{Pd}{Protein\ intake};\ kF = \frac{Fd}{Fat\ intake}$$

2.6. Nitrogen excretion

Nitrogen intake ($\mathbf{N_i}$) was calculated multiplying the N feed content by FI. Body nitrogen retained ($\mathbf{N_R}$) was obtain from total N content in carcass and feathers. The N_R value was subtracted from the N_i to calculate the apparent nitrogen excretion ($\mathbf{AN_e}$) as described by Bregendahl et al. (2002). The AN_e relative to the N_i i.e., AN_e/N_i, was calculated to obtain the apparent nitrogen excretion coefficient ($\mathbf{AN_eC}$).

2.7. Carcass traits

On day 39, three birds per replicate with BW close to the group average weight were selected, fasted for 12 h, weighed, then euthanized via cervical dislocation to evaluate carcass yields (total carcass yield, breast yield and abdominal fat). The breast (without skin) was removed and weighed for breast yield determination. The abdominal fat pad was weighed and expressed relative to BW. The carcass yield, liver, and abdominal fats were expressed relative to the BW, while breast weight (without skin) was calculated relative to the carcass weight (without shanks, head, and neck).

2.8. Apparent ileal nutrient digestibility coefficients

On day 40, five birds per replicate were euthanized via cervical dislocation. Digesta were flushed from the distal ileum using distilled water (Sakomura and Rostagno, 2016), pooled per replicate, stored at −80°C (ColdLab CL374–86V, USA), lyophilized (Edwards Supermudulyo-220 freeze dryer, USA), and ground (Marconi MA 350, Brazil) prior to analysis. Samples were analyzed for DM (AOAC, 2005: method 920.39), CP (AOAC, 2005: method 2001.11), and GE (IKA C2000 basic, Germany), while the AAs in both digesta and diet were determined by AMINOLab® (Evonik Operations GmbH, Germany).

The indigestible marker (titanium dioxide) in both the feed and digesta was recovered according to the method of Myers et al. (2004). Briefly, the samples (0.25 g) were weighed into 100 ml test tubes, with one empty tube serving as the blank. 3 g of $CuSO_4$ (catalyst) and 7 ml of H_2SO_4 were added, and the solutions were digested at 420ºC (temperature was gradually increased from 200ºC to 300ºC, and finally to 420ºC at a 30-minute interval, then left to digest for 2 h or until persistent light green coloration). The digested samples were cooled for 30 min, after which 5 ml H_2O_2 was added. The solution was transferred into a beaker, and distilled water was added to take the volume to 50 ml and then filtered using Whatman® 541 filter papers. Absorbance was measured at 410 nm (Beckman DU 640; USA). Titanium concentration was calculated from the standard curve: $Y_{[titanium\ conc.]} = 5.961 * X_{[wavelength]}$ (Intercept = 0; $R^2 = 0.99$). The apparent digestibility values were calculated as follows.

$$AIDC = \frac{(N_{diet}/TiO_{2diet}) - (N_{dig.}/TiO_{2dig.})}{(N_{diet}/TiO_{2diet})}$$

Where **AIDC** is apparent ileal digestibility coefficient, $\mathbf{N}_{diet}$ is nutrient or energy in diet, $\mathbf{N}_{dig.}$ is nutrient or energy in digesta, $\mathbf{TiO_2}_{\ diet}$ is the titanium dioxide concentration in the diet, $\mathbf{TiO_2}_{\ dig.}$ is the titanium dioxide concentration in the digesta.

The indigestibility factor (**IF**) and apparent ileal digestible energy (**AIDE**) were calculated from equations adapted from Lu et al. (2020).

$$IF = TiO_{2diet}/TiO_{2dig.}$$

$$AIDE(MJ/kg\ DM) = GE_{diet} - (GE_{dig.} * IF)$$

Where IF is the indigestibility factor, $TiO_{2\ diet}$ is the titanium dioxide concentration in the diet, $TiO_{2\ dig.}$ is the titanium dioxide concentration in the digesta, $\mathbf{GE}_{diet}$ is the gross energy in diet, and $\mathbf{GE}_{dig.}$ is the gross energy in digesta.

2.9. Statistical analysis

The data obtained were subject to normality and homoscedasticity of errors using the Cramer-Von Mises and Brown and Forsythe tests at 5 %, respectively. The data were analyzed as a two-way ANOVA in a 3 × 3 experimental arrangement using the SAS® ODA Software (SAS Institute Inc., Cary, NC, USA). Means were compared using Tukey test at 5 % level of probability. At the grower II and finisher phases, BW at d 18 and BW at d 28 were used as covariates, respectively, for analyses of the growth performance parameters.

3. Results

3.1. Growth performance

The growth performance of broiler chickens fed reduced CP diets of varying ingredient sources at different growth phases is presented in Table 5.

3.1.1. Grower I phase (8–18 d)

During the grower I phase, the interaction between diet and CP levels did not have significant effect on BW and BWG, but significantly affected FI ($P = 0.007$) and FCR ($P = 0.028$). The FI of birds fed with corn or sorghum-wheat diets was similar across all CP levels. In contrast, birds fed with wheat-corn diet at control CP level consumed 5.7 % more feed than those fed with LCP40 diet. The FCR of broiler chickens fed with corn or sorghum-wheat diets was lower for control and LCP20 compared with those fed LCP40 diet, while the birds fed with wheat-corn diet showed no differences between the CP levels evaluated.

3.1.2. Grower II phase (18–28 d)

In this phase, the interaction between diets and CP levels did not significantly impact BW, BWG, and FCR, but significantly ($P = 0.047$) affected the FI. The FI of broiler chickens fed with corn or sorghum-wheat diets were similar across the evaluated CP levels. Nonetheless, the birds fed with wheat-corn diet at LCP40 exhibited higher (5.3 %) FI than those fed control CP.

Diets and CP levels significantly influenced BW, BWG, and FCR ($P < 0.05$). Broiler chickens fed with corn-based diets showed higher BWG ($P = 0.009$), than those fed sorghum-wheat diets while the birds fed wheat-corn diets had similar BWG to those fed corn or sorghum-wheat diets. However, corn yielded 3.8 % lower FCR ($P < 0.001$) than those fed wheat-corn or sorghum-wheat diets.

3.1.3. Finisher phase (28–38 d)

During the finisher phase, the BW, BWG, and FI were significantly ($P < 0.05$) influenced by the interaction of diet and CP levels. While the BW, BWG and FI seemed to be similar across all treatments, the BWG of the birds fed sorghum-wheat diets with LCP40 was significantly lower compared to the other treatments.

The FCR of the birds fed with corn-based diet was 5.7 % and 4.1 % lower ($P < 0.001$) than those fed wheat-corn or sorghum-wheat diets, respectively, while that of birds fed control CP was higher than those fed with LCP20 or LCP40 diets. The similarity in FCR values for control and LCP20 at the grower I and grower II phases, while different ($P < 0.05$) at the finisher phase could indicate that 20 g/kg CP reduction poses no threat to growth performance of broiler chickens at earlier stages of growth but could be debilitating at latter stages.

3.1.4. Cumulative growth performance (8–38 d)

The cumulative growth performance result (Table 6) shows that all the parameters evaluated (BW, BWG, FI, and FCR) were not significantly influenced by the interaction between diets and CP levels. However, the main effects (diets and CP levels) significantly ($P < 0.05$) impacted BW, BWG, FI, and FCR.

The BW and BWG of birds fed with corn-based diet were higher compared with those fed with wheat-corn or sorghum-wheat diets. Broiler chickens fed with corn-based diets showed FI similar to those fed with sorghum-wheat diet, but 2 % lower than those fed with wheat-corn diet on average. Furthermore, the FCR of birds fed with corn-based diet was lowest, followed by those fed with sorghum-wheat, while the birds fed with wheat-corn diets showed the highest FCR.

Although reducing the CP level by 20 g/kg did not have a negative effect on the BW, BWG, and FI of broiler chickens, as their values were comparable to those fed the control CP, the FCR increased ($P < 0.05$) as the CP level decreased.

3.2. Body composition

Table 7 shows the body composition of broiler chickens fed reduced CP diets of varying ingredient sources. It was observed that the interaction of diets and CP levels had significant ($P < 0.05$) impact on all the evaluated parameters, except protein deposition. Protein and fat intake reduced ($P < 0.05$) as the levels of CP reduce for all diet types, although birds fed with wheat-corn diets at control and LCP20 diets have similar fat intake. Also, birds fed with sorghum-wheat diets with 40 g/kg of CP reduction had lowest protein intake.

The fat deposition of broiler chickens was observed to increase as the level of CP decreased, for all diet types. The fat deposition increased by 16.5 %, 8.6 %, and 20.2 %, from control to 20 g/kg CP reduction diet, in corn, wheat-corn, and sorghum-wheat diets, respectively. Wheat-corn diets therefore seem to deposit less fat compared with corn or sorghum-wheat when dietary protein is reduced by 20 g/kg.

For all diet types, ratio of protein deposition of the broiler chickens to intake increased ($P < 0.05$) with decreased CP level. The

Table 5
Growth performance of broiler chickens fed reduced CP diets of varying ingredient combinations at different growth phases.

Treatments			Grower I (8–18 d)				Grower II (18–28 d)				Finisher (28 – 38 d)			
		d8 BW	d18 BW	BWG	FI	FCR	d28 BW	BWG	FI	FCR	d38 BW	BWG	FI	FCR
Diets	CP Levels	g/b					g/b				g/b			
Corn	Control[1]	185	746	561	695^{ab}	1.24^{c}	1635	906	1271^{b}	1.40	2738^{abc}	1140^{abc}	1829^{bc}	1.61
	LCP20[2]	185	749	563	704^{ab}	1.25^{c}	1630	901	1274^{b}	1.41	2743^{ab}	1144^{ab}	1860^{abc}	1.63
	LCP40[3]	185	715	529	710^{ab}	1.34^{a}	1566	837	1288^{b}	1.54	2685^{bc}	1086^{bc}	1861^{abc}	1.71
Wheat & Corn	Control	185	746	560	721^{a}	1.29^{bc}	1611	883	1276^{b}	1.44	2733^{abc}	1134^{abc}	1887^{abc}	1.67
	LCP20	185	736	551	701^{ab}	1.27^{bc}	1611	882	1309^{ab}	1.49	2714^{abc}	1115^{abc}	1946^{a}	1.75
	LCP40	185	704	519	680^{b}	1.31^{ab}	1575	846	1348^{a}	1.60	2642^{cd}	1043^{cd}	1901^{ab}	1.82
Sorghum & Wheat	Control	185	727	543	684^{ab}	1.26^{bc}	1594	866	1269^{b}	1.47	2772^{a}	1174^{a}	1926^{a}	1.64
	LCP20	185	735	550	705^{ab}	1.28^{bc}	1601	872	1289^{ab}	1.48	2698^{bc}	1099^{bc}	1900^{abc}	1.73
	LCP40	185	699	514	691^{ab}	1.35^{a}	1541	812	1276^{b}	1.57	2603^{d}	1005^{d}	1806^{c}	1.79
SEM		0.30	6.80	6.90	8.20	0.016	12.60	12.20	11.90	0.016	24.40	19.60	17.90	0.025
Main effects														
Diets														
	Corn	185	736^{a}	551^{a}	703	1.277	1610^{a}	882^{a}	1277	1.45^{b}	2722	1123	1850	1.65^{b}
	Wheat & Corn	185	729^{ab}	543^{ab}	700	1.289	1599^{ab}	870^{ab}	1310	1.51^{a}	2696	1098	1912	1.75^{a}
	Sorghum & Wheat	185	721^{b}	535^{b}	693	1.295	1579^{b}	850^{b}	1278	1.51^{a}	2691	1092	1877	1.72^{a}
CP Levels														
	Control	185	740^{a}	555^{a}	700	1.26	1613^{a}	885^{a}	1272	1.44^{b}	2747	1149	1881	1.64^{c}
	LCP20	185	740^{a}	555^{a}	703	1.27	1614^{a}	885^{a}	1290	1.46^{b}	2718	1120	1902	1.70^{b}
	LCP40	185	706^{b}	521^{b}	694	1.33	1560^{b}	832^{b}	1304	1.57^{a}	2643	1045	1856	1.78^{a}
SEM		0.20	3.90	4.00	4.70	0.009	7.30	7.00	6.90	0.009	14.10	11.30	10.30	0.014
P-value														
	Diets	0.970	0.002	0.002	0.305	0.134	0.0091	0.009	0.001	< 0.001	0.092	0.092	0.001	< 0.001
	CP Levels	0.766	< 0.001	< 0.001	0.322	< 0.001	0.001	0.001	0.046	< 0.001	< 0.001	< 0.001	0.099	< 0.001
	Diet * CP Levels	0.891	0.566	0.604	0.007	0.028	0.632	0.632	0.047	0.354	0.013	0.013	0.001	0.256

^{abcd}Means with different superscripts across the columns are significantly ($P < 0.05$) different.
BW, Body weight; BWG, Body weight gain; FI, Feed intake; FCR, Feed Conversion Ratio
1Control CP
2LCP20: Control CP – 20 g/kg
3LCP40: Control CP – 40 g/kg

Table 6
Cumulative growth performance of broiler chickens fed reduced CP diets of varying ingredient combinations (8 – 38d).

Treatments		d8 BW	d38 BW	BWG	FI	FCR
Diets	CP Levels	g/b		g/d		
Corn	Control[1]	185	2799	68.76	99.84	1.45
	LCP20[2]	185	2802	68.87	101.37	1.47
	LCP40[3]	185	2572	64.39	100.29	1.56
Wheat & Corn	Control	185	2762	67.82	102.55	1.51
	LCP20	185	2734	67.08	103.61	1.55
	LCP40	185	2573	62.84	101.45	1.62
Sorghum & Wheat	Control	185	2779	68.24	101.34	1.49
	LCP20	185	2708	66.39	102.08	1.54
	LCP40	185	2509	61.16	97.58	1.60
SEM		0.30	24.40	24.40	29.10	0.013
Main effects						
Diets						
	Corn	185	2744^{a}	67.34^{a}	100.50^{b}	1.49^{c}
	Wheat & Corn	185	2690^{b}	65.92^{b}	102.53^{a}	1.56^{a}
	Sorghum & Wheat	185	2665^{b}	65.26^{b}	100.34^{b}	1.54^{b}
CP Levels						
	Control	185	2780^{a}	68.29^{a}	101.24^{ab}	1.48^{c}
	LCP20	185	2748^{a}	67.45^{a}	102.34^{a}	1.52^{b}
	LCP40	185	2572^{b}	62.79^{b}	99.76^{b}	1.59^{a}
SEM		0.20	14.10	14.10	16.80	0.007
P-value						
	Diets	0.970	< 0.001	< 0.001	0.001	< 0.001
	CP Levels	0.766	< 0.001	< 0.001	0.001	< 0.001
	Diet * CP Levels	0.891	0.142	0.143	0.081	0.286

^{abc}Means with different superscripts across the columns are significantly ($P < 0.05$) different.
BW, Body weight; BWG, Body weight gain; FI, Feed intake; FCR, Feed Conversion Ratio
[1]Control CP
[2]LCP20: Control CP – 20 g/kg
[3]LCP40: Control CP – 40 g/kg

broiler chickens fed with corn-based diets at control CP level had lower protein deposition to intake ratio ($P = 0.011$) than both LCP20 and LCP40. The birds fed with wheat-corn diets had similar protein deposition to intake ratio across all CP levels. Similarly, for all diet types, the fat deposition to intake ratio also increased ($P < 0.05$) as the dietary CP level reduced. Broiler chickens fed corn or sorghum-wheat diets at 40 g/kg of CP reduction showed lower fat deposition to intake ratio than those fed with 20 g/kg of CP reduction.

The CP levels significantly ($P < 0.05$) influenced the protein deposition in the broiler chickens. The protein deposition reduced ($P < 0.05$) as the CP levels decreased, although the values recorded for control (13.16 g/d) and LCP20 (12.91 g/d) diets are similar, which follows the result of growth performance where the control had similar result to that of LCP20 diet.

3.3. *Apparent nitrogen excretion*

The interaction between diets and CP levels had no significant effect on the evaluated parameters (Table 8), except nitrogen intake ($P < 0.001$). Irrespective of the diet type, the nitrogen intake decreased progressively as the CP level decreased.

The CP level significantly ($P < 0.05$) impacted the apparent nitrogen excretion, nitrogen retention, and the apparent nitrogen excretion coefficient. The apparent nitrogen excretion decreased ($P < 0.001$) as the CP level decreased, as the broiler chickens fed with control CP level excreted the highest amount of nitrogen and lowest with 40 g/kg of CP reduction. The nitrogen retained was similar for birds fed control and LCP20 but higher than LCP40 group. Interestingly, the coefficient of apparent nitrogen excretion was lower and similar for birds fed 20 and 40 g/kg of CP reduction compared to control CP diets, indicating that birds tend to excrete more nitrogen with increased nitrogen intake.

3.4. *Carcass traits*

The carcass yield and liver were not significantly affected by the interaction between diets and CP levels (Table 9). However, the empty BW, carcass weight, abdominal fat, and breast yield of broiler chickens were influenced ($P < 0.05$) by the interaction between diets and CP level. Birds fed corn or wheat-corn diets at control or 20 g/kg reduced CP levels had higher ($P < 0.05$) empty BW than those fed with 40 g/kg of CP reduction. In contrast, broiler chickens fed sorghum-wheat diet at control CP level showed higher body weight than birds fed with 20 or 40 g/kg of CP reduction.

The carcass weight of broiler chickens fed all diet types were similar for control or LCP20, while the lowest ($P < 0.05$) value was observed for those fed LCP40. The abdominal fat of broiler chickens increased ($P < 0.001$) as the CP level decreased, with corn or sorghum-wheat diets at control and 20 g/kg CP reduction having lower abdominal fat than those fed LCP40 diets. The breast yield was

Table 7
Total body composition of broiler chickens fed reduced CP diets of varying ingredient combinations.

Treatments		Intake		Deposition		Ratio	
Diets	CP Levels	Protein (g/d)	Fat (g/d)	Pd (g/d)	Fd (g/d)	kP (g/g)	kF (g/g)
Corn	Control[1]	26.14[a]	8.74[b]	12.83	7.70[d]	0.49[b]	0.88[d]
	LCP20[2]	23.17[b]	7.58[d]	13.41	8.97[abc]	0.58[a]	1.19[c]
	LCP40[3]	21.63[c]	6.33[e]	12.77	9.04[abc]	0.59[a]	1.42[b]
Wheat & Corn	Control	25.52[a]	8.39[c]	13.56	8.70[bcd]	0.54[ab]	1.07[c]
	LCP20	23.53[b]	8.42[c]	12.77	9.45[ab]	0.54[ab]	1.12[c]
	LCP40	20.95[c]	6.38[e]	11.95	9.05[abc]	0.57[a]	1.41[b]
Sorghum & Wheat	Control	25.66[a]	9.82[a]	13.11	7.92 [cd]	0.51[b]	0.80[d]
	LCP20	23.05[b]	8.18[c]	12.55	9.52[ab]	0.55[ab]	1.18[c]
	LCP40	19.82[d]	6.05 [f]	11.78	10.05[a]	0.59[a]	1.66[a]
SEM		0.168	0.056	0.305	0.295	0.013	0.042
Main effects							
Diets							
	Corn	23.64	7.55	13.00	8.57	0.55	1.64
	Wheat & Corn	23.34	7.73	12.76	9.07	0.55	1.20
	Sorghum & Wheat	22.84	8.02	12.48	9.17	0.55	1.21
CP Levels							
	Control	25.78	8.98	13.16[a]	8.11	0.51	0.92
	LCP20	23.25	8.06	12.91[a]	9.32	0.56	1.16
	LCP40	20.80	6.26	12.17[b]	9.38	0.58	1.50
SEM		0.097	0.033	0.176	0.171	0.008	0.024
P-value							
	Diets	< 0.001	< 0.001	0.0912	0.017	0.914	0.264
	CP Levels	< 0.001	< 0.001	<0.001	< 0.001	< 0.001	< 0.001
	Diet * CP Levels	< 0.001	< 0.001	0.059	0.02	0.011	< 0.001

[abcd]Means with different superscripts across the columns are significantly ($P < 0.05$) different.
Pd – Protein deposition; Fd – Fat deposition; kP – Ratio of protein deposition to intake; kF - Ratio of fat deposition to intake
[1]Control CP
[2]LCP20: Control CP – 20 g/kg
[3]LCP40: Control CP – 40 g/kg

Table 8
Nitrogen excretion of broiler chickens fed reduced CP diets of varying ingredient combinations.

Treatments		N_i (g/d)	N_R (g/d)	AN_e (g/d)	AN_eC (g/g)
Diets	CP Levels				
Corn	Control[1]	4.17[a]	2.05	2.12	0.51
	LCP20[2]	3.70[b]	2.15	1.56	0.42
	LCP40[3]	3.47[c]	2.04	1.43	0.41
Wheat & Corn	Control	4.16[a]	2.19	1.99	0.48
	LCP20	3.77[b]	2.04	1.72	0.46
	LCP40	3.37[c]	1.87	1.49	0.44
Sorghum & Wheat	Control	4.12[a]	2.10	2.02	0.49
	LCP20	3.67[b]	2.01	1.67	0.45
	LCP40	3.17[d]	1.88	1.29	0.41
SEM		0.030	0.0656	0.068	0.018
Main effects					
Diets					
	Corn	3.78	2.08	1.70	0.45
	Wheat & Corn	3.77	2.03	1.73	0.46
	Sorghum & Wheat	3.65	2.00	1.66	0.45
CP Levels					
	Control	4.15	2.11[a]	2.04[a]	0.49[a]
	LCP20	3.71	2.07[a]	1.65[b]	0.44[b]
	LCP40	3.34	1.93[b]	1.40[c]	0.42[b]
SEM		0.017	0.038	0.039	0.010
P-value					
	Diets	< 0.001	0.239	0.359	0.649
	CP Levels	< 0.001	0.002	< 0.001	< 0.001
	Diet * CP Levels	< 0.001	0.161	0.089	0.209

[abcd]Means with different superscripts across the columns are significantly ($P < 0.05$) different.
N_i – Nitrogen intake; N_R – Nitrogen retained; AN_e – Apparent nitrogen excretion; AN*e*C - Apparent nitrogen excretion coefficient
[1]Control CP
[2]LCP20: Control CP – 20 g/kg
[3]LCP40: Control CP – 40 g/kg

Table 9
Carcass traits of broiler chickens fed reduced CP diets of varying ingredient combinations.

				[4]Relative fasted body weight, %			[5]Relative carcass weight, %
Treatments		Fasted body weight (g)	Carcass weight (g)	Carcass yield	Abdominal fat	Liver	Breast yield
Diets	CP Levels						
Corn	Control[1]	2578[a]	1950[ab]	75.64	0.83[d]	1.79	36.36[abc]
	LCP20[2]	2600[a]	1978[a]	76.07	0.91 [cd]	1.83	37.29[a]
	LCP40[3]	2458[c]	1854[c]	75.44	1.24[ab]	2.05	36.23[abc]
Wheat & Corn	Control	2557[ab]	1930[ab]	75.48	0.80[d]	1.87	35.22[bcd]
	LCP20	2528[abc]	1910[abc]	75.55	1.14[b]	2.00	36.79[ab]
	LCP40	2376[d]	1773[d]	74.60	1.13[bc]	2.13	35.19 [cd]
Sorghum & Wheat	Control	2578[a]	1946[ab]	75.52	0.88[d]	1.87	36.28[abc]
	LCP20	2492[bc]	1881[bc]	75.46	0.92 [cd]	1.92	36.00[abc]
	LCP40	2344[d]	1743[d]	74.33	1.36[a]	2.03	34.02[d]
SEM		17.74	16.22	0.320	0.054	0.042	0.412
Main effects							
Diets							
	Corn	2545	1927	75.71[a]	1.00	1.89[b]	36.63
	Wheat & Corn	2487	1871	75.21[ab]	1.02	2.00[a]	35.74
	Sorghum & Wheat	2471	1857	75.12[b]	1.05	1.94[ab]	35.43
CP Levels							
	Control	2571	1941	75.51[a]	0.83	1.85[b]	35.95
	LCP20	2540	1923	75.70[a]	0.99	1.92[b]	36.70
	LCP40	2392	1790	74.81[b]	1.25	2.07[a]	35.15
SEM		10.24	9.365	0.185	0.031	0.024	0.238
P-value							
	Diets	< 0.001	< 0.001	0.018	0.366	0.003	0.001
	CP Levels	< 0.001	< 0.001	<0.001	< 0.001	< 0.001	< 0.001
	Diet * CP Levels	0.014	0.010	0.500	< 0.001	0.473	0.008

[abcd]Means with different superscripts across the columns are significantly ($P < 0.05$) different.
[1]Control CP
[2]LCP20: Control CP – 20 g/kg
[3]LCP40: Control CP – 40 g/kg
[4]% relative fasted body weight
[5]% relative carcass weight

similar across all treatment interactions, with the lowest yield observed in birds fed sorghum-wheat of 40 g/kg CP reduction. Birds fed corn-based diets had similar breast yield for all CP levels despite reduction of CP up to 40 g/kg of CP reduction, while wheat-corn and sorghum-wheat only recorded similar values for control and LCP20. The relative liver weights ($P = 0.003$) of birds fed corn-based diet was lower than those fed wheat-corn diet, while the value recorded for the sorghum-wheat diet was similar to the other two diets. Control and LCP20 resulted in lower relative liver weight compared with birds fed with LCP40.

3.5. Apparent ileal nutrient digestibility coefficients

3.5.1. CP and energy

The CP digestibility coefficient, energy digestibility coefficient, and AME of the broiler chickens were not significantly influenced by the interaction between diets and CP levels (Table 10). However, diet type influenced ($P < 0.05$) all parameters evaluated, while the CP levels influenced ($P < 0.001$) the CP digestibility. The CP digestibility of broiler chickens fed corn or wheat-corn diets was similar and higher compared with birds fed with sorghum-wheat diet. Also, birds fed control and LCP20 showed lower CP digestibility compared with LCP40. The energy digestibility coefficient of broiler chickens fed corn-based diet was lower compared with those fed wheat-corn or sorghum-wheat diets. In contrast, broilers fed corn-based had higher AME compared with the birds fed the other two diets.

3.5.2. Amino acids

While the apparent digestibility of 13 (methionine, lysine, threonine, arginine, leucine, leucine, valine, histidine, glycine, serine, alanine, aspartate, and glutamate) of the 16 AAs considered in this study were not significantly influenced by the interaction between diets and CP levels, three (cysteine, phenylalanine, and proline) were significantly ($P < 0.05$) impacted (Tables 11.1 and 11.2). Across all diets, the digestibility coefficients of cysteine ($P = 0.033$), phenylalanine ($P = 0.006$), and proline ($P = 0.011$) were similar for birds fed with control, LCP20 and LCP40 diets, respectively. Also, for all the AA digestibility coefficients (cysteine, phenylalanine, and proline) of broiler chickens affected by the interaction of diet and CP levels, corn and wheat-corn yielded similar and best digestibility at all CP levels.

Table 10
Apparent ileal nutrient digestibility coefficients of broiler chickens fed reduced CP diets of varying ingredient combinations at d 40.

Treatments		Digestibility coefficients		
Diets	CP Levels	CP	Energy	AIDE (MJ/kg DM)
Corn	Control[1]	0.79	0.73	11.64
	LCP20[2]	0.82	0.76	11.64
	LCP40[3]	0.82	0.76	11.52
Wheat & Corn	Control	0.79	0.67	10.54
	LCP20	0.77	0.65	10.37
	LCP40	0.83	0.68	10.26
Sorghum & Wheat	Control	0.75	0.67	11.09
	LCP20	0.76	0.70	10.83
	LCP40	0.79	0.73	11.02
SEM		0.013	0.019	0.297
Main effects				
Diets				
	Corn	0.81^{a}	0.75^{a}	11.60^{a}
	Wheat & Corn	0.80^{a}	0.67^{b}	10.39^{c}
	Sorghum & Wheat	0.76^{b}	0.70^{b}	10.98^{b}
CP Levels				
	Control	0.78^{b}	0.69	11.09
	LCP20	0.78^{b}	0.70	10.95
	LCP40	0.81^{a}	0.72	10.93
SEM		0.008	0.011	0.172
P-value				
	Diets	<0.001	< 0.001	< 0.001
	CP Levels	0.002	0.150	0.761
	Diet * CP Levels	0.367	0.557	0.975

[abcd]Means with different superscripts across the columns are significantly ($P < 0.05$) different.
CP– Crude protein; AIDE – Apparent ileal digestible energy; DM – Dry matter
[1]Control CP
[2]LCP20: Control CP – 20 g/kg
[3]LCP40: Control CP – 40 g/kg

Table 11.1
Apparent ileal amino acid digestibility coefficients of broiler chickens fed reduced CP diets of varying ingredient combinations at d 40.

Treatments		Methionine	Cysteine	Lysine	Threonine	Arginine	Isoleucine	Leucine	Valine
Diets	CP Levels								
Corn	Control[1]	0.95	0.73^{ab}	0.89	0.79	0.92	0.86	0.85	0.84
	LCP20[2]	0.95	0.73^{ab}	0.89	0.79	0.91	0.85	0.85	0.84
	LCP40[3]	0.96	0.73^{ab}	0.92	0.83	0.93	0.89	0.86	0.87
Wheat & Corn	Control	0.91	0.73^{ab}	0.87	0.76	0.88	0.84	0.81	0.81
	LCP20	0.92	0.73^{ab}	0.86	0.75	0.86	0.81	0.78	0.79
	LCP40	0.94	0.79^{a}	0.91	0.81	0.90	0.88	0.83	0.85
Sorghum & Wheat	Control	0.90	0.70^{b}	0.86	0.73	0.87	0.79	0.76	0.76
	LCP20	0.91	0.70^{b}	0.89	0.76	0.87	0.81	0.78	0.79
	LCP40	0.94	0.68^{b}	0.91	0.79	0.89	0.84	0.78	0.82
SEM		0.005	0.016	0.008	0.013	0.007	0.010	0.012	0.011
Main effects									
Diets									
	Corn	0.95^{a}	0.73	0.90^{a}	0.80^{a}	0.92^{a}	0.87^{a}	0.85^{a}	0.85^{a}
	Wheat & Corn	0.92^{b}	0.75	0.88^{b}	0.78^{b}	0.88^{b}	0.84^{b}	0.81^{b}	0.82^{b}
	Sorghum & Wheat	0.92^{b}	0.69	0.88^{b}	0.76^{b}	0.88^{b}	0.81^{c}	0.77^{c}	0.79^{c}
CP Levels									
	Control	0.92^{b}	0.72	0.87^{b}	0.76^{b}	0.89^{ab}	0.83^{b}	0.81	0.80^{b}
	LCP20	0.92^{b}	0.72	0.88^{b}	0.77^{b}	0.88^{b}	0.83^{b}	0.80	0.81^{b}
	LCP40	0.94^{a}	0.73	0.92^{a}	0.81^{a}	0.90^{a}	0.87^{a}	0.82	0.85^{a}
SEM		0.003	0.009	0.004	0.008	0.004	0.006	0.007	0.006
P-value									
	Diets	< 0.001	<0.001	<0.001	<0.001	< 0.001	< 0.001	< 0.001	< 0.001
	CP Levels	< 0.001	0.386	< 0.001	< 0.001	0.001	< 0.001	0.167	< 0.001
	Diet * CP Levels	0.057	0.033	0.191	0.548	0.507	0.103	0.199	0.073

[abcde]Means with different superscripts across the columns are significantly ($P < 0.05$) different.
[1]Control CP
[2]LCP20: Control CP – 20 g/kg
[3]LCP40: Control CP – 40 g/kg

Table 11.2
Apparent ileal amino acid digestibility coefficients of broiler chickens fed reduced CP diets of varying ingredient combinations at d 40.

Treatments		Histidine	Phenylalanine	Glycine	Serine	Proline	Alanine	Aspartate	Glutamate
Diets	CP Levels								
Corn	Control[1]	0.85	0.88[ab]	0.77	0.84	0.80[bcd]	0.85	0.85	0.88
	LCP20[2]	0.84	0.87[ab]	0.75	0.83	0.81[bc]	0.86	0.83	0.88
	LCP40[3]	0.85	0.88[ab]	0.80	0.83	0.80[bc]	0.86	0.84	0.89
Wheat & Corn	Control	0.84	0.85[bc]	0.76	0.82	0.83[ab]	0.80	0.81	0.88
	LCP20	0.80	0.83 [cd]	0.73	0.79	0.82[bc]	0.75	0.76	0.87
	LCP40	0.84	0.90[a]	0.82	0.82	0.88[a]	0.80	0.78	0.92
Sorghum & Wheat	Control	0.81	0.81[d]	0.75	0.78	0.77 [cd]	0.74	0.78	0.83
	LCP20	0.79	0.82 [cd]	0.75	0.77	0.78 [cd]	0.77	0.77	0.85
	LCP40	0.80	0.85[bc]	0.82	0.76	0.76[d]	0.78	0.76	0.85
SEM		0.013	0.009	0.013	0.012	0.011	0.013	0.013	0.008
Main effects									
Diets									
	Corn	0.85[a]	0.88	0.77	0.83[a]	0.81	0.86[a]	0.84[a]	0.88[a]
	Wheat & Corn	0. 83[b]	0.86	0.77	0.81[b]	0.84	0.78[b]	0.78[b]	0.89[a]
	Sorghum & Wheat	0.80[c]	0.83	0.77	0.77[c]	0.77	0.76[b]	0.77[b]	0.84[b]
CP Levels									
	Control	0.83	0.85	0.76[b]	0.81	0.80	0.80	0.81	0.86[b]
	LCP20	0.81	0.84	0.74[b]	0.79	0.80	0.79	0.79	0.87[b]
	LCP40	0.83	0.88	0.81[a]	0.80	0.81	0.81	0.80	0.88[a]
SEM		0.006	0.005	0.007	0.007	0.006	0.007	0.007	0.005
P-value									
	Diets	< 0.001	< 0.001	0.944	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	CP Levels	0.075	< 0.001	< 0.001	0.228	0.451	0.139	0.096	0.001
	Diet * CP Levels	0.363	0.006	0.103	0.406	0.011	0.127	0.590	0.068

[abcd]Means with different superscripts across the columns are significantly ($P < 0.05$) different.
[1]Control CP
[2]Control CP – 20 g/kg
[3]Control CP – 40 g/kg

Corn had the highest digestibility coefficient for all the AAs that were significantly influenced by the diet types, except glutamate that had similar digestibility value for corn and wheat-corn diets. While the digestibility coefficients of birds fed wheat-corn and sorghum-wheat were similar but lower for methionine, lysine, threonine, arginine, alanine, and aspartate, the digestibility values of the branched chain AAs (isoleucine, leucine, valine), histidine, and serine were significantly ($P < 0.05$) different for the three diet types, with birds fed sorghum-wheat having the poorest digestibility coefficients while those fed corn had the highest (best). While the digestibility coefficients of glycine were similar for corn, wheat-corn, and sorghum-wheat, serine digestibility was different among the diet types.

The birds fed control and 20 g/kg of CP reduction diets had similar but lower methionine, lysine, threonine, arginine, isoleucine, valine, glycine, and glutamate digestibility, compared with diets of 40 g/kg CP reduction.

4. Discussion

Studies (Chrystal et al., 2021; Liu et al., 2021) have consistently identified corn as the superior choice for implementing reduced CP diets in broiler chicken nutrition and our present investigation supports this. However, given that diverse ranges of cereal combinations are used worldwide (Ravindran, 2013), it is imperative to consider the limits of other cereals other than corn, such as sorghum, wheat, and their combinations, when exploring the limits of dietary protein reduction in broiler diets.

During the early stages (8 – 28 days) of the birds, the FI and FCR remained comparable across all dietary groups, even with a reduction of up to 20 g/kg CP but got worse at the finisher phase (28 – 38 d). Presence of kafirin and condensed tannins in sorghum (Liu et al., 2015) and non-starch polysaccharide (**NSP**) fractions in wheat (Ravindran and Amerah, 2009) has been attributed to the differences usually observed in performance of birds fed reduced protein diets; however, the uniformity in early-stage performance observed in the current study raises intriguing questions, as the birds were able to perform optimally when younger. Liu et al. (2014) reported a 5 % and 9 % higher nitrogen (protein) digestibility in corn-based diets compared to wheat-based and sorghum-based diets in their study. Perhaps, the declined performance observed in birds fed wheat-corn and sorghum-wheat diets when older could be attributed to inability of the available protein to support their growth as they grew older.

Liu et al. (2021) reported in their review that CP can be reduced in broiler chicken diets by up to 3 % and this was supported by the study of Chrystal et al. (2021) when they found that birds fed 2.9 % reduced dietary CP had similar FI and FCR to those fed control CP. Although the result of the current study corroborates the aforementioned reports when the broiler chickens were young (d 8 – 28), the slightly increased feed intake of the wheat-corn medium CP diet at the finisher phase caused a slight discrepancy in the feed conversion ratio, thus resulting in higher FCR with 20 g/kg CP reduction at the finisher (28 – 38 d) phase and overall (8 – 38 d) period. Although our result for growth performance does not totally resonate with the reports of Chrystal et al. (2021) and Liu et al. (2021), it is similar to the result of some studies (Maynard et al., 2021; van Harn et al., 2019) that found differences in the FCR of birds fed diets of up to

20 g/kg and 40 g/kg CP reduction. While Chrystal et al. (2021) designed their experiment to have only wheat or corn, and not a combination, which was also the view of Liu et al. (2021), other experiments (Maynard et al., 2021; van Harn et al., 2019) had ingredient combinations (wheat and corn) similar to our study. Therefore, the results obtained could be attributed to the ingredient combinations employed in this study. Considering the result obtained for the cumulative growth performance where the BW, BWG, and FI were similar for control and 20 g/kg dietary CP reduction, and other parameters of this study supporting 20 g/kg CP reduction, the result of the FCR alone might not be enough to conclude that 20 g/kg CP reduction is not sustainable.

The decrease in protein and fat intake as dietary protein decreases is an expected outcome, given that FI appears to remain relatively constant as dietary CP levels decline (Table 7). Simultaneously, the fat content of the diet also decreases, as evident from Tables 1, 2, and 3. Therefore, it is logical to anticipate that a reduction in dietary protein and fat content would correspondingly lead to a decrease in their intake. The decline in protein deposition as CP levels decrease aligns with the growth performance trend during the early stages (days 8–28). Notably, the values recorded for control (13.16 g/d) and 20 g/kg CP reduction (12.91 g/d) were statistically similar during this phase. The lowest protein deposition in the birds occurred when dietary CP was reduced by 40 g/kg. Waldroup (2007) proposed that the subpar performance of broilers on reduced CP diets could be attributed to an insufficient nitrogen pool for synthesizing non-essential AAs, AA imbalances, and the deamination of excess AAs, leading to the accumulation of excessive ammonia (Selle et al., 2020). Although our study ensured that all essential AA requirements were met, the reduced protein deposition observed in birds fed the lowest CP level may be a consequence of potential imbalances.

Li et al. (2019) opined that extra glucose produced after the consumption of an excessive amount of carbohydrates by mammals is used to create glycogen, which is then stored in the liver. However, if the glucose concentration is more than necessary to produce glycogen and energy, the leftover glucose molecules are converted to fat (Zhang et al., 2010). Thus, excess starch (glucose) borne from the reduction of dietary CP of broiler chickens is converted to fat. This process with other fates of dietary glucose under conditions of chronically high energy is well described by Alemany (2011) and Li et al. (2019). According to Gous et al., (1990)), broiler chickens fed reduced CP diets consume more energy than protein and deposit the excess energy as lipid. The progression of the fat deposition with reducing CP level follows the trend of the abdominal fat in the study, which is reasonable, as the reduction in dietary CP reduces dietary fat (and decreased heat increment [**HI**]) but increases dietary starch composition as shown in the diet composition for the broiler chickens (Tables 1, 2, and 3). The result of the present study is similar to those presented by several authors (Allameh and Toghyani, 2019; Chrystal et al., 2021, 2020b; Hilliar et al., 2020) that reported increased abdominal fat pad, and also to that of Namroud et al. (2008) who found increased fat deposition and abdominal fat pad with decreased dietary CP in broiler chickens' diets. For all diet types (ingredient combinations), the increased ratio of protein deposition to intake with decreased CP level could be attributed to an innate mechanism activated by the birds to improve the efficiency of utilizing the dietary protein thus coping with the diminishing dietary protein. The ratio of fat deposition to intake also increased as the dietary CP level reduces, and most of the values are above 1. This buttresses the fact that excess starch from reduced CP diets is metabolized to fat in broiler chickens.

Protein deposition in broiler chickens is limited by their genetic potential, and excess N is excreted (Woyengo et al., 2023). In this study, the N excretion was not determined from the excreta but rather calculated as an apparent excretion from difference between the body N retention and intake. Reducing dietary CP of broiler chickens by 20 g/kg and 40 g/kg in this study reduced the apparent nitrogen excretion by 19 % and 31 %, respectively. This result corroborates several studies (Hofmann et al., 2019; Lemme et al., 2019; Macelline et al., 2020; van Harn et al., 2019), showing that reducing dietary CP in broiler nutrition is one of the ways to reduce the contribution of poultry to carbon footprint, as opined by Liu et al. (2021). Although the apparent N excretion reduces with reduced CP level, the coefficient of apparent N excretion (relative to N intake) in this study only differed between control and reduced CP diets. There was no significant difference between the N excretion of the two reduced CP levels. This implies that lesser N is excreted when dietary protein reduces, which supports the overall aim of reduced protein in broiler diets.

The impact of reduced dietary CP on the carcass yield of broiler chickens has been reported in several studies (Brink et al., 2022; Hilliar et al., 2020; Lemme et al., 2019; van Harn et al., 2019), albeit with consideration of different yield parameters. In this study, for all three ingredient combinations (diet types), it was found that the live and carcass weights are similar for control and 20 g/kg protein reduction. This follows the trend recorded for the BWG of the growth performance, thus supporting the fact that 20 g/kg protein reduction, if supplemented with the limiting AAs, is not inimical. Although the carcass yield was not significantly affected by diet and CP level interaction, the highest yields were recorded for control and 20 g/kg CP reduction. The breast yielded similar values for corn-based diets despite the reduction of dietary CP up to 40 g/kg. In comparison, wheat-corn and sorghum-wheat only recorded similar values for control and moderate CP reduction (up to 20 g/kg reduction). This once again supports the claim of Chrystal et al. (2021) that a corn-based diet is best for reduced CP in broiler chickens.

Limited literature is available concerning the impact of reduced protein diets on the liver of broiler chickens. In our study, we observed an increase in liver size as the CP level decreased. This may indicate an augmented workload on the liver, stemming from the conversion of excess starch (glucose) into fatty acids, a process intensified by the higher inclusion of grains in reduced CP diets. Regardless of the specific dietary composition, the abdominal fat in broiler chickens increased with decreasing CP levels, signifying that the greater starch content resulting from reduced CP led to increased fat deposition. This consistent rise in abdominal fat as CP levels decreased aligns with the findings of other studies of Allameh and Toghyani (2019), Chrystal et al. (2021), (2020b), Hilliar et al. (2020), Namroud et al. (2008), among others. Despite formulating the diet with similar apparent metabolizable energy values, we observed that the effective energy (**EE**) values of the diets differed (increased as the CP level decreases), due to the decrease in the heat increment of the diets. The EE is similar to the net energy (**NE**) system (Barzegar et al., 2020). It has been shown in pigs (Batorek-Lukač et al., 2021; Moreira et al., 2021), and laying hens (Barzegar et al., 2019), that fat deposition increases with increased NE. Perhaps, formulating reduced CP diets in broilers using either the EE or NE system (considering the optimal value of the control CP group as standard) may be helpful to combat the increased fat deposition of reduced CP diets in broiler nutrition, thus allowing more energy for transamination and deamination of excess AAs (Namroud et al., 2008), rather than starch metabolism to fat.

In this study, corn-based and wheat-corn-based diets demonstrated superior protein digestibility coefficients compared to sorghum-wheat diets in broiler chickens. This aligns with the findings of Liu et al. (2014), who reported higher protein digestibility in corn compared to wheat and sorghum. Our results confirm the superior digestibility of corn, which corresponds to previous findings. The improved performance of the wheat-based diet compared to sorghum, however, may be attributed to the small amount of corn present in the wheat-corn diet. Although the apparent CP digestibility increases as the CP level reduces, the control and 20 g/kg CP reduction have similar values. Regarding energy digestibility, corn outperformed the other feed ingredients in this study.

The similar AA (cysteine, phenylalanine, and proline) digestibility coefficients recorded for birds fed corn-based diets at either control, LCP20 or LCP40, compared with the other diets supports the superiority of corn while feeding reduced CP diets to broiler chickens. It has been demonstrated that the ileal digestibility of isoleucine, valine, and phenylalanine were best for corn, compared with sorghum or wheat (Lemme et al., 2004), which the current result corroborates for all the AAs tested. The increased AA digestibility observed with decreased CP levels observed in this study has been reported by several studies using either corn-based (Chrystal et al., 2020b, 2020c), wheat-corn (Chrystal et al., 2021), or wheat-sorghum (Hilliar et al., 2020) diets. Although Chrystal et al. (2020c) attributed this to the increased unbound proportion in the formulation as dietary CP reduces, compared to the protein-bound proportion, Hilliar et al. (2020) reported that this reflects the increased inclusion of feed-grade crystalline AAs in reduced CP diets. While the arguments are relevant, there is also a possibility that the need for the birds to meet up with requirements of the limiting nutrients could have pushed them to improve nutrient utilization, and consequently CP digestibility in the reduced CP diets. This is supported by the fact that control and LCP20 in the current study yielded similar results, which corresponds with the growth performance, carcass trait, nitrogen excretion, CP digestibility, and protein deposition results, indicating that the birds could not have struggled with 20 g/kg CP reduction, but a critical reduction (above 20 g/kg) could be debilitating.

The results of the study indicate that corn remains the best-suited energy source for reduced CP broiler diet, but the performance of wheat-corn and sorghum-wheat diets were not far off. Perhaps supplementing the wheat-corn and sorghum-wheat diets with xylanase and phytase could have pushed the performance to be on par with corn-based diet. While the fat deposition of broiler chickens fed a sorghum-wheat diet with a 20 g/kg CP reduction was comparable to those fed corn or wheat-corn diets of the same CP level, the cumulative FCR of the sorghum-wheat diet was slightly better than that of the wheat-corn diet. Despite this, the carcass weight and breast yield of both diets (wheat-corn and sorghum-wheat) were similar with a 20 g/kg CP reduction. Therefore, although their yields are not comparable to corn, the result suggests that it is possible to feed wheat-corn or sorghum-wheat diets to broiler chickens at a 20 g/kg CP reduction. Furthermore, 20 g/kg dietary CP reduction reduced soybean meal inclusion, on average, by 20.57 %, 27.24 %, and 23.23 %, in corn, wheat-corn, and sorghum-wheat diets, respectively. Although profit is the main driving factor of the poultry industry, FCR may not be the only factor to consider when concluding on the optimal level to which CP can be reduced in broiler diets.

5. Conclusion

The result of this study suggests that reducing dietary crude protein in broiler diets by 20 g/kg allows the reduction of nitrogen excretion without negatively impacting carcass yield, breast yield, digestibility (crude protein and amino acid), and protein deposition. Furthermore, feeding broilers with combinations of reduced protein wheat-corn or sorghum-wheat diets has the potential to yield performance similar to corn. Also, further novel feed formulation and dietary strategies that consider the interrelationship between the starch digestion rate (slowly or rapidly digestible), starch:protein digestive dynamics, amino acid digestion rate and energy metabolism could help better understand and perhaps improve nutrient utilization and consequently performance of birds on reduced protein diets when feed cereal grains other than corn are utilized.

CRediT authorship contribution statement

Damilola U. Kareem: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Juliano César P. Dorigam**: Conceptualization, Project administration, Methodology, Funding acquisition, Writing - review & editing. **Matheus P. Reis**: Conceptualization, Methodology, Writing - review & editing. **Rony Riveros**: Investigation, Data curation, Writing - review & editing. **Audasley T. Fialho**: Investigation, Formal Analysis. **Larissa M. Pereira**: Investigation, Formal Analysis. **Rosiane S. Camargos**: Investigation, Formal Analysis. **Bruno B. Leme**: Investigation, Formal Analysis. **Nilva K. Sakomura**: Conceptualization, Methodology, Funding acquisition, Supervision, Project administration, Writing - review & editing.

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Declaration of Competing Interest

The authors declare no financial or personal relationships that could 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. Although, the second author, Juliano Cesar P. Dorigam is an employee of the funding body of this research, Evonik Operations GmbH, Hanau-Wolfgang, Germany, his involvement was transparent and does not compromise the integrity of the findings.

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