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Effect of Source and Processing on Maize Grain Quality and Nutritional Value for Broiler Chickens: 2. Milling Technique and Particle Size

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ABSTRACT

In a 2×2×3 factorial study, the effect of milling technique (hammer vs. roller) with differing particle sizes (fine or coarse) of maize from three sources (Downs, Emerald or Moree) on growth performance, ileal digestibility and intestinal microbial profiles of broiler chickens (from 1-21 days post-hatch) was investigated. A total of 420 day-old male Cobb chicks were randomly allocated to 12 treatments of 5 replicates (seven birds per replicate) in brooder cages set up in an environmentally controlled room. The maize grain was finely ground using a hammer mill or roller mill with a 2 mm screen or coarsely ground through a 4 mm screen. Feed intake up to 7 days of age was higher ($p<0.003$) on the diet containing finely roller-milled grain than on the coarsely milled grain but no effect found on d21. Live weight was affected by the source of maize ($p<0.04$). The FCR to d21 was improved ($p<0.042$) on the Moree maize that was finely roller milled. The relative weight of proventriculus plus gizzard ($p<0.01$) and liver ($p<0.01$) were higher in diets containing coarsely milled grain than finely milled grain at 7 but not 21 days. Nutrient digestibility was affected due to maize source ($p<0.01$) and particle size reduction ($p<0.01$). These results suggest that fine grinding some sources of maize would be beneficial in terms of improvement in FI, LW and weight of proventriculus and gizzard in early ages as well as nutrient digestibility at a later age.

Key words: Maize source, processing technique, particle size, nutritional value, broiler performance

INTRODUCTION

The nutritive value of grain largely depends on its quality, which is dependent, among other factors, on variety, season, source and harvest conditions. In addition to the quality, the utilization of energy from maize by broiler chickens depends on the preparation and processing of feed, particularly the particle size and milling technique (Reece *et al.*, 1985; Parsons *et al.*, 2006). Coarse grain particle has been found to induce the development of gastro-intestinal organs particularly gizzard in young chicks, which ultimately contributes to improved productivity (Nir *et al.*, 1994).

The season, location and variety of grains can have large effects on starch, soluble and insoluble NSP, protein and AME of wheat, triticale and rye (Metayer *et al.*, 1993; Cowieson, 2005). Other cereals, for example, maize, sorghum, barley and oats were also relatively inconsistent in quality across season and sites (Hughes and Choct, 1999). The climatic conditions can also be responsible for the variations of starch content and granular structure, composition and hardness of grains (Tester, 1997; Tester *et al.*, 1995).

The percentage of fine particles obtained after grinding depends on the hardness of the grain, with a higher percentage of fine particles from softer grains (Carre *et al.*, 2005). A harder endosperm gives larger particles with more irregular shapes, while a soft endosperm produces smaller sized particles (Rose *et al.*, 2001). This effect may be responsible for the better broiler performance reported with mash diet containing small particles based on hard wheat (Rose *et al.*, 2001; Pirgozliev *et al.*, 2003).

Gizzard development is strongly dependent on the feed particle size (Nir *et al.*, 1995; Engberg *et al.*, 2002). A coarse particle size stimulates better development of the gizzard, leading to increased digestion of grain. However, there is evidence that smaller particle size with an increased surface area improves access for digestive enzymes for the digestion of nutrients (Waldroup, 1997) and presumably lower energy expenditure on crushing or grinding (Jurgens, 1993).

Two key milling techniques, hammer and roller milling, are employed by the feed mill industry and both have a bearing on the physical makeup of components of the grain (Little, 1997). Traditionally, the hammer mill is used to reduce the particle size of the grains, while roller milling has been applied mainly to produce coarser feeds (Koch, 1996; Waldroup, 1997). Roller mills can, however, produce fine particles of similar uniformity compared to those produced by hammer mills. In either case a range of particle sizes is produced depending on a number of factors, including the type of grain used, the speed and power of motor, screen size used, feed rate and speed of air flow through the mill (Martin, 1985; McCracken, 2002). Within a grain type, grinding in the same mill type under similar conditions may result in different particle sizes due to variations in endosperm hardness (Lentle *et al.*, 2006).

There are reports that chickens fed on maize-based diets ground with a roller mill have superior weight gain and feed conversion ratio compared to chickens fed the same diet ground in a hammer mill (Reece *et al.*, 1985), however the results are not consistent (Douglas *et al.*, 1990). The influence of particle size appeared to be confounded by the complexity of the diet and nature of feed processing, such as milling, pelleting and crumbling (Goodband *et al.*, 2002). There is broad agreement that the uniformity of the diet is important for optimum performance of broilers, especially those raised in close confinement using automated feeding equipment (McCoy *et al.*, 1994).

The present study was designed to investigate the effects of milling technique and particle size on the nutritive value and feed utilization of maize grain from different sources and on gastrointestinal physiology and performance of broiler chickens.

MATERIALS AND METHODS

Experimental design and bird management: In the present study, a 2×2×3 factorial experiment (Table 1) was designed to investigate the effect of milling technique (hammer vs. roller) with differing particle sizes (fine or coarse) of maize from three sources (Downs, Emerald or Moree) on nutrient composition, growth performance, ileal digestibility and intestinal microbial profiles of broiler chickens up to 21 days of age in 2010. A total of 420 day-old male Cobb broiler chicks (Baiada Poultry Pty. Ltd., Tamworth, NSW, Australia), weighing 37.83±0.04 g, were randomly allocated to 12 treatments with 5 replicates (7 birds per replicate) in brooder cages (600×420×23 cm) set up in an environmentally controlled room.

Table 1: Dietary treatments

	Hammer mill (H)		Roller mill (R)	
Maize source	Fine (<2 mm)	Coarse (2-4 mm)	Fine (<2 mm)	Coarse (2-4 mm)
Downs (DO)	DOHF=T1	DOHC=T2	DORF=T3	DORC=T4
Emerald (EM)	EMHF=T5	EMHC=T6	EMRF=T7	EMRC=T8
Moree (MO)	MOHF=T9	MOHC=T10	MORF=T11	MORC=T12

DOHF: Downs hammer milled fine, DOHC: Downs hammer milled coarse, DORF: Downs roller milled fine, DORC: Downs roller milled coarse, EMHF: Emerald hammer milled fine, EMHC: Emerald hammer milled coarse, EMRF: Emerald roller milled fine, EMRC: Emerald roller milled coarse, MOHF: Moree hammer milled fine, MOHC: Moree hammer milled coarse, MORF: Moree roller milled fine, MORC: Moree roller milled coarse

Table 2: Particle size distribution (%) of roller-milled and hammer-milled grain from three sources of maize passed through a 2 mm screen

		Hammer milling		Roller milling	
Maize source	Particle size class (mm)	Coarse particle	Fine particle	Coarse particle	Fine particle
Downs	>2	33	17	59	4
	<2	67	83	41	96
Emerald	>2	34	17	58	5
	<2	66	83	42	95
Moree	>2	37	13	57	6
	<2	63	87	43	94

The sources and properties of the grains used in this experiment were described in the first paper of this series. Before diet preparation, a short screening test was conducted by sieving the milled material through a 2 mm sieve in order to determine the particle size of grains of the three cultivars after grinding through hammer and roller mills (Table 2). There was no difference between the three sources of maize in the particle size produced by each milling technique.

The semi-purified diet used was a coarsely and finely ground maize-based diet without microbial enzyme supplementation. The finely ground diet was prepared using a hammer mill or roller mill with a 2 mm screen and, for the coarsely ground diet, a 4 mm screen was used.

The ingredient and nutrient composition of the diet fed is shown in Table 3. This was fed from hatch to the end of study at 21 days of age. An indigestible marker Celite (Celite Corporation, Lompoc, CA, 93436) was incorporated to assess the nutrient digestibility. The birds were brooded at an initial temperature of 33°C, which was reduced gradually to 25±1°C by 21 days. Sixteen hours of lighting per day was provided throughout the trial period. All birds were provided with the experimental diet as mash and water *ad libitum*.

On days 7 and 21, one and three birds, respectively were randomly selected from each replicate, weighed and humanely killed by cervical dislocation. The abdominal cavity was opened and the small intestine was ligated and removed. The contents of the gizzard, jejunum, ileum and caeca were collected into plastic containers for pH and digestibility (ileum content only) measurement. The ileal digesta were frozen immediately after collection and freeze-dried (Martin Christ Gerfriertrocknungsanlagen, GmbH, Osterode am Harz, Germany). They were then ground in a small coffee grinding machine and stored at -4°C in airtight containers until chemical analyses were performed.

Table 3: Ingredient and nutrient composition of broiler starter diet

Ingredients	Conc. (g kg^{-1})	Nutrient composition	Conc. (g kg^{-1})
Maize	722.7	ME poultry (MJ kg^{-1})	12.8
Soycomil R[1] (65% CP)	218.7	Crude protein	210.0
Limestone (38% Ca)	15.1	Lysine	13.2
Vegetable oil	10.0	Methionine	5.4
Dicalcium phosphate	14.1	Arginine	13.6
Sodium bicarbonate	4.6	Methionine+cystine	8.8
Lysine- HCl	2.1	Histidine	5.7
DL-methionine	2.0	Threonine	8.5
Salt	1.7	Calcium	10.0
Choline chloride (70% choline)	2.0	Available phosphorus	4.2
Broiler premix[2]	2.0	Sodium	2.0
Celite	5.0	Choline	2.06

[1]Soycomil R: Soycomil R is a unique feed grade soy protein concentrate meeting the high quality standards of the today's animal feed industry, [2]Supplied per kg of diet (mg): Vitamin A (as all-trans retinol), 3.6 mg, cholecalciferol, 0.09 mg, vitamin E (as d-a-tocopherol), 44.7 mg, vitamin K_3, 2 mg, thiamine, 2 mg, riboflavin, 6 mg, pyridoxine hydrochloride, 5 mg, vitamin B_{12}, 0.2 mg, biotin, 0.1 mg, niacin, 50 mg, D-calcium pantothenate, 12 mg, folic acid, 2 mg, Mn, 80 mg, Fe, 60 mg, Cu, 8 mg, I, 1 mg, Co, 0.3 mg and Mo, 1 mg

Animal ethics: The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No.: AEC 08/002). Health and animal husbandry practices complied with the Code of Practice for the Use of Animals for Scientific Purposes issued by the Australian Bureau of Animal Health (NHMRC, 1990).

Measurement and analyses:

Growth performance: Feed Intake (FI) and Live Weight (LW) on a cage basis were recorded at weekly intervals for determination of average FI and LW. Mortality was recorded as it occurred and feed conversion ratio (FCR; feed intake/weight gain) was corrected for mortality.

Visceral organ weight: The body weight and the weight of the proventriculus, gizzard and small intestine (the region from the distal end of the gizzard to 1 cm above the ileo-caecal junction) with contents, pancreas, bursa of Fabricius, yolk sac, spleen and liver were recorded at days 7 and 21. The relative organ weight was subsequently calculated as an indication of mass per unit of body weight (g/100 g of b.wt.).

Acid insoluble ash content and nutrient digestibility: The concentration of Acid Insoluble Ash (AIA) in feed and freeze-dried ileal digesta was determined after ashing the samples and treating the ash with boiling 4 M HCl, following the methods described by Vogtman *et al.* (1975) and Choct and Annison (1990).

The ileal digestibility of protein, gross energy and starch of feeds and freeze-dried ileal digesta was calculated and related to the concentration of the AIA. Diets and ileal digesta were analyzed for protein, gross energy using standard AOAC (2002) methods and starch was determined with the Megazyme assay kit (Megazyme International Ireland, Bray Business Park, Bray, Ireland) as described by Mccleary *et al.* (1994). The digestibility coefficient of nutrient was calculated using the equation:

$$\text{Digestibility Coefficient} = 1 - \frac{\text{Digesta nutrient (g kg}^{-1}\text{)/Digesta AIA (g kg}^{-1}\text{)}}{\text{Diet nutrient (g kg}^{-1}\text{)/Diet AIA (g kg}^{-1}\text{)}}$$

where, AIA x acid insoluble ash.

Particle size characteristics in gizzard content: At 21 days of age the pooled (on cage basis) dry matter contents of gizzard were determined by the gravimetric method which is described in Bhuiyan *et al.* (2012) of this series. Further, the particle size of gizzard content was determined by sieving method through a 1 mm screen, after oven-drying (105°C) overnight and cooling at room temperature.

Enumeration of gut microbial community: Fresh intestinal contents, weighing around 1 g, from the ileum and caeca were transferred into 15 mL MacCartney bottles containing 10 mL of anaerobic broth and used to assess microbial profiles.

Lactic acid bacteria were enumerated on MRS agar (Oxoid, CM0361) incubated under anaerobic conditions at 39°C for 48 h. Coliform (red colonies) and lactose-negative *Enterobacteria* (colourless colonies) were counted on MacConkey agar (Oxoid, CM 0007) incubated aerobically at 39°C for 24 h as red and colourless colonies, respectively. Lactobacilli were enumerated on Rogossa agar (Oxoid, CM 0627) after anaerobic incubation at 39°C for 48 h. Total anaerobic bacteria were counted using anaerobic roll tubes containing 3 mL of Wilkins-Chalgren anaerobic agar (Oxoid, CM 0619) incubated at 39°C for 7 days. Numbers of *C. perfringens* (Cp) were counted on Tryptose-Sulfite-Cycloserine and Shahidi-Ferguson Perfringens agar base (TSC and SFP) (Oxoid, CM 0587 OPSP) mixed with egg yolk emulsion (Oxoid, SR0047) and Perfringens (TSC) selective supplement (Oxoid, SR0088E) according to the pour-plate technique, where plates were overlaid with the same agar after spreading the inoculum and incubated anaerobically at 39°C for 24 h. An anaerobic AnaeroGen™ sachet (AN0025A, Oxoid Ltd, Hampshire, UK) was used to generate the anaerobic environment (<1% O_2 and 9-13% CO_2) for all anaerobically incubated agar plates.

The enumeration of microbial profiles was conducted only on chickens raised on diets containing fine hammer-milled and coarse roller-milled grains. This was done mainly to reduce the number of replicates analysed but the groups were chosen to reflect differences in particle size and milling techniques. Roller-milling at a fine setting yielded more fine particles, more than hammer-milling at a fine setting. After incubation, colonies formed on the respective media were carefully counted, converted into logarithmic equivalents ($\log_{10}$) and expressed as number of Colony Forming Units (CFU) per gram of wet intestinal content.

Statistical analysis: Data for each day of sampling were analysed separately. The performance data such as FI, LW, FCR, relative weight of visceral organs, intestinal pH, nutrient digestibility and gut microbial community were analysed using the General Linear Models (GLM) procedure of SPSS, Version 17.0.0 (SPSS, 2009) for the main effects of milling technique, particle size and source of grain, along with their interactions. Separation of means within a significant effect was done by Duncan's Multiple Range Test (DMRT) through post-hoc procedure of SPSS. Significance levels were set at $p \leq 0.05$ unless otherwise specified. The data were analysed according to the following model:

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

Where:

Y_{ij} = Observed dependent variable
μ = Overall mean
α_i = Fixed effect of treatment
β_j = Random effect for replicate
ε_{ij} = Residual error for treatment

RESULTS

Gross responses: Feed intake to 7 days of age was affected by particle size but this was significant ($p<0.003$) only for maize from Downs that was roller milled, in which intake was higher in the diet containing finely milled grain than for the coarsely milled grain (Table 4). The interaction between milling technique and particle size on FI was also significant ($p<0.003$). In general, feed intake was higher in diets containing finely milled grain than coarsely milled grain (118.7 vs. 112.6 g bird^{-1}). Live weight at 7 days of age was affected by the source of maize but this was significant ($p<0.040$) only for maize from Moree and Downs that was roller milled, in which live weight was higher on the diet containing finely milled grain than for the finely hammer milled Emerald maize. The

Table 4: Feed Intake (FI), Live Weight (LW) and FCR of broiler chickens at 7 days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources[1]

Treatments					
Source	Milling	Particle	FI (g bird^{-1})	LW (g bird^{-1})	FCR (g: g)
Downs	Hammer	Coarse	116.1^{abc}	116.3^{a}	1.48
		Fine	112.9^{abc}	111.3^{ab}	1.54
	Roller	Coarse	108.8^{bc}	108.7^{ab}	1.54
		Fine	124.4^{a}	117.2^{a}	1.57
Emerald	Hammer	Coarse	117.1^{abc}	110.5^{ab}	1.62
		Fine	117.4^{abc}	104.3^{b}	1.78
	Roller	Coarse	106.1^{c}	106.4^{ab}	1.55
		Fine	116.9^{abc}	114.6^{ab}	1.53
Moree	Hammer	Coarse	117.6^{abc}	115.6^{ab}	1.52
		Fine	120.6^{ab}	111.5^{ab}	1.67
	Roller	Coarse	109.8^{bc}	113.1^{ab}	1.46
		Fine	119.9^{ab}	118.4^{a}	1.50
Pooled SEM			1.09	0.10	0.02
Model P			<0.01	<0.07	<0.09
Source of variation					
Source			ns	<0.040	ns
Milling			ns	ns	0.06
Particle			<0.003	ns	0.08
Source×milling			ns	ns	0.09
Source×particle			ns	ns	ns
Milling×particle			<0.003	<0.002	ns
Source×milling ×particle			ns	ns	ns

[1]Each value represents the mean of 5 replicates for each treatment group, Values with unlike superscripts within each column are significantly different ($p<0.05$), ns: Non-significant

Table 5: Feed Intake (FI), Live Weight (LW) and FCR of broiler chickens at 21 days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources[1]

Treatments					
Source	Milling	Particle	FI (g bird^{-1})	LW (g bird^{-1})	FCR (g: g)
Downs	Hammer	Coarse	845.8	597.2	1.51^{ab}
		Fine	851.5	606.7	1.50^{ab}
	Roller	Coarse	831.0	579.1	1.54^{ab}
		Fine	843.1	582.0	1.55^{ab}
Emerald	Hammer	Coarse	857.2	565.6	1.63^{a}
		Fine	827.8	546.4	1.63^{a}
	Roller	Coarse	822.5	568.6	1.55^{ab}
		Fine	820.0	597.9	1.47^{b}
Moree	Hammer	Coarse	871.4	596.7	1.56^{ab}
		Fine	862.1	584.0	1.58^{ab}
	Roller	Coarse	879.3	610.6	1.54^{ab}
		Fine	812.5	587.8	1.48^{b}
Pooled SEM			6.94	4.73	0.01
Model p			ns	ns	ns
Source of variation					
Source			ns	0.062	ns
Milling			ns	ns	ns
Particle			ns	ns	ns
Source×milling			ns	ns	<0.042
Source×particle			ns	ns	ns
Milling×particle			ns	ns	ns
Source×milling×particle			ns	ns	ns

[1]Each value represents the mean of 5 replicates for each treatment group, Values with unlike superscripts within each column are significantly different ($p<0.05$), ns: Non-significant

interaction between milling technique and particle size was also significant ($p<0.002$). In particular, live weight was lowest (109.0 g bird^{-1}) on diets containing Emerald maize than diets containing Moree and Downs. There was no significant effect or interaction between maize source and milling technique on FCR up to 7 days of age.

Up to 21 days of age, there was no significant effect of maize source, milling technique, particle size or interaction between these factors on FI and LW (Table 5). However, LW was marginally higher ($p<0.062$) on the diet containing Moree maize (594.8 g bird^{-1}) than diets containing Downs (591.3 g bird^{-1}) and Emerald (569.6 g bird^{-1}) maize. Feed conversion ratio up to this age was improved ($p<0.042$) on the Moree maize that was finely roller milled. The poorest FCR was observed in birds on the hammer milled Emerald maize diets.

Visceral organ weight: At day 7, the relative weight of small intestine was marginally increased ($p<0.06$) due to interaction between milling technique and particle size (Table 6). The relative weight of pancreas was affected by an interaction between maize source and milling technique but this was significant ($p<0.02$) only when the group on finely milled Downs's maize was compared to the group on Emerald, when both sources were hammer milled. The relative weight of liver was highest ($p<0.01$), in the group on the finely roller milled Moree maize and lowest on diet with Downs maize that was hammer milled to a fine texture or coarsely roller milled (5.3 vs. 4.1 g/100 g of body weight).

Table 6: Relative weight of visceral organs (g/100 g of body weight) of broiler chickens at 7 days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources[1]

Treatments									
Source	Milling	Particle	Small intes[2]	Pancreas	Liver	Pro+gizz[3]	Spleen	Bursa	Yolk sac
Downs	Hammer	Coarse	10.0	0.51^{abc}	4.4^{ab}	8.1^{abc}	0.08^{ab}	0.12	0.16
		Fine	11.9	0.59^{a}	4.1^{b}	8.5^{abc}	0.07^{b}	0.13	0.03
	Roller	Coarse	10.9	0.47^{bc}	4.2^{b}	8.8^{abc}	0.08^{ab}	0.13	0.09
		Fine	10.7	0.51^{abc}	4.9^{ab}	7.5^{c}	0.10^{ab}	0.15	0.09
Emerald	Hammer	Coarse	10.0	0.46^{c}	4.9^{ab}	7.9^{abc}	0.08^{ab}	0.14	0.04
		Fine	11.1	0.53^{abc}	4.4^{ab}	7.6^{bc}	0.11^{a}	0.15	0.03
	Roller	Coarse	11.1	0.56^{abc}	4.4^{ab}	8.9^{ab}	0.10^{ab}	0.13	0.06
		Fine	10.1	0.58^{ab}	4.7^{ab}	7.9^{bc}	0.07^{b}	0.13	0.34
Moree	Hammer	Coarse	9.2	0.52^{abc}	4.9^{ab}	8.0^{abc}	0.08^{ab}	0.15	0.10
		Fine	9.8	0.51^{abc}	4.3^{b}	7.9^{abc}	0.09^{ab}	0.15	0.07
	Roller	Coarse	10.9	0.54^{abc}	4.7^{ab}	9.3^{a}	0.07^{b}	0.14	0.07
		Fine	10.9	0.56^{abc}	5.3^{a}	7.7^{bc}	0.09^{ab}	0.14	0.07
Pooled SEM			0.21	0.01	0.08	0.13	0.01	0.01	0.02
Model P			ns	ns	ns	<0.07	ns	ns	ns
Source of variation									
Source			ns	ns	ns	ns	ns	ns	ns
Milling			ns	ns	ns	ns	ns	ns	ns
Particle			ns	0.08	ns	<0.01	ns	ns	ns
Source×milling			ns	<0.02	ns	ns	ns	ns	0.09
Source×particle			ns	ns	ns	ns	ns	ns	ns
Milling×particle			0.06	ns	<0.01	<0.01	ns	ns	ns
Source×milling×particle			ns	ns	ns	ns	<0.03	ns	ns

[1]Each value represents the mean of 5 replicates for each treatment group, [a,b,c]Values with unlike superscripts within each column are significantly different ($p<0.05$), [2]Small intestines with digesta, [3]Proventriculus and gizzard with digesta, ns: Non-significant, SEM: Standard error of mean

There was an effect of particle size on the relative weight of proventriculus plus gizzard but this was significant ($p<0.01$) only when the groups on coarse roller milled Moree maize was compared to the group on finely milled (roller) Downs. In general, the relative weight of proventriculus plus gizzard was higher in diets containing coarsely milled grain than finely milled grain (8.5 vs. 7.9 g/100 g of body weight).

The interaction between milling technique and particle size on the relative weight of proventriculus plus gizzard was also significant ($p<0.01$). There was a significant three-way interaction between the effect of sources, milling technique and particle size on relative weight of spleen. On diets based on Emerald, spleen weight was reduced when the hammer and roller milled, fine diet was fed. Moreover, on the diet based on grain from Downs, spleen weight was reduced with hammer and roller milled fine diet. For Moree, the fine roller milled diet caused this reduction. The relative weight of yolk sac was highest in chickens on diets containing finely roller milled maize from Emerald.

At 21 days of age, there was no significant effect of maize source, milling technique, particle size or interaction between the factors on relative of weight of small intestine and proventriculus plus gizzard (Table 7). However, there was an effect of milling technique and particle size on the relative weight of pancreas but this was significant ($p<0.01$) only for chickens on maize from

Table 7: Relative weight of visceral organs (g/100 g of b.wt.) of broiler chickens at 21 days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources[1]

Treatments								
Source	Milling	Particle	Small intes.[2]	Pan-creas	Liver	Pro+gizz[3]	Spleen	Bursa
Downs	Hammer	Coarse	6.8	0.36^{abc}	3.5	2.9	0.08	0.18
		Fine	6.1	0.34^{bc}	3.1	2.8	0.10	0.21
	Roller	Coarse	6.1	0.33^{bc}	4.0	2.9	0.11	0.21
		Fine	6.0	0.38^{abc}	3.0	2.7	0.11	0.23
Emerald	Hammer	Coarse	6.1	0.42^{a}	3.4	3.0	0.09	0.19
		Fine	5.9	0.34^{bc}	3.3	2.6	0.09	0.21
	Roller	Coarse	6.7	0.35^{abc}	4.1	2.9	0.10	0.18
		Fine	6.4	0.39^{abc}	3.6	2.7	0.12	0.23
Moree	Hammer	Coarse	6.4	0.34^{bc}	3.4	2.9	0.12	0.18
		Fine	5.8	0.31^{c}	3.1	3.0	0.10	0.22
	Roller	Coarse	6.2	0.37^{abc}	3.6	3.1	0.09	0.18
		Fine	6.4	0.41^{ab}	3.2	2.7	0.10	0.21
Pooled SEM			0.09	0.01	0.08	0.06	0.00	0.01
Model p			ns	0.07	0.07	ns	ns	ns
Source of variation								
Source			ns	ns	ns	ns	ns	ns
Milling			ns	ns	0.07	ns	ns	ns
Particle			ns	ns	<0.01	ns	ns	<0.02
Source×milling			ns	ns	ns	ns	<0.02	ns
Source×particle			ns	ns	ns	ns	ns	ns
Milling×particle			ns	<0.01	ns	ns	ns	ns
Source×milling×particle			ns	ns	ns	ns	ns	ns

[1]Each value represents the mean of 5 replicates for each treatment group, [a,b]Values with unlike superscripts within each column are significantly different (p<0.05), [2]Small intestines weight considered with digesta, [3]Proventriculus plus gizzard weight considered without digesta, ns: Non-significant, SEM: Standard error of mean

Emerald that was hammer milled, in which the relative weight of pancreas was higher in the diet based on coarsely milled grain than finely milled grain. Generally, the relative weight of liver was higher (p<0.01) in chickens raised on diets containing coarse particle compared to those on fine diets (3.7 vs. 3.2 g/100 g of body weight). In addition, the relative weight of the liver was marginally affected (p<0.07) by milling technique, being higher in chickens on roller milled diets than on hammer milled diets. The relative weight of the bursa was significantly affected (p<0.02) by particle size, with generally higher values in chickens fed the finely milled diets than those on coarsely milled diets (0.22 vs. 0.19 g/100 g of body weight).

Intestinal pH: Generally, there was no significant effect of maize source, milling and particle size or interactions between these factors on the pH value of the intestinal contents from the gizzard, ileum and caecum at 21 days of age. In general, the pH ranged from 2.9-3.3 in the gizzard, 6.3-6.9 in the ileum and 6.8-7.1 in the caeca.

Nutrient digestibility: The ileal digestibility of protein was not affected by maize source and milling technique but this variable was affected (p<0.01) by particle size, the digestibility of protein being higher in birds on diets containing finely (0.82) milled grain than coarsely (0.79) milled grain (Table 8). There was a significant effect of maize source (p<0.01) and particle size (p<0.03) but not

Table 8: Ileal protein, gross energy and starch digestibility of broiler chickens at 21 days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources of maize[1]

Treatments					
Source	Milling	Particle	Protein	Gross energy	Starch
Downs	Hammer	Coarse	0.80[ab]	0.72[ab]	0.93[b]
		Fine	0.81[ab]	0.76[a]	0.95[ab]
	Roller	Coarse	0.77[b]	0.68[b]	0.94[ab]
		Fine	0.81[ab]	0.73[ab]	0.94[ab]
Emerald	Hammer	Coarse	0.79[ab]	0.72[ab]	0.97[ab]
		Fine	0.82[ab]	0.78[a]	0.98[a]
	Roller	Coarse	0.81[ab]	0.77[a]	0.96[ab]
		Fine	0.83[a]	0.77[a]	0.95[ab]
Moree	Hammer	Coarse	0.79[ab]	0.75[a]	0.97[ab]
		Fine	0.81[ab]	0.77[a]	0.97[ab]
	Roller	Coarse	0.80[ab]	0.77[a]	0.97[ab]
		Fine	0.82[a]	0.76[a]	0.96[a]
Pooled SEM			0.004	0.006	0.004
Model p			ns	<0.02	ns
Source of variation					
Source			ns	<0.01	<0.01
Milling			ns	ns	ns
Particle			<0.01	<0.03	ns
Source×milling			ns	ns	ns
Source×particle			ns	ns	ns
Milling×particle			ns	ns	ns
Source×milling×particle			ns	ns	ns

[1]Each value represents the mean of 5 replicates for each treatment group; [a,b]Values with unlike superscripts within each column are significantly different ($p<0.05$), ns: Non-significant

of milling technique on gross energy digestibility, with higher values in chickens raised on diets based on Emerald and Moree maize (0.76) than those on Downs (0.72). In general, gross energy digestibility was higher on diets containing finely milled grain than coarsely milled grain (0.76 vs. 0.73). There was no significant interaction between the main effects on gross energy digestibility. Generally, starch digestibility was higher ($p<0.01$) on diets containing maize from Emerald (0.96) and Moree (0.97) than Downs (0.94). There was no significant interaction between the main effects on starch digestibility at 21 days of age.

Particle size and dry matter of gizzard contents: The grain particle size characteristics of gizzard contents at 21 days of age are shown in Table 9. There was a significant effect of feed particle size ($p<0.01$) but not of maize source and milling technique on the proportion of particle sizes in gizzard contents. For birds fed finely ground diets, 26% of the material found in the gizzard was less than 1 mm while for the coarsely ground diets, 19% of the gizzard content was fine and the rest was coarse (>1 mm). The dry matter of gizzard contents was higher ($p<0.01$) on diets based on Moree (39.6) and Downs (38.6) maize than Emerald (36.2) and also higher on coarse (38.8) than on fine (37.4) particle diets. There was no significant interaction between main effects on particle size or dry matter of gizzard contents.

Table 9: Proportion of particle size classes in the gizzard content (on a dry weight basis) of broiler Chickens at day 21 days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources of maize[1]

Treatment					
Sources	Milling	Particle	Fine particles (<1 mm)	Coarse particles (>1 mm)	Dry matter (%)
Downs	Hammer	Coarse	0.20^{bc}	0.80^{ab}	38.0^{abcd}
		Fine	0.23^{abc}	0.77^{abc}	38.7^{abcd}
	Roller	Coarse	0.16^{c}	0.84^{a}	40.0^{abc}
		Fine	0.26^{ab}	0.74^{bc}	37.6^{abcd}
Emerald	Hammer	Coarse	0.21^{bc}	0.79^{ab}	36.2^{cd}
		Fine	0.24^{abc}	0.76^{abc}	36.9^{bcd}
	Roller	Coarse	0.20^{bc}	0.80^{ab}	36.7^{bcd}
		Fine	0.26^{ab}	0.74^{bc}	35.1^{d}
Moree	Hammer	Coarse	0.19^{bc}	0.81^{ab}	41.2^{a}
		Fine	0.25^{ab}	0.75^{abc}	38.6^{abcd}
	Roller	Coarse	0.19^{bc}	0.81^{ab}	40.7^{ab}
		Fine	0.31^{a}	0.69^{c}	37.8^{abcd}
Pooled SEM			0.009	0.009	0.390
Model p			<0.039	<0.036	<0.026
Source of variation					
Source			ns	ns	0.001
Milling			ns	ns	ns
Particle			0.001	0.001	0.05
Source×milling			ns	ns	ns
Source×particle			ns	ns	ns
Milling×particle			ns	ns	ns
Source×milling×particle			ns	ns	ns

[1]Each value represents the mean of 5 replicates for each treatment group, Values with unlike superscripts within each column are significantly different ($p<0.05$), ns: Non significant

Table 10: Bacterial counts ($\log_{10}$ CFU g^{-1} digesta) in ileal digesta of broiler chickens at 21st days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources of maize

Treatments						
Source	Milling	Particle	Anaerobic	Lactic acid	*C. perfringens*	Entero-bacteria
Downs	Hammer	Fine	7.4	7.8	3.1	4.0
	Roller	Coarse	8.1	6.9	3.6	4.3
Emerald	Hammer	Fine	7.9	7.9	3.2	3.9
	Roller	Coarse	7.8	8.2	3.1	4.0
Moree	Hammer	Fine	7.9	8.2	3.0	4.1
	Roller	Coarse	8.2	8.3	3.3	4.2
Pooled SEM			0.089	0.260	0.104	0.051
Model p			ns	ns	ns	ns
Source of variation						
Source			ns	ns	ns	ns
Milling and particle			<0.09	ns	ns	<0.08
Source×milling and particle			ns	ns	ns	ns

C. *perfringens*: *Clostridium perfringens*, ns: Non significant

Gut microflora: Overall, there was marginally stronger effect of maize source, milling technique and particle size or interactions between these main effects on different types of bacterial populations in the ileal contents at 21 days of age (Table 10). However, the number of anaerobic

Table 11: Bacterial counts (log_{10} CFU g^{-1} digesta) in caecal digesta of broiler chickens at 21 days of age given finely and coarsely ground maize based diets obtained by hammer or roller milling from various sources of maize

Treatments						
Source	Milling	Particle	Anaerobic	Lactic acid	*C. perfringens*	Entero-bacteria
Downs	Hammer	Fine	8.7	9.1	7.4	6.1
	Roller	Coarse	8.7	9.3	7.2	5.7
Emerald	Hammer	Fine	8.7	9.0	7.5	6.0
	Roller	Coarse	8.6	9.1	7.3	5.3
Moree	Hammer	Fine	8.8	8.9	7.3	6.0
	Roller	Coarse	8.8	9.1	7.3	5.7
Pooled SEM			0.051	0.062	0.122	0.063
Model p			ns	ns	ns	ns
Source of variation						
Source			ns	ns	ns	ns
Milling and particle			ns	ns	<0.05	ns
Source×milling and particle			ns	ns	ns	ns

C. perfringens: *Clostridium perfringens*, ns: Non significant

bacteria (8.0 vs. 7.7 log_{10} CFU g^{-1} digesta) and enterobacteria (4.2 vs. 4.0 log_{10} CFU g^{-1} digesta) was marginally increased ($p<0.09$ and $p<0.08$, respectively) in chickens on diets containing coarsely roller milled grain than in those on finely hammer milled grain. The number of *Clostridium perfringens* was numerically higher in the ileal content of birds on the roller-milled, coarse particle diet than those on hammer-milled, fine particle diet except on the diet based on Emerald maize.

In general, the bacterial populations in caecal contents were not affected by maize source, milling technique and particles or the interaction between these factors (Table 11). An exception was observed in chickens on the finely hammerBmilled diets, with generally higher ($p<0.05$) population of *C. perfringens* than birds on the coarsely rolled grain.

DISCUSSION

Gross responses: The present study revealed that the FI and LW of broiler chicks was affected by the source of maize, particle size and their interactions in mash form although these effects waned with age. Nir *et al*. (1994) found similar responses and stated that the particle sizes and texture of diets affect the performances of broiler chickens considerably. Higher feed intake on the fine particle size diet up to day 7, but not at a later age. This may be due to undeveloped small beaks at early age and agree with Moran (1982) and Portella *et al*. (1988), who suggested that the particle size of maize consumed by birds was related to beak development and the age of the birds.

Milling technique had no effect on overall performance, but the interaction between milling technique and particle size affected FI and LW at seven days of age. This finding coincides with the report of Reece *et al*. (1985), who observed that chickens fed with mash diets containing maize ground with a roller mill grew faster and more efficiently than those fed on diets containing maize ground with a hammer mill. This may be due to a higher proportion of coarse particles in hammer-milled diets compared to roller-milled diets. In addition, the source of maize made had an effect on LW at an early age (1-7 days) of the birds. This may be a result of compositional differences between the varieties and this is supported by the findings of Cowieson (2005), who reported that the chemical composition and nutritional value of maize differs from batch to batch, resulting in a high degree of variation in its energy value for poultry. Performance was generally below breed standards probably due to the semi-purified nature of the diets, although, nutrient balance was ensured.

Visceral organ weight: In the present study there was an impact of particle size on the relative weights of the proventriculus-gizzard, liver and bursa. In particular, there was an increase in the relative weight of the proventriculus/gizzard and liver in early age for chickens on coarse particle diets, while the relative weight of the immune organ, the bursa, was increased by the fine particles of diets at grower stage. Similar results were recorded by Nir *et al.* (1994), Svihus *et al.* (1997), who acknowledged that gizzard weight of broilers became significantly greater when they were fed diets with coarser particles than when they were fed fine mash. Gizzard development can be achieved by manipulating the feed particle size of diets and this can be employed as a nutritional strategy (Nir *et al.*, 1995; Engberg *et al.*, 2002).

Particle size distributions in gizzard content: The distribution of ingested feed particles in the gizzard contents was influenced by the source of maize, the proportion of coarse and fine particles as well as the DM content of the diet. Significantly, higher DM in gizzard contents was observed in birds fed diets based on the Downs and Moree maize than Emerald maize. This may be due to the initial moisture content of individual sources, or perhaps due to variation in the particle size distribution of the milled grain. The proportion of particle sizes in the gizzard reflected the original particle distribution of the diets used, confirming minimal pre-ventricular processing of diets by birds.

Nutrient digestibility: There was a significant effect of grain source on ileal digestibility of energy and starch, this being highest in birds on diets based on Emerald and Moree maize. This variation may be due to differences in chemical composition of grain from different sources. Moreover, protein and energy digestibility was increased in chicks fed on fine particle diets. These findings are in contrast to the report by Parsons *et al.* (2006), who found that feeding medium to coarse particle maize improved nutrient digestion; most likely due to better gizzard development and function. In this study, the CP and gross energy digestibility was improved on the fine particle diet, maybe due to increase in the surface area available for enzyme activity, allowing more nutrients to be digested and absorbed through the small intestine (Cumming, 1994; Forbes and Covasa, 1995).

In this study, there was no significant effect of milling technique on nutrient digestibility; however, particle size appears to be more important. In a study by Weurding *et al.* (2001), milling method (hammer or roller) had no effect on the extent of starch digestion but enhanced its digestion rate and maximum digestibility resulted from roller-milled maize. The differences in starch digestibility due to maize variety may be due to the variable starch composition of the grain sources.

Gut microflora: There was no significant change in bacterial populations in the gut of chickens due to maize source, milling method or particle size. However, the population of *C. perfringens* was affected by interactions between milling technique and particle size. This observation is supported to some extent by the study of Engberg *et al.* (2002) who mentioned that the structural properties of feed, grain particle size and feed formulation can influence the intestinal microflora of poultry. For example, an increase in retention time in the gizzard and more acidic gizzard pH may not only kill ingested enteric pathogens, but also increase the fermentation by symbiotic bacteria in the crop that act as seed stock to colonize the lower digestive tract and competitively exclude pathogens (Engberg *et al.*, 2004).

In the current study, there was a significant reduction in population of *C. perfringens* in caecal content due to roller milling and coarse particle size. This is expected as a well developed gizzard is associated with improvement in gut motility (Ferket, 2000) and may prevent pathogenic bacteria such as *C. perfringens* from entering the small intestine (Bjerrum *et al.*, 2005). Such diets may reduce the risk of coccidiosis and other enteric diseases (Engberg *et al.*, 2004; Bjerrum *et al.*, 2005).

CONCLUSION

The present study showed that the source, milling and particle size of grains are important in mash diets in terms of bird performance, essentially in early life. In particular, fine particle size resulted in an increase in FI and one of the maize sources (Moree) enhanced LW at the same growth phase. However, FCR was improved on diets with roller-milled maize from Emerald and Moree in the third week of age. Large particle size had an initial stimulatory effect on the gizzard and subsequent effects on the liver and bursa.

Overall, the nutritive value and digestibility of starch were higher in this experiment than under the commercial practice where a fixed level of maize (72%) was used in diet. It may not certain if theses response will remain the same, regardless of level of maize inclusion or supplementation with appropriate microbial enzymes with roller milling. So, further studies covering the inclusion level of maize with different milling technique and with enzyme supplement in broiler diets are needed to explore more of this current study.

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