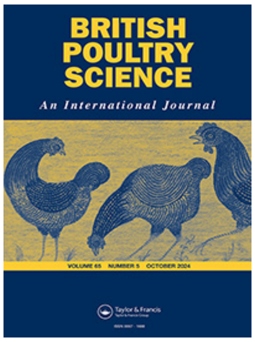

British Poultry Science

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/cbps20

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To cite this article: M. Naeem, E. J. Burton, D. V. Scholey, A. Alkhtib & S. Broadberry (2024) Efficacy of maize differing in particle size in low-density protein diets fed to broilers from day 1 to 21 of age, British Poultry Science, 65:5, 615-624, DOI: 10.1080/00071668.2024.2356655

To link to this article: https://doi.org/10.1080/00071668.2024.2356655

Published online: 24 May 2024.

BRITISH POULTRY SCIENCE
2024, VOL. 65, NO. 5, 615–624
https://doi.org/10.1080/00071668.2024.2356655

Efficacy of maize differing in particle size in low-density protein diets fed to broilers from day 1 to 21 of age

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ABSTRACT

1. This study evaluated the efficacy of maize differing in particle size in low-density protein diets on performance, the digestive tract and litter characteristics in broilers. Four dietary treatments; control commercial diet with a typical crude protein content 22.50% (CON); CON + 20% maize with particle size 350 µm (M350), crude protein 19.90%; CON + 20% maize with particle size 2600 µm (M2600), crude protein 19.90%; CON + 20% whole maize (WM), crude protein 19.90%, were fed *ad libitum* to broiler chicks up to 21 d of age.
2. No differences in body weight gain, feed intake and FCR were found between the WM and CON. WM increased body weight gain compared to M350 and M2600. M350, M2600 and WM increased ($p = 0.004$) gizzard relative weight compared with CON on day 14. Both WM and M2600 reduced ($p = 0.001$) gastric isthmus diameter on d 14 and 21 compared with CON and M350.
3. No differences were seen in mucosa for the *Lamina propria* and the extent of *Tunica muscularis* of gizzard on d 21 and ileum mucosal depth on d 14 between WM and CON diets. However, WM reduced villus-to-crypt ratio compared with CON on d 21. The M350 reduced ($p < 0.05$) gizzard digesta particle size compared with CON, M2600 and WM on d 14.
4. Both WM and M350 decreased ($p < 0.05$) nitrogen excretion compared to birds fed CON. Feeding WM increased nitrogen efficiency compared with M350 and M2600 diets, but was similar to birds fed CON. Feeding M350, M2600 or WM decreased ($p < 0.05$) litter moisture and footpad dermatitis (FPD) scores compared with results from birds fed CON.
5. Overall, diluting the protein level in broiler diets with whole maize appeared better than fine or coarse maize in terms of growth performance, digestive tract development, nitrogen excretion and litter parameters. This may lead to economic benefits by reducing grinding costs and dependence on rich protein resources contributing to sustainable meat production and food security.

ARTICLE HISTORY
Received 27 November 2023
Accepted 11 April 2024

KEYWORDS
Low-protein; particle size; environmental footprint; food security; sustainability

Introduction

Up to 70% of the overall cost of producing chicken goes into feeding the animals. About 95% of the overall cost is spent on supplying necessary protein and energy (Ravindran 2013). Protein sources used for poultry diets can be expensive (Jahan et al. 2006). Plant protein sources are being used in diets for animals and humans, creating competition between them (Liu et al. 2015). Reducing the protein content in poultry diets can be a useful strategy to lower the excretion of nutrients, especially nitrogen, which improves the environmental footprint (Greenhalgh et al. 2020). Providing low-protein diets to poultry could be effective nutritional management to achieve this target.

Studies have shown that poultry growth performance can be impaired by a decrease in protein content in diets, especially when there are no extra amino acids added to balance the diets (Incharoen et al. 2010; Kamran et al. 2008; Laudadio et al. 2012; Lemme et al. 2019). However, it has been reported that supplementing extra amino acids regained any lost performance (Bennett and Classen 2003; Chrystal et al. 2020; Ullrich et al. 2018; van Harn et al. 2019). Diluting the protein content in poultry diets is a nutritional strategy that can lower content in diets if the diluent has low protein.

Cereal grains *e.g.*, wheat and maize, are normally high in carbohydrate but low in protein (Leeson and Summers 2001) which can be used to dilute protein density. Researchers have reported impaired growth performance of poultry when diets were diluted with an increasing inclusion level of whole wheat or crumbled wheat (Bennett and Classen 2003; Bennett et al. 1995).

Economic costs may be reduced by decreasing the grinding cost of feed ingredients during milling as they can be supplied in a coarse form or including them whole in poultry feeds. It has been demonstrated that coarse particles are ground into finer forms in the gizzard (Hetland, Svihus, and Olaisen 2002) and that this has no effect on transit rate through the digestive system (Svihus et al. 2002). This suggests that, from a nutritional standpoint, chicken can be fed coarser grain than currently. Reece et al. (1986) showed that increasing the sieve size from 4.76 to 6.35 mm lowered the energy cost of hammer mill grinding maize from 2.9 to 2.2 kWh/ton.

Recently, particle size has been reported to improve performance of poultry by promoting foregut development, which plays a vital role in the digestion and utilisation of nutrients (Amerah et al. 2008; Zaefarian et al. 2016). Studies conducted on the particle size of structural components *e.g.*, grains or fibrous material (cereal hulls and husks) evaluated the effect of particle size on poultry performance in standard-level protein diets and reported beneficial effects (Amerah, Ravindran, and Lentle 2009; Lv et al. 2015). For

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This article has been corrected with minor changes. These changes do not impact the academic content of the article.

example, Lv et al. (2015) fed broilers mash diets containing fine, medium and coarse particles from 1 to 40 d of age and found that medium and coarse particle sizes resulted in higher ($p < 0.05$) body weight, daily gain and daily feed intake compared with fine particles. Similarly, Mahdavi Sadati et al. (2022) fed broiler chickens with ground, pelleted and whole wheat and observed that whole wheat improved ($p < 0.05$) feed conversion ratio (FCR) during starter, grower and finisher phases.

It can be hypothesised that growth performance of broilers fed low protein diets may be mitigated by increasing the efficiency of the digestive tract with the inclusion of structural components (coarse or whole grains). Hence, the current study was conducted to investigate the efficacy of maize differing in particle size in diluting the protein density of broiler diets, as maize is much lower in protein.

Materials and methods

Ethical statement

The experiment was approved by the Ethical Review Committee of the School of Animal, Rural and Environmental Sciences, Nottingham Trent University (Approval Code: ARE 894) and carried out at the Poultry Research Unit of Nottingham Trent University.

Bird husbandry

A total of 336 male broiler chicks (Ross 308) were obtained from PD Hook Hatchery (Cote, Oxfordshire, UK) and allocated to 28 pens containing 12 chicks each. Four dietary treatments were randomly assigned, giving seven pens per treatment. The experimental pens (each with floor area of 0.64 m^2) were bedded on softwood shavings approximately 3 cm in depth. All the chicks had *ad libitum* access to feed and water throughout the experimental period of 21 d. Birds were fed with starter crumbs for the first week (d 0–7) and then grower pellets for the other 2 weeks (d 8–21). A 1 h dark period was provided on d 1 then increased each day by 1 hour up to 6 h maintained from d 6 to 21. On chick placement, the temperature was set at 32°C which was then reduced by 0.5°C every day up to 21.5°C, on d 21. Temperature and minimum ventilation requirements were met throughout the experimental period, according to Ross 308 guidelines (Aviagen 2018). Twice-a-day checks were made to make sure birds were comfortable with eating and watering, and ventilation and temperature were adequate. Minimum and maximum temperatures within 24 h were recorded.

Dietary treatments

Commercially formulated and manufactured diets served as the control in the starter and grower phases and were obtained from the local feed mill (GLW Feeds, Loughborough, UK). Separate maize (fine or coarse ground or whole grain) was obtained from the same feed mill. A geometric mean diameter (GMD) of 350 µm was obtained by grinding whole maize using a hammer mill fitted with a screen size of 1.00 mm while a GMD of 2600 µm using a hammer mill fitted with a screen size of 7.5 mm.

The addition of maize replaced 20% of the control diet (CON) resulting in a total of four diets; CON (commercial pellet diet, crude protein 22.5%), M350 (CON +20% maize of GMD 350 µm, crude protein 19.90%), M2600 (CON +20% maize of GMD 2600 µm, crude protein 19.90%) and WM (CON +20% whole maize, crude protein 19.90%). Post-pellet inclusion of maize was performed to eliminate any confounding effect of heat treatment or partial grinding on cereal particle size due to the pelleting process. This has been reported to reduce the particle size of pelleted feed significantly in previous studies (Amerah et al. 2007b; Naeem et al. 2024; Svihus et al. 2004). The composition of basal diets is provided in Table 1.

Particle size calculation

Particle analysis for maize, gizzard digesta and residual feed was performed by particle size distribution obtained through dry or wet sieving using a vibratory shaker (Retsch®, Germany) stacked with seven sieves. The sieves were placed in the sequence being the largest on top (4.0, 2.0, 1.0, 0.5, 0.25, 0.125 and 0.063 mm) with a feed sample weight of 100 g, sieving time of 15 min, and amplitude 3.0 mm/g. Particle size as geometric mean diameter (GMD) was calculated by the method described by Baker and Herrman (2002) as follows;

Average diameter of the two sieves $(d_i) = (d_u \times d_o)^{1/2}$

$$\text{GMD} = \log^{-1}\left[\sum(W_i \log d_i) \div \sum W_i\right]$$

where d_i is the diameter of the i^{th} sieve in the stack, d_u the diameter opening through which particles will pass (sieve proceeding i^{th}), d_o the diameter opening through which particles will not pass (i^{th} sieve), W_i the weight of the sample retained on each sieve and d_{gw} the geometric mean diameter (GMD).

Growth performance, nitrogen utilisation, footpad dermatitis scores, and litter sampling

The growth performance of broilers was calculated by measuring the feed intake, body weight gain and feed conversion ratio (FCR) for each pen. The FCR was not adjusted for mortality to represent field conditions. Any mortality and weight of dead chickens were recorded daily. Nitrogen efficiency and excretion were calculated following a method described by Belloir et al. (2017). Footpad dermatitis (FPD) scoring was performed by following the guidance of Aviagen (de Jong and van Harn 2012) on d 21. Within each pen, a litter sample (approximately 50 g each) was picked from five points on d 20; corners and centre, and mixed well to create one homogenous sample for moisture and nitrogen analysis.

Bird sampling and digestive organ measurements

Two birds, representing the average weight of the pen, were sampled from each pen by cervical dislocation on d 14 and 21 of age. Immediately post-euthanasia, the digestive organs from each bird were excised and digesta contents were removed carefully from the proventriculus, gizzard and intestinal sections to record their empty weights. A vernier calliper was used to measure the gastric isthmus

Table 1. Ingredients (%) and calculated nutritional composition of basal starter and grower with analysed nutrient values given in brackets where available.

Ingredient	Basal stater (days 0–7)	Basal grower (days 8–21)
Wheat	42.94	46.50
Soybean meal	23.30	34.50
Rapeseed (whole)	–	6.60
Maize	15.00	–
Maize DDGS	–	4.76
Wheat feed	5.00	–
Barley	5.00	–
Sunflower meal	4.00	–
Soyabean oil	1.20	3.45
Calcium carbonate	0.97	0.85
Monocalcium phosphate	0.97	–
Dicalcium phosphate	–	1.85
Salt	0.27	0.30
Lysine-HCl	0.37	0.39
Methionine-DL	0.26	0.30
Premix[1]	0.50	0.50
Sodium bicarbonate	0.10	–
Elancoban G200	0.05	–
L-Threonine	0.03	–
Roxazyme G2G liquid 35.7%	0.03	–
Hiphos (L) 10000 (Liquid)	0.01	–
Calculated Nutrients		
DM (%)	88 (87.32)	88 (88)
CP (%)	23 (22.50)	22 (22.50)
ME	12MJ/kgDM	12.5MJ/kgDM
Crude fat (%)	3.60	(5.02)
Fibre (%)	4.63	(2.78)
Ash (%)	5 (4.49)	5 (4.74)
Lysine (%)	1.32	1.32
Methionine (%)	0.49	0.65
Methionine + Cystine	0.95	0.87
Threonine (%)	0.86	0.77
Isoleucine (%)	0.86	0.78
Valine (%)	0.96	0.86
Arginine (%)	1.37	1.23
Tryptophan (%)	0.20	0.18
Leucine (%)	1.41	1.27
Sodium (%)	0.14	(0.13)
Available phosphorus (%)	0.62	(0.44)
Calcium (%)	0.81	(0.64)

[1]Premix per kg of the diet: Vitamins; A: 8000IU; D3: 5000IU; E:0.075 g; Trace elements; Calcium iodate:3.1 mg (iodine:2.0 mg); Copper sulphate: 80 mg (copper 3b405:27 mg); Iron sulphate: 83.5 mg (Iron 3b103); Zootechnical additives; Axtra PHY 10 000 L: 0.01%; Ronozyme cereal WX-MG MP E1607 in cat N of directive 70/524/EEC: 0.029%; Maxiban G160 (80 mg/kg Narasin and 80 mg/kg Nicarbazin to provide 50 mg/kg Narasin and 50 mg/kg Nicarbazin in final feed as an aid in the prevention of coccidiosis in broiler chickens).

circumference, and then its diameter was calculated. The empty weight and length of the small intestine (duodenum, jejunum, and ileum) were measured.

Histology of gizzard and ileum

Cross sections of the gizzard wall were excised from the right cranioventral thick muscle of the gizzard wall adjacent to the pyloric junction to maintain consistency. Cross-sections were excised from the distal 5 cm part of the ileum preceding the ileo-caecal junction. All sections were washed with distiled water and fixed in Bouin's fixative for 6 h then transferred to 70% ethanol for processing. All fixed sections were embedded with paraffin wax on a tissue processor (HistoCore, Leica Microsystems, Milton Keynes, UK) and embedding station (HistoCore, Leica Microsystems, Milton Keynes, UK). Samples were dehydrated using graded alcohols (70, 90 and 100%) at ambient temperature, cleared in a xylene bath and carefully infiltrated with paraffin wax at 56°C. Embedded samples were processed using a rotary microtome processer (Leica 1215, Leica Microsystems, Milton Keynes, UK) with disposable blades by cutting 8-µm-thick slices. Slices were stained with haematoxylin and eosin and examined under a light microscope. Histological measurements of the gizzard and ileum were taken using an arbitrary-line tool of the Olympus Soft Image Solution programme (Tokyo, Japan) with calibration on an Olympus microscope (BX52). Measurements were taken from the two thickest and two thinnest layers from cross sections of the gizzard wall and the average was used for data analysis, whereas the villus height, width, crypt and mucosal depth were measured from six healthy and unbroken villi from each cross section on the slides and the average was used for data analysis.

Tensile strength

The tensile strength of the gizzard was measured using TA. XT plus Texture Analyzer 100 (Stable Micro Systems, Surrey, UK). The tearing or shearing force was measured in Newtons (N) using a 100 kg load cell at a speed of 25 mm per minute, following the method published by Abdollahi et al. (2019).

Chemical analysis

Chemical analysis of the diets and litter was performed using standard procedures published by the Association of Official Analytical Chemists (AOAC 1990). Dry matter (method 930.15), and ash content (method 942.05) of the feed were determined by standard methods. Nitrogen content was determined by the combustion method (Dumas) using a nitrogen analyser (Gerhardt, Germany) following a standard method (AOAC 968.06). Crude protein was calculated by multiplying nitrogen using a conversion factor of 6.25.

Statistical analysis

Analysis of variance was used to analyse data according to the following model.

$$Y_{ij} = M + TRT_i + B_j + E_{ij}$$

where Y_{ij} was the response variable, M the overall mean, TRT_i the effect of dietary treatment, B_j the effect of block and E_{ij} the residual. Data were analysed using the analytical software JMP Statistical Discovery 2020 from the SAS Institute, NC, USA. Means were compared by protected LSD test at $p \leq 0.05$.

Results

Particle size analysis of maize

The particle size distribution of maize included in the diets is provided in Figure 1.

Growth performance

The results given in Table 2 show that feeding low-protein diet with maize of GMD 350 µm (M350) and low protein diet with maize of GMD 2600 µm (M2600) significantly reduced body weight gain and feed intake of broiler chickens at the end of the trial compared with control (CON). However, there was no difference ($p > 0.05$) in body weight gain and feed intake between low protein diet with whole maize (WM) and CON fed groups. Treatments did affect the FCR of broiler chickens at the end of the trial.

One bird died on treatment M2600 in each phase of starter (d 0–7) and grower (d 7–14) whereas, two birds died when fed treatment WM between d 14–21. Mortality was in the normal range recorded at the Poultry Research Unit at Nottingham Trent University.

Gastrointestinal tract

The results presented in Table 3, show that, on d 14, proventriculus and gizzard absolute weights from birds fed CON and WM were similar ($p > 0.05$). However, feeding WM increased gizzard weight significantly compared with M350 and M2600 treatments on d 14. The dietary treatments M350 and M2600 reduced ($p < 0.05$) small intestine absolute weight compared with CON, however, there was no difference between WM and CON d on d 14. Feeding WM significantly increased ($p < 0.05$) the gizzard to proventriculus ratio compared with those fed CON, M350 and M2600 on d 14. No significant difference ($p > 0.05$) in absolute weights of any digestive organ was observed on d 21 among treatments.

Feeding diets M350 and M2600 increased ($p < 0.05$) proventriculus weight compared with CON and WM on d 14,

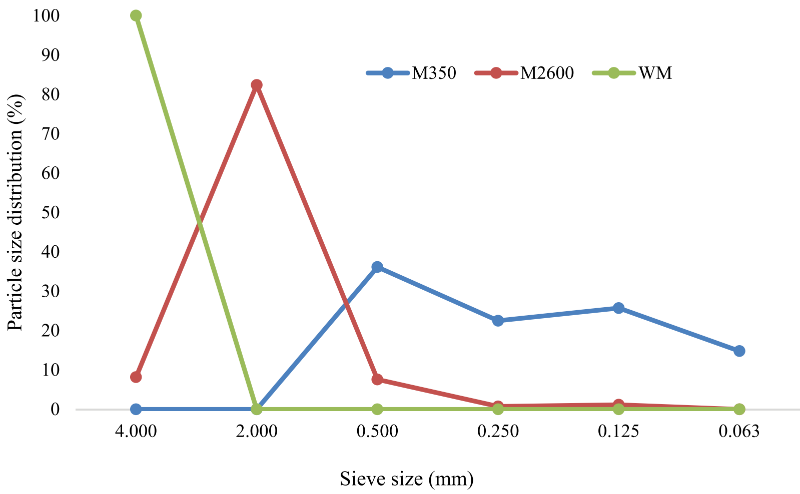

Figure 1. Particle size distribution of maize included in the diets.

Table 2. Effect of typical and low protein diets with fine, coarse, and whole maize on growth performance from day 1 to 21.

	Treatment[1]					
Parameter	CON	M350	M2600	WM	SEM[2]	*P*-value
Initial body weight (g)	40.04	40.11	40.22	40.12	0.265	0.971
Final body weight (g)	1004[a]	665.9[c]	768.0[b]	959.4[a]	22.14	<0.001
Body weight gain (g)	964.1[a]	625.8[c]	727.8[b]	919.3[a]	22.06	<0.001
Feed intake (g)	1223[a]	914.6[c]	1054[bc]	1165[ab]	49.33	0.002
Feed conversion ratio (g/g)	1.273	1.471	1.462	1.262	0.072	0.087

[1]CON: Commercial diet, crude protein 22.50%, M350: CON + 20% maize of GMD 350 µm crude protein 19.90%, M2600: CON + 20% maize of GMD 2600 µm crude protein 19.90%, WM: CON + 20% whole maize crude protein 19.90%.
[2]SEM: Standard error of means.
[a,b,c]Means within a row with different superscripts differ significantly ($p \leq 0.05$).

Table 3. Effect of typical and low protein diets with fine, coarse, and whole maize on gastrointestinal tract organs.

	Treatment[1]					
Parameter	CON	M350	M2600	WM	SEM[2]	*P*-value
Absolute weights (g)						
D14 Proventriculus	2.794[a]	2.245[b]	2.290[b]	2.393[ab]	0.138	0.044
D14 Gizzard	11.15[ab]	9.649[c]	9.900[bc]	11.57[a]	0.488	0.031
D14 Small intestine	19.85[a]	14.65[c]	16.37[bc]	17.92[ab]	0.915	0.006
D14 Gizzard: proventriculus (g/g)	4.055[b]	4.322[b]	4.315[b]	4.866[a]	0.166	0.020
D21 Proventriculus	4.407	4.429	4.202	4.642	0.200	0.505
D21 Gizzard	18.40	18.87	18.64	18.96	1.079	0.983
D21 Small intestine	26.91	28.09	24.83	24.65	1.174	0.148
D21 Gizzard: proventriculus (g/g)	4.169	4.226	4.454	4.165	0.189	0.675
Relative weights (g/kg BW)						
D14 Proventriculus	6.049[b]	7.225[a]	6.990[a]	5.950[b]	0.253	0.003
D14 Gizzard	24.31[b]	31.15[a]	30.07[a]	29.00[a]	1.191	0.004
D14 Small intestine	43.26[b]	47.22[ab]	50.44[a]	44.54[b]	1.741	0.043
D21 Proventriculus	4.580	4.962	4.962	4.845	0.206	0.527
D21 Gizzard	19.04	21.06	21.95	19.86	1.146	0.318
D21 Small intestine	27.78[bc]	31.26[a]	29.01[ab]	25.78[c]	1.037	0.012
Measurements						
D14 Gastric isthmus diameter (mm)	7.020[a]	6.450[a]	5.650[b]	5.695[b]	0.232	0.001
D14 Small intestine (cm)	135.8	121.4	125.2	126.5	5.380	0.313
D21 Gastric isthmus diameter (mm)	7.515[a]	7.401[a]	6.466[b]	6.380[b]	0.203	0.001
D21 Small intestine (cm)	152.8	153.7	152.1	152.0	3.674	0.987
Intestinal density (g/100 cm)						
D14 Small Intestine	14.56[a]	12.09[c]	13.18[bc]	14.19[ab]	0.465	0.006
D21 Small Intestine	17.62[ab]	18.20[a]	16.26[b]	16.15[b]	0.567	0.049

[1]CON: Commercial diet, crude protein 22.50%, M350: CON + 20% maize of GMD 350 µm crude protein 19.90%, M2600: CON + 20% maize of GMD 2600 µm crude protein 19.90%, WM: CON + 20% whole maize crude protein 19.90%.
[2]SEM: Standard error of means.
[a,b,c]Means within a row with different superscripts differ significantly ($p \le 0.05$).
BW: Body weight.
Small intestine consists of duodenum, jejunum and ileum.

whereas gizzard relative weight was increased by feeding M350, M2600 and WM compared with CON on d 14. There was no difference in the small intestine relative weight observed between birds receiving WM and CON, however, this was lower ($p < 0.05$) than seen for the M2600 diet on d 14. The results showed that small intestine relative weight on d 21 was similar ($p > 0.05$) between birds fed WM and CON, however, M350 increased this parameter compared to CON and WM. Feeding M2600 and WM significantly decreased ($p < 0.05$) gastric isthmus diameter compared with birds fed CON and M350 at the age of d 14 and 21.

Birds fed M350 and M2600 had decreased ($p < 0.05$) small intestine density compared with those fed CON; however, no difference ($p > 0.05$) was observed between those receiving WM and CON on d 14. However, on d 21, this was reduced for birds fed M2600 and WM compared with the M350 treatment, whereas no difference was found between WM and CON treatments.

Histology, tensile strength and gizzard digesta particle size

The results in Table 4 show that there was no statistical difference observed in koilin depth between samples taken from birds fed CON, M350, and WM on 14 d of age. Feeding M350 and WM decreased ($p < 0.05$) mucosal depth of *Lamina propria* compared to those receiving CON. Sub-mucosal depth on M350 and WM decreased ($p < 0.05$) compared to CON and M350 on d 14. The same trend was observed for *Tunica muscularis* on d 14. No difference ($p > 0.05$) was observed in mucosal depth and extent of *Tunica muscularis* between dietary treatments M2600, WM and CON on d 21.

Feeding M350 and M2600 decreased ($p < 0.05$) ileum mucosal depth compared to birds receiving CON on d 14; however, there was no difference between the WM and CON groups. Villus-to-crypt ratio was reduced ($p < 0.05$) for those fed M350 and WM compared with CON, but there was no difference in comparison to the M2600 diet observed on d 21.

The tensile strength of the gizzard and ileum was not affected significantly ($p > 0.05$) by dietary treatments at any age. However, numerically, the tensile strength of the ileum from birds fed WM increased by 13.45% compared with CON on d 14, whereas feeding WM increased gizzard and ileum tensile strength by 7.85 and 17.24%, respectively, compared with CON on d 21.

Feeding M350 reduced gizzard digesta particle size compared to M2600, WM and CON treatments, but there was no difference ($p > 0.05$) in the gizzard digesta GMD between birds fed M2600, WM or CON on d 14. With age, gizzard digesta particle size evened out and no more effects of treatment on gizzard digesta GMD were observed.

Nitrogen efficiency, litter characteristics, and footpad scores

The results (Table 5) indicated that feeding M350 and WM reduced nitrogen excretion significantly compared with CON. Birds receiving WM increased nitrogen efficiency compared with those on M350 and M2600, however, compared with CON, this was not significantly different ($p > 0.05$). All the low protein diets; M350, M2600 and WM, significantly decreased litter moisture and footpad dermatitis scores compared to birds fed CON.

Discussion

The study was conducted to determine any impaired growth performance of broilers fed low-protein diets without supplementing extra synthetic amino acids or increasing protein

Table 4. Effect of typical and low-protein diets with fine, coarse, and whole maize on gizzard histology and ileum histology (μm), tensile strength (N), and gizzard digesta particle size (μm).

Parameter	Treatment[1] CON	M350	M2600	WM	SEM[2]	*P*-value
Gizzard histology (μm)						
D14 Koilin	373.1[b]	335.5[b]	440.7[a]	327.8[b]	21.48	0.006
D14 Mucosa of lamina propria	538.4[a]	359.3[b]	442.2[ab]	384.9[b]	36.70	0.014
D14 Submucosa	181.8[a]	118.4[bc]	164.6[ab]	107.2[c]	17.84	0.023
D14 The extent of tunica muscularis	1093[a]	813.1[b]	1048[a]	820.0[b]	66.65	0.011
D21 Koilin	401.6	494.8	416.7	371.3	31.52	0.070
D21 Mucosa of lamina propria	528.8[ab]	551.5[a]	400.8[c]	423.9[bc]	40.60	0.040
D21 Submucosa	177.6	176.5	110.4	123.1	23.28	0.113
D21 The extent of tunica muscularis	1108[ab]	1223[a]	927.9[b]	918.3[b]	75.85	0.029
Ileum histology (μm)						
D14 Villus height	545.8	561.8	517.9	570.4	38.71	0.785
D14 Crypt depth	151.5	136.3	143.1	140.4	10.21	0.757
D14 Mucosal depth	186.3[a]	150.2[b]	135.4[b]	166.2[ab]	11.80	0.039
D14 Villus: crypt	3.693	4.356	3.725	4.101	0.327	0.439
D21 Villus height	697.2	614.3	656.0	631.8	22.87	0.095
D21 Crypt depth	123.0	140.1	136.6	147.3	8.155	0.231
D21 Mucosal depth	287.4	262.2	254.0	301.2	25.10	0.530
D21 Villus: crypt	5.799[a]	4.461[b]	4.834[ab]	4.396[b]	0.329	0.027
Tensile strength (N)						
D14 Gizzard	9.789	8.609	8.931	9.563	1.804	0.963
D14 Ileum	1.294	1.077	1.184	1.468	0.168	0.418
D21 Gizzard	14.01	9.485	11.94	15.11	2.207	0.318
D21 Ileum	1.433	1.559	1.611	1.680	0.168	0.763
Gizzard digesta particle size						
D14 GMD (μm)	1572[a]	1375[b]	1583[a]	1678[a]	62.24	0.021
D21 GMD (μm)	1640	1685	1773	1745	49.33	0.259

[1]CON: Commercial diet, crude protein 22.50%, M350: CON + 20% maize of GMD 350 μm crude protein 19.90%, M2600: CON + 20% maize of GMD 2600 μm crude protein 19.90%, WM: CON + 20% whole maize crude protein 19.90%.
[2]SEM: Standard error of means.
[a,b,c]Means within a row with different superscripts differ significantly ($p \leq 0.05$).
The extent of tunica muscularis: Koilin+ Mucosa of lamina propria+ Submucosa.

Table 5. Effect of typical and low-protein diets with fine, coarse, and whole maize on nitrogen excretion and efficiency (D0–21), and welfare variables.

Parameter	Treatment[1] CON	M350	M2600	WM	SEM[2]	*P*-value
N excretion (g/bird)	16.08[a]	10.97[b]	12.45[ab]	10.42[b]	1.650	0.026
N Efficiency (%)	63.91[ab]	62.91[b]	63.60[b]	72.59[a]	3.022	0.036
Excreta and litter characteristics, and footpad scores						
D20 Litter N (%)	2.931	2.470	2.501	2.641	0.125	0.068
D20 Litter moisture (%)	15.40[a]	8.174[b]	8.374[b]	9.335[b]	1.566	0.013
D21 Footpad scores	0.357[a]	0.143[b]	0.071[b]	0.001[b]	0.070	0.013

[1]CON: Commercial diet, crude protein 22.50%, M350: CON + 20% maize of GMD 350 μm crude protein 19.90%, M2600: CON + 20% maize of GMD 2600 μm crude protein 19.90%, WM: CON + 20% whole maize crude protein 19.90%.
[2]SEM: Standard error of means.
[a,b,c]Means within a row with different superscripts differ significantly ($p \leq 0.05$).

content. Further, it was examined whether this could be mitigated by the inclusion of structural components; *e.g.*, coarse or whole grains, which could help develop the digestive tract and utilise the nutrients more efficiently. A low CP diet with whole maize showed improved weight gain compared to low CP diets, with fine or coarse maize. The current study showed that a low protein diet with WM produced comparable growth performance results to the standard protein diet (CON).

Previous studies reported that a 2 to 3% reduction in crude protein content did not compromise growth of broilers, but further reductions (5% or more) resulted in inferior performance (Aftab et al. 2006; Belloir et al. 2017). Xu et al. (2015a) observed that body weight gain was improved by 1.1%, feed conversion ratio by 0.8% and gizzard relative weight by 7.4% compared to a negative control diet when 50% of the fine ground maize was replaced with a coarse form. Similarly, Naderinejad et al. (2016) observed a 0.8% improvement in body weight gain, 2.5% in feed intake and 2.6% in FCR when all the maize in the diets was coarsely rather than finely ground. When Kheravii et al. (2017) used 100% coarse maize in the diet, weight gain was improved by 2%, feed intake by 0.6% and gizzard relative weight by 14.6% compared with a negative control diet using fine maize. The current study showed low CP diet with whole maize improved the weight gain by 46.9% and 26.3% and FCR by 16.6% and 15.84% compared to low CP diets with fine and coarse maize, respectively. Previously Naeem et al. (2023a) found no difference in FCR when they diluted diets with 20% whole wheat, though weight gain was reduced. However, diluting a diet with 5% coarse oat hulls, Naeem et al. (2023b) found that performance was mitigated in terms of feed intake, body weight gain and FCR. Bennett et al. (2002) included whole wheat at varying inclusion rates into pellet and mash diets with no dilution and found that weight gain was significantly decreased, FCR was increased with increasing levels of whole wheat. However, in another study, Bennett et al. (1995) diluted diets with whole and crumbled wheat and found that body weight was not affected, but poorer FCR was observed with the inclusion of whole wheat. With protein dilution in poultry pullet diets (30% ground or whole wheat) Covasa and Forbes (1994) observed no effect on final body weight but increased feed consumption when using ground wheat in diets. The current study found no difference in boy weight gain, feed intake or FCR in birds fed a standard protein diet or a low CP diet containing whole maize. Feed intake in the current study was not affected between birds fed WM or CON diets but overall feed intake was lower on the low-protein diet with maize of GMD 350 µm (diet M350) which may have caused lower weight gain.

These results disagree with previous findings of Leeson et al. (1991) who reported that broilers consumed 40% more feed when diets were diluted with rice hulls in order to meet their nutritional requirements (Hill and Dansky 1954; Zubair and Leeson 1994). Similarly, Zubair and Leeson (1994) diluted diets with 50% oat hulls and rice hulls and found significantly reduced body weight of broilers. The increased feed intake which was likely an attempt to maintain nutrient intake. The current study did not show increased feed intake contrary to these findings. This may have been due to the differences in feed ingredients as maize was used to dilute diets that had good energy and protein levels, compared to rice or oat hulls.

According to Nir et al. (1994), coarse corn particles significantly reduced growth performance compared to medium and fine particles up to d 7 of age. Impaired growth performance in young broilers given coarse particle maizee, such as decreased weight and lower FCR, might be attributed to their inability to adequately grind coarse particles due to an immature gizzard (Douglas et al. 1990; Lott et al. 1992). Previous research on the influence of particle size on growth performance in older birds has been contradictory and inconsistent. According to Amerah et al. (2007b), varied wheat particle sizes (839 or 1164 µm) in pelleted diets did not influence growth performance. Furthermore, Naderinejad et al. (2016) found that pelleted diets containing varied maize particle sizes (490, 651 or 796 µm) did not influence growth performance. However, Lott et al. (1992) discovered that, when the particle size of maize was reduced from 1196 to 716 µm, birds performed better. Furthermore, Chewning et al. (2012) reported that reducing maize particle size improved body weight up to 21 d of age, but not at 44 d. However, the particles used in those investigations were quite small (267 and 570 µm) compared to the current trial. Xu et al. (2015b) found that replacing 50% of fine particles (294 µm) with coarse particles (1362 µm) of maize enhanced feed conversion and weight growth compared to fine particle size alone, in agreement with the current results.

Increased particle size in feed has been reported to have beneficial effects on the digestive tract of poultry, especially the gizzard which has been associated with better utilisation of nutrients due to enhanced activity (Amerah et al. 2007a). The low CP diet with whole maize increased gizzard relative weight by 19.29% on d 14 of age and 4.31% on d 21 compared to the standard CP diet. The comparable performance for birds fed low CP diets containing whole maize and standard CP diets, and the improved performance of birds given a whole-maize low CP diet compared to fine and coarse maize low CP diets in the current study was most likely due to improved gizzard development. The structure of the diet is critical for gizzard growth (Choct 2009; Naderinejad et al. 2016). Increased gizzard activity leads to more complete feed grinding and a longer retention period, allowing the digesta to reach a certain size before passing through the pyloric sphincter (Hetland et al. 2003). Particle size of the low CP diet with whole maize was the largest but the gizzard digesta particle size analysis showed comparable results to the standard protein diet containing no maize. Theoretically, gizzard digesta particle size for birds fed WM must be larger than that CON, but no difference was observed between them. It can be speculated that the gizzard functioned efficiently on a low CP diet with whole maize. This might have increased gizzard volume and the frequency of contractions, resulting in muscle adaptation to deal with the increased grinding required. The low CP diet containing whole maize increased gizzard tensile strength by 7.85% on d 21 compared to the standard CP diet, which might be due to more grinding action in the presence of whole grains (Liu et al. 2015). The gizzard is considered a pacemaker that controls the intake of feed, plays a role in reflux action and creates beneficial effects on gut integrity (Svihus 2011). This supports the findings of the current study, as feeding a low CP diet with whole maize increased ileum tensile strength by 13.45% and 17.24% on d 14 and 21 of age, respectively, compared to the standard CP diet. Furthermore, the gastric isthmus diameter on d 14 and 21 of age was reduced by 18.88% and 15.10%, respectively, feeding a low CP diet containing whole maize compared to the standard CP diet. Interestingly, the gizzard-to-proventriculus ratio was higher in birds fed the low-protein diet with whole-maize inclusion which agreed with previous findings on the prevention of proventricular dilation (Jones and Taylor 2001). As the proventriculus and gizzard work together, a dilated proventriculus is not always desirable because the gizzard might work as a transit organ rather than for grinding (Svihus 2011). Furthermore, a dilated proventriculus can lead to carcass condemnation as it causes contamination in the meat processing line (Amer 2021).

Increased relative weight of the small intestine was observed in birds fed low CP diets containing fine and coarse maize compared to standard CP and low CP containing whole maize diets. This may have been due to an adjustment made by the birds according to nutrient requirements or gizzard development and its efficiency.

Feeding the whole maize, low CP diet resulted in decreased mucosal depth of the lamina propria on d 14 of age compared to the standard CP diet. This may have been due to less utilisation of the whole maize grains. This difference was evened out at the age of 21 d, which could have been due to more use of larger particles of feed which was shown by the particle size analysis of the residual feeds (data not shown). A whole maize, low CP diet decreased the *Tunica muscularis* of the gizzard on d 14 and 21 of age compared to the standard CP diet. One possible reason for this may be the abrasion of the koilin thickness, which was reduced by 12.14% on d 14 and 7.54% on d 21, due to more grinding action of whole maize (Akester 1986). Numerically, WM increased ileum villus height by 4.51% and villus-to-crypt ratio by 11.05% compared to CON on d 14, and crypt depth by 1.76% and mucosal depth by 4.80% on d 21 compared with birds fed CON. This might have provided an enlarged surface area for the absorption of nutrients resulting in better nutrient utilisation, growth and FCR (Ravindran et al. 2006).

The effect of particle size on gut histology was not consistent in previous studies. This may have been due to the different composition of the diets, particle size or even sections of the gut that have been studied. For example, Novotný et al. (2023) fed broilers with coarse (1111.26 µm), medium (959.89 µm) and fine (730.48 µm) particles. They observed that coarse feed resulted in increased villus height and deeper crypts in the duodenum compared with other treatments but, in the jejunum, the same treatment resulted in decreased values compared with other treatments. However, the villus-to-crypt ratio was higher in jejunum on coarse treatment compared with other treatments. Regarding the ileum, these researchers found that the coarse diet caused increased villus height and villus-to-crypt ratio compared with fine or medium particle size, contrary to the current trial, as there was no effect of dietary treatment observed on villus height on d 14 and 21 of age. However, the villus-to-crypt ratio was decreased in birds fed the whole maize, low-protein diet compared with the standard diet. The structural adaptation of intestinal villi in broiler chickens can be influenced by factors related to both the macro- and micro-structure of the feed (Amerah et al. 2007b; Qaisrani et al. 2014). However, there is limited research on the impact of feed particle size on intestinal morphology. Previous studies have typically compared pelleted and mashed forms of feed (Amerah et al. 2007b; Naderinejad et al. 2016; Zang et al. 2009). The findings of Naderinejad et al. (2016) aligned more closely with those of Amerah et al. (2007b), as they indicated a positive effect on the villus height and crypt depth ratio in broilers fed a pelleted compared to a mash diet. Ege et al. (2019), in their study involving laying hens fed a pelleted diet, observed increased villus height and width, along with an elevated ratio between villus height and crypt depth.

Bird welfare is an important issue in broiler production and litter quality is considered a good indicator (Greenhalgh et al. 2020). Wet or poor-quality litter can hamper growth performance as well as compromise bird welfare associated with foot pad dermatitis and associated conditions (Dunlop et al. 2016; Lemme et al. 2019; van Harn et al. 2019). Footpad dermatitis (FPD) is contact condition that occurs on the plantar surface of a bird's feet and has been directly associated with wet litter (de Jong and van Harn 2012, de Jong et al. 2012, 2015). The current study resulted in 13.6% higher nitrogen efficiency, 35.2% less nitrogen excretion, 9.9% less litter nitrogen, 39.4% less litter moisture and 99.7% fewer footpad scores on a whole-maize low CP diet compared to a standard CP diet. Greenhalgh et al. (2020) showed that a reduction of dietary crude protein from 189 to 169 g/kg reduced footpad dermatitis scores from 85.5 to 35.0 - an improvement of 59%. Reduction in crude protein in poultry diets can promote better litter quality and enhance bird welfare by resulting in low litter moisture (Brink et al. 2022; Elwinger and Svensson 1996; Ferguson et al. 1998). High levels of crude protein in diets have been linked to increased water intake and elevated excreta moisture (Hilliar et al. 2020), as well as higher nitrogen excretion (Lemme et al. 2019). The reduction in water excretion observed with whole maize may be attributed to several plausible reasons. First, whole grains may hold a substantial amount of water causing prolonged retention time for digesta coupled with a greater water-holding capacity in the gut. This would enhance water reuse in the caeca and minimise water excretion. Second, birds with structural dietary components *e.g.*, coarse or whole grains, may have their gizzard operating as a pacemaker organ for digestion and absorption. This could optimise water absorption, resulting in less being excreted in manure, as birds would not have an excessive urge to drink. The lower footpad dermatitis score for birds fed the whole maize, low CP diet may be attributed to the reduced moisture content of the litter in the current study (Taira et al. 2014). Higher water intake has been previously reported for birds fed pellet diets compared with mash diets (Brink et al. 2022).

The current study showed that whole maize can be used successfully to dilute protein in broiler diets and develop the gastrointestinal tract efficiently to mitigate poor performance on low protein diets. However, no chemical analysis to assess composition and non-statistical analysis of particle size of residual diets remain the limitations of this study. Feed particle size analysis before and after feeding did not provide statistically analysed outcomes and conclusive information about feed selection or flicking behaviour of broilers due to mixing fresh diets with residual diets within each treatment. Numerically, particle size difference was +100.7%, +78.6%, +85.6%, and +50.2% at the end of the first week; +4.4%, +6.6%, +17.6% and −0.6% at the end of the second week; and +0.7%, −4.1%, 2.7% and −3.7% at the end of the third week between new and residual diets for CON, M350, M2600 and WM treatments, respectively. The numerical increase in particle size of residual feeds especially for the first of age showed that broiler chickens exhibited a trend to select smaller particles than larger ones in the starter phase. With age, the broiler chickens were capable of handling the larger feed particles more efficiently.

Conclusions

Diluting protein content in broiler diets by around 10% with whole maize did not affect broiler performance adversely for up to 21 d of age. However, using maize with GMD of 350 or 2600 µm negatively affected growth performance, specifically weight gain. Whole maize-diluted diets were able to mitigate any performance losses from low-density protein diets and outperformed fine or coarsely ground forms without compromising FCR, weight gain or feed intake. The inclusion of whole maize in low protein diets showed potential in improving digestive efficiency, reducing nitrogen excretion and enhancing welfare parameters, including litter moisture and footpad dermatitis. This feeding approach, particularly where maize is available, may reduce feed costs by reducing grinding requirements and dependence on traditional protein-rich resources in chicken production. This can contribute towards sustainable meat production, food security and lowering the environmental footprint from farms.

Acknowledgments

Special thanks are due to NTU Poultry Research Unit technicians; Ben Gadsby, Ashley Maxey, Kate Wilshaw, Agne Cerniauskaite, and John Olifent, for helping in running this study.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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