Response of broiler chickens to diets containing artificially dried high-moisture maize supplemented with microbial enzymes

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Abstract

The effect of feeding high-moisture maize grains dried in the sun or artificially in a forced draught oven at 80, 90 or 100 °C for 24 hours and supplemented with microbial enzymes (Avizyme 1502 and Phyzyme XP) on growth performance, visceral organs, tissue protein, enzyme activity and gut development was investigated in a broiler growth trial. Feed intake (FI) up to 21 days decreased as a results of oven drying of grains whereas supplementation with microbial enzymes increased FI compared to the non-enzyme diets (881.1 *vs*. 817.2 g/bird). The highest FI (900 g/bird) was found only in sun-dried grain diets. There was no effect of grain drying temperature or enzyme supplementation on FI when assessed at 7 days of age. Up to day 21 there was a reduction in live weight (LW) with increase in grain drying temperature while supplementation with enzymes significantly improved LW only on the diets containing sun-dried grains (731 g/bird) and grains dried at 90 °C (634 g/bird). Live weight was significantly higher in chickens on the enzyme supplemented diets than on diets without enzymes (638 *vs*. 547 g/bird). The feed conversion ratio (FCR) at this age was poorer with an increase in grain drying temperature but improved when the diets were supplemented with enzymes (1.48 *vs*. 1.62 g/g). There was an increase in the relative weight of the small intestine and liver with an increase in grain drying temperature at day 21 but there was no difference in the relative weight of these organs when the diets were supplemented with enzymes. Only the activities of the alkaline phosphatase at day 7 and maltase and sucrase at days 7 and 21 increased as a result of grain drying treatment but not by microbial enzyme supplementation. The ileal digestibility of gross energy, protein and starch was not significantly changed with an increase in grain drying temperature or by enzyme supplementation. The concentrations of ileal formic and acetic acids and caecal propionic and valeric acids were significantly increased by an increase in grain drying temperature but not affected by the microbial enzyme supplementation. The populations of lactic acid and lactobacilli bacteria in the ileal content were reduced on diets containing enzymes but were not affected by an increase in grain drying temperature. In the caecal content, the total anaerobic bacterial count was higher in birds on diets supplemented with microbial enzymes (8.1 *vs*. 7.8 log_{10} *cfu x*/g digesta). The resident lactic acid bacteria population also increased as a result of an increase in grain drying temperature. From results of the current study, diets based on sun-dried maize or maize dried at 90 °C provided comparatively better gross response. It may be inferred that there was a positive response to addition of microbial enzymes. Overall, it may be assumed that, for broiler chickens, there is little or no difference in the nutritive value of sun-dried grain and grains artificially dried at 90 °C.

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Introduction

In many parts of the world, especially in humid areas, maize is harvested at a relatively high moisture content, with a view to minimizing damage in the field when left to dry naturally. The grain is then subjected to artificial drying, which may result in loss of quality such as an increase in retrograde starch (Brown, 1996). The retrograde starch is caused by high temperature heating of grains followed by storage at a lower temperature. The digestibility of cereal grains is influenced by the starch component, especially the ratio between amylose and amylopectin (McDonald *et al.*, 1995). Noy & Sklan (1994) stated that about 15% of maize starch is known to remain undigested up to the terminal ileum and is assumed to be resistant to digestion. This presents an opportunity for the use of exogenous enzymes as is done with wheat and other temperate cereals. Starch in high moisture grain also anneals due to heat processing. It is not well known

how much of these processes occur during routine production and processing of maize.

Due to the amorphous nature of its structure, amylopectin is more readily digested than the amylose. Moreover, this readily available amylopectin content may increase due to artificial drying of high moisture grain and conversely amylose content may decrease. This was confirmed by Bhuiyan *et al.* (2010), but it is not known if further improvements could be achieved through supplementation of microbial enzymes.

The normal structure (spherical, 10 – 16 microns across) of starch granules with protein bodies and matrix may be altered easily and can create a favourable environment for enzymatic digestion (Taylor & Belton, 2002). However, the normal structure of starch granules may also change, becoming more shrunken if dried at a high temperature, and maize quality can be compromised (Bhuiyan *et al.*, 2010). The research of Panigrahi *et al*. (1996), who evaluated the effects of artificial drying of grains that had stack-burn and reduction in *in vitro* digestibility, showed that the quality of diets with stack burned/overheated maize grain tends to negatively affect broiler performance. There are many reasons advanced for these reductions in quality such as denaturation of heat-labile vitamins and damage to proteins via an interaction with reducing sugars (Maillard reaction) and retrograde starch formation after heat processing (Panigrahi *et al.*, 1996; Iji *et al.*, 2003; Cowieson, 2005).

Exogenous enzymes have been used for many years to break down cell walls in feed ingredients, to reduce the viscosity of digesta and to improve the digestibility of carbohydrates and proteins (Bedford & Moran, 1996). Generally they are used to improve the nutritive value of wheat- and barley-based diets, but some enzyme preparations are also used to increase the nutritional value of maize and its by-products (Boros *et al.*, 2004; Cowieson, 2005). However, in some investigations the positive effect of enzymes was not registered (Peric *et al.*, 2002; Iji *et al.*, 2003). There is little in the literature about the addition of enzyme mixture to diets based on artificially dried maize, especially if the grains were harvested with high-moisture.

The aim of this trial was to evaluate the response of high-moisture maize grain to artificial drying and supplementation with microbial enzymes. These investigations were carried out considering feed intake (FI), body weight (BW) and feed conversion ratio (FCR), as well as the physiological mechanisms behind these responses.

Materials and Methods

A 2 x 4 factorial experiment was performed to study the effect of drying high-moisture maize at different temperatures (sun-dried and artificially dried at 80, 90 or 100 °C) and the addition of microbial enzymes to the diet on the nutritive value of the grain and physiological responses in broiler chickens. Three hundred and eighty-four (384) day-old male Cobb broiler chicks (Baiada Poultry Pty. Ltd, Tamworth, NSW, Australia), weighing 40.1 ± 0.08 g, were randomly assigned to 48 cages ($600 \times 420 \times 23$ cm each) in four-tier battery brooders, housed in an environmentally controlled house. Each of the eight treatments was randomly assigned to six cages with eight birds per cage. The birds were initially brooded at a temperature of 33 °C, but this was gradually reduced to 24 ± 1 °C at 21 days of age when the feeding trial ended. Sixteen hours of lighting per day were provided throughout the trial period.

Maize grain obtained from Inverell in northern New South Wales, Australia (2009 planting year; at the end of April) was used in this experiment. The maize contained a moisture content of 23% and was split into four batches. The first batch was dried in the sun for three days until the moisture content dropped to about 13%. The other three batches were dried artificially for 24 hours using a forced draught oven at 80, 90 or 100 °C. After drying, the dry matter (DM) content was 95.0, 96.3 and 98.0%, respectively.

Four basal diets were formulated to meet minimum National Research Council (1994) nutrient recommendations for broiler chickens (Table 1). The experimental diets were semi-purified, comprising of more than 70% maize grain: sun-dried, or dried at 80, 90 or 100 °C. Each of these diets was fed as such or supplemented with enzymes at a rate of 0.5 g/kg of Avizyme 1502 (containing amylase 800, xylanase 1200, protease 8000 U/g) and 0.1 g/kg of Phyzyme XP (1000 FTU). Both these enzymes were supplied by Danisco Animal Nutrition, UK. An indigestible marker, titanium dioxide (TiO_2), was incorporated in the diets to assess nutrient digestibility of the feed. The diets were formulated to be iso-energetic and iso-nitrogenous, and fed for 21 days. Feeds and water were available to the birds *ad libitum*.

On days 7 and 21, two birds and three birds, respectively from each cage, randomly selected, were weighed and humanely killed by cervical dislocation. The abdominal cavity was opened and the small intestine was ligated and removed. The content of the ileum and caecum was collected in labelled plastic

containers for the determination of nutrient digestibility and short-chain fatty acids (SCFA) content. Approximately 1 g of ileal and caecal samples was collected separately into pre-prepared McCartney bottles containing anaerobic broth for the enumeration of microbial populations. The McCartney bottles and plastic containers containing digesta samples were kept at –20 °C pending bacterial enumeration and analysis of SCFA. For the determination of the TiO_2 as well as nutrient digestibility, the digesta from the ileum were pooled on a cage basis, homogenized and stored at –20 °C. Later, the samples were freeze-dried, ground through a small coffee grinding and stored in airtight containers at –4 °C for the chemical analysis of TiO_2, gross energy, starch and protein.

Table 1 Broiler starter diet[1] composition (g/kg)

	Diet 1 (sun-dried)	Diet 3 (80 °C)	Diet 5 (90 °C)	Diet 7 (100 °C)
Ingredients				
Maize	71.0	72.6	72.6	73.9
Soycomil K (67% CP)	22.6	21.7	21.6	21.2
Chlorine chloride	0.1	0.1	0.1	0.1
Sodium bicarbonate	0.1	0.1	0.1	0.1
Vegetable oil	2.3	1.2	1.2	0.0
Limestone	1.3	1.3	1.3	1.3
Dicalcium phosphate	1.7	1.7	1.7	1.7
Salt	0.3	0.3	0.3	0.3
Lysine	0.01	0.09	0.1	0.1
Methionine	0.19	0.17	0.2	0.2
Titanium oxide	0.5	0.5	0.5	0.5
Vitamin mineral pemix[2]	0.2	0.2	0.2	0.2
Total	100	100	100	100
Nutrients composition				
Crude protein (g/kg)	210.1	211.0	211.0	211.2
ME (MJ/kg diet)	12.7	12.8	12.8	12.8
Lysine (g/kg)	11.4	11.8	12.6	11.4
Methionine (g/kg)	5.4	5.2	5.0	5.4
Methionine + cystine (g/kg)	6.9	6.8	6.8	6.8
Threonine (g/kg)	8.5	8.2	8.2	8.2
Calcium (g/kg)	10.0	9.9	9.9	9.9
Avail. phosphorus (g/kg)	4.2	4.2	4.2	4.2

[1] Diets 2, 4, 6 and 8 corresponded to diets 1, 3, 5 and 7, respectively but contained ; 0.5 g Avizyme [TM] 1502/kg and 0.1 g Phyzyme XP/kg feed (Danisco Animal Nutrition, UK);
[2] Supplied per kg of diet (g): 3.6 mg vitamin A (as all-trans retinol); 0.09 mg cholecalciferol; 44.7 mg vitamin E (as d-α-tocopherol); 2 mg vitamin K_3; 2 mg thiamine; 6 mg riboflavin; 5 mg pyridoxine hydrochloride; 0.2 mg vitamin B_{12}; 0.1 mg biotin; 50 mg niacin; 12 mg D-calcium pantothenate; 2 mg folic acid; 80 mg Mn; 60 mg Fe; 8 mg Cu; 1 mg I; 0.3 mg Co; 1 mg Mo.
ME – Metabolizable energy.

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No.: AEC09/004). 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 (National Health and Medical Research Council, 1990).

Live weights, live weight gain (LWG) and FI were recorded on a cage basis at weekly intervals. Mortality was recorded and FCR was corrected for mortality. The body weight and the weight of the proventriculus, gizzard and small intestine with content, 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 body weight).

To examine the digestive enzyme activity and protein concentration, the jejunal tissue was processed as described by Shirazi-Beechey *et al.* (1991). Sub-samples of the homogenate were taken, put into Eppendorf tubes (Eppendorf South Pacific, North Ryde, Australia) and stored in a freezer (–20 °C) pending enzyme analysis. The pancreas was processed in a similar procedure to the jejunum except that Milli-Q water (Millipore Australia, North Ryde, Australia) was used instead of a buffer, and the entire tissue was homogenized (Nitsan *et al.*, 1974). The specific activities of jejunal and pancreatic enzymes were assessed by incubation with fixed substrate concentrations as standardized for poultry by Iji *et al.* (2001b). On the jejunal homogenates, the assays were conducted for mucosal protein content and activities of alkaline phosphatase (EC 3.1.3.1), maltase (EC 3.2.1.20) and sucrase (EC 3.2.1.10). For the pancreas, assays were conducted for protein content and chymotrypsin amidase (EC 3.4.21.1). Specific activities of enzymes were measured according to the methods previously described for other species (Holdsworth, 1970; Serviere-Zaragoza *et al.*, 1997) after standardization for poultry. The concentration of protein in both the jejunal mucosa and pancreatic tissue was measured using the Coomassie dye-binding procedure described by Bradford (1976). The raw data outputs were processed using the recommended Lowry Software (Mcpherson, 1985) before running the statistical analysis.

The analytical method described by Jensen *et al.* (1995) was adopted with modifications for the analysis of organic acid (SCFA, lactic acid and succinic acid) concentrations. Before running on a Varian CP3400 CX gas chromatograph (Varian Analytical Instruments, Palo Alto, CA, USA), sample vials were kept in a heating block at 80 °C for 20 min and left at room temperature for 48 h. Total organic acid concentration was derived as the sum of all the organic acids observed in a sample, expressed as mg/g digesta after $\log_{10} +1$ transformation.

The TiO_2 content of the digesta and diet samples was measured according to the method of Short *et al.* (1996). Aliquots of the solutions obtained and of similarly prepared standard solutions were analyzed using a Hitachi 150-20 UV spectrophotometer (Hitachi Science Systems Ltd., Ibaraki, Japan), measuring the absorbance at 410 nm. The TiO_2 content, measured in mg/mL, was determined from the standard curve and converted to mg/g of the sample. The TiO_2 marker was used to calculate the digestibility coefficient. Diets and ileal digesta were analyzed for protein and gross energy using standard AOAC (2002) methods and starch was determined with a Megazyme assay kit (Megazyme International Ireland, Bray Business Park, Bray, Ireland) as described by McCleary *et al*. (1994). The digestibility coefficient of nutrients was calculated using the following equation:

Digestibility coefficient = 1 – [{digesta nutrient (g/kg DM)/digesta TiO_2 (g/kg DM)}/{diet nutrient (g/kg DM)/diet TiO_2 (g/kg DM)}]

Fresh intestinal content weighing *ca*. 1 g from the ileum and caeca was transferred into 15 mL MacCartney bottles containing 10 mL of an anaerobic broth. The suspension was homogenized for 2 min in CO_2-flushed plastic bags using a bag mixer (Interscience, St. Norm, France) and then serially diluted in 10-fold increments in anaerobic broth according to the procedure described by Miller & Wolin (1974) and Engberg *et al.* (2004). One millilitre of the homogenized suspension was then transferred into 9 mL of anaerobic broth and serially diluted from 10^{-1} to 10^{-5} for ileum samples and 10^{-1} to 10^{-6} for caecal samples. From the last three diluted samples, 0.1 mL each was plated on the appropriate medium (10 mL) for enumeration of microbial populations.

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 collarless 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 seven days. Number of *C. perfringens* (*C.p*) was counted on a Tryptose-Sulfite-Cycloserine and Shahidi-Ferguson

Perfringens agar base (TSC & SFP) (Oxoid, CM 0587 OPSP) mixed with egg yolk emulsion (Oxoid, SR0047) and a 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. 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.

The data were analysed using the multiple regression options of SPSS, Version 17.0.0 (SPSS Inc, 2009) for the main effects of increasing drying temperature and enzyme supplementation (Morris, 1998). The data were also subjected to GLM analysis and reported where there was an interaction between drying temperature and microbial enzyme supplementation. Data for organic acid concentrations were log-transformed ($\log_{10}$ +1) prior to analyses. Separation of means within a significant effect was conducted using Duncan's Multiple Range Test (DMRT) through the post hoc procedure of SPSS. Differences between mean values were considered significant at P ≤0.05, unless otherwise specified.

Results

The gross response of chickens fed with the experimental diets is shown in Table 2. Up to seven days of age, there was no effect of grain drying temperature and enzyme supplementation on FI. However, the FI was marginally higher (P <0.08) in diets with enzymes than in unsupplemented diets (127.0 *vs*. 122.9 g/bird). Live weight at this age was affected (P <0.01, R^2 = 0.25) by drying temperature but the trend was dependent on microbial enzyme supplementation. The microbial enzyme also improved (P <0.01, R^2 = 0.25) LW only on the diets containing sun-dried grains. The FCR was significantly improved (P <0.03, R^2 = 0.18) by increasing grain drying temperature up to 90 °C. In general, FCR up to 7 days of age was better (P <0.03) on enzyme-supplemented diets than on diets without the microbial enzyme (1.56 *vs*. 1.64 g feed/g gain).

Table 2 Feed intake (FI), live weight (LW) and feed conversion ratio (FCR) of broiler chickens at 7 and 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperatures with or without enzymes[1]

Treatment		FI (g/bird)		LW (g/bird)		FCR (g feed/g gain)	
Drying temp.	Enzyme	Day 7	Day 21	Day 7	Day 21	Day 7	Day 21
Sun drying	–	125.5	832.4[b]	115.9[cd]	557.2[cde]	1.67	1.61
	+	130.2	966.6[a]	130.4[a]	731.3[a]	1.45	1.40
80 °C	–	125.7	841.9[b]	119.0[bc]	546.7[de]	1.59	1.66
	+	120.9	844.3[b]	116.2[bcd]	619.7[bc]	1.60	1.46
90 °C	–	123.3	831.2[b]	118.6[bc]	579.8[bcd]	1.58	1.55
	+	130.9	863.1[b]	125.8[ab]	634.7[b]	1.53	1.45
100 °C	–	117.0	763.3[c]	107.7[d]	505.1[e]	1.73	1.66
	+	126.2	850.4[b]	116.5[bcd]	567.5[cde]	1.66	1.62
Pooled SEM		1.23	10.32	1.37	11.76	0.02	0.02
Model P		0.08	<0.01	<0.01	<0.01	<0.01	<0.01
Source of variation							
Drying temp.		NS	<0.01	<0.01	<0.01	<0.03	<0.02
Enzyme		0.09	<0.01	<0.01	<0.01	<0.03	<0.01
Drying temp. × Enzyme		NS	<0.02	NS	<0.03	NS	<0.07

[1] Each value represents the mean of six replicates; [a, b, c, d, e] Values with unlike superscripts within each column are significantly different (P <0.05); NS - Non-significant; SEM - Standard error of mean.

Feed intake up to day 21 declined (P <0.01, R^2 = 0.39) with an increase in drying temperature of grains, while supplementation with microbial enzymes increased (P <0.01, R^2 = 0.39) FI, but this was significant only on the diets based on sun-dried maize and the maize dried at 100 °C (Table 2). The interaction in FI between drying temperature and enzyme was also significant (P <0.02). In general, enzyme supplementation resulted in a higher FI compared to non-enzyme diets (881.1 *vs.* 817.2 g/bird). At 21 d of age there was a reduction (P <0.01, R^2 = 0.49) in LW with an increase in grain drying temperature while supplementation with microbial enzymes (P <0.01, R^2 = 0.49) improved LW only on the diets containing sun-dried grains and grains dried at 90 °C. Generally, LW was higher (P <0.01) in chickens on the enzyme-supplemented diets than on diets without enzymes (638.3 *vs.* 547.2 g/bird). Furthermore, the interaction between drying temperature and enzyme on LW was significant (P <0.03). The FCR at this age was reduced (P <0.02, R^2 = 0.37) with an increase in grain drying temperature while the FCR improved (P <0.01, R^2 = 0.37) on diets when supplemented with enzyme. Feed conversion ratio was also better (P <0.01) with diets containing enzymes than without enzymes (1.48 *vs.* 1.62 g feed/g gain).

At day 7, the relative weight of the small intestine was not significantly affected by increase in grain drying temperature but was decreased (P <0.04, R^2 = 0.11) in chickens on diets that were supplemented with microbial enzymes (Table 3). The relative weights of the proventriculus plus gizzard, pancreas, liver, spleen and bursa of fabricius were not significantly affected by an increase in grain drying temperature with or without enzyme supplementation.

Table 3 Relative weight of visceral organs (g/100 g of body weight) of broiler chickens at seven days of age on the different diets based on sun-dried maize or artificially dried at different temperatures with or without enzymes[1]

Treatment		Small intest.[2]	Proven.+ gizzard[2]	Pancreas	Liver	Spleen	Bursa of fabricius
Drying Temp.	Enzyme						
Sun drying	–	11.4	9.0	0.40	4.5	0.08	0.15
	+	11.1	9.0	0.46	5.0	0.10	0.14
80 °C	–	10.2	8.6	0.47	5.3	0.08	0.15
	+	9.9	8.6	0.43	5.1	0.09	0.16
90 °C	–	10.6	8.3	0.44	5.1	0.10	0.18
	+	9.9	8.8	0.47	5.3	0.09	0.15
100 °C	–	11.2	9.0	0.47	4.9	1.66	0.15
	+	10.0	8.7	0.48	5.3	0.09	0.14
Pooled SEM		0.17	0.13	0.01	0.12	0.20	0.01
Model P		<0.09	NS	NS	NS	NS	NS
Source of variation							
Drying temperature		NS	NS	NS	NS	NS	NS
Enzyme		<0.04	NS	NS	NS	NS	NS

[1] Each value represents the mean of six replicates; [2] Organs were weighed with content;
NS - Non-significant; SEM - Standard error of mean.

At day 21, there was a significant increase (P <0.04, R^2 = 0.10) in the relative weight of the small intestine with an increase in grain drying temperature while there was no change in the relative weight of this tissue due to supplementation with microbial enzymes (Table 4). The relative weight of the pancreas was not affected by grain drying temperature but was increased (P <0.01, R^2 = 0.24) in chickens on diets supplemented with enzymes. At this age the relative weight of the liver was significantly increased (P <0.01, R^2 = 0.14) with an increase in grain drying temperature but this effect was absent in chickens on diets containing microbial enzymes. There was no significant effect of grain treatments with or without enzyme on the relative weight of the spleen and bursa of fabricius at the same day of age.

Table 4 Relative weight of visceral organs (g/100 g of body weight) of broiler chickens at 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperatures with or without enzymes[1]

Treatment Drying temp.	Enzyme	Small intest[2]	Proven+ Gizzard[2]	Pancreas	Liver	Spleen	Bursa of fabricius
Sun drying	–	6.0	4.6	0.37	2.8	0.09	0.20
	+	5.8	4.6	0.30	2.8	0.08	0.18
80 °C	–	6.1	4.3	0.41	3.0	0.08	0.21
	+	6.1	4.5	0.32	3.0	0.08	0.19
90 °C	–	6.5	4.5	0.45	3.1	0.10	0.16
	+	6.0	4.4	0.32	3.1	0.09	0.22
100 °C	–	6.2	4.5	0.36	3.1	0.09	0.20
	+	6.4	4.8	0.36	3.0	0.08	0.20
Pooled SEM		0.07	0.07	0.01	0.04	0.00	0.01
Model P		NS	NS	<0.01	NS	NS	NS
Source of variation							
Drying temperature		<0.04	NS	NS	<0.01	NS	NS
Enzyme		NS	NS	<0.01	NS	0.09	NS

[a, b] Values with unlike superscripts within each column are significantly different (P <0.05);
[1] Each value represents the mean of six replicates; [2] Organs were weighed with content;
NS - Non-significant; SEM - Standard error of mean.

Table 5 Tissue protein content and activities of digestive enzymes in the pancreas and jejunum of broiler chickens at seven days of age on the different diets based on sun-dried maize or artificially dried at different temperatures with or without enzymes[1]

Treatment Drying temp.	Enzyme	Pancreas Protein[2]	CA	Jejunum Protein[2]	AP	Maltase (ηmol/mg protein)	Sucrase
Sun drying	–	259.8	1.4[b]	283.6	3.1	174.1[bc]	13.0[b]
	+	312.0	2.6[a]	310.8	2.8	151.1[c]	11.2[b]
80 °C	–	260.8	2.0[ab]	349.2	2.7	216.0[abc]	17.7[ab]
	+	251.6	2.3[ab]	329.2	3.0	273.9[a]	22.4[a]
90 °C	–	299.3	1.8[ab]	307.0	3.0	216.7[abc]	16.5[ab]
	+	266.7	1.9[ab]	311.6	3.5	236.3[ab]	17.7[ab]
100 °C	–	263.9	1.9[ab]	310.0	3.0	211.6[abc]	16.7[ab]
	+	242.1	2.2[ab]	277.9	4.9	277.2[a]	22.5[a]
Pooled SEM		8.85	0.001	8.79	0.18	10.02	0.95
Source of variation							
Drying temp.		NS	NS	NS	<0.03	<0.01	<0.02
Enzyme		NS	NS	NS	<0.08	NS	NS
Drying temp. × Enzyme		NS	<0.02	NS	NS	NS	NS

[a, b, c] Values with unlike superscripts within each column are significantly different (P <0.05);
[1] Each value represents the mean of six replicates;
[2] (mg/g tissue); CA - Chymotrypsin amidase (ηmol/mg protein), AP - Alkaline phosphatase (μmol/mg protein);
NS - Non-significant; SEM - Standard error of mean.

At 7 days of age pancreatic tissue protein content and chymotrypsin amidase activity were not affected by grain drying temperature or enzyme supplementation (Table 5). However, there was a significant interaction between the effects (P <0.02) of drying temperature and enzyme on the activity of chymotrypsin amidase.

In the jejunum there was no significant variation on tissue protein content at day 7 due to an increase in grain drying temperature, enzyme supplementation or any interactions between these factors (Table 5). The activity of alkaline phosphatase was significantly increased (P <0.03, R^2 = 0.16) with an increase in grain drying temperature while supplementation with microbial enzymes also marginally increased (P <0.08, R^2 = 0.16) the activity of this enzyme. The activity of maltase increased significantly (P <0.01, R^2 = 0.18) with an increase in grain drying temperature but this effect was absent on diets supplemented with microbial enzymes. The activity of sucrase also increased (P <0.02, R^2 = 0.15) with an increase in grain drying temperature but this activity was not affected by microbial enzyme supplementation.

At day 21 the pancreatic protein content, chymotrypsin amidase activity, jejunal protein content and alkaline phosphatase activity were not significantly affected by increases in grain drying temperature, enzyme supplementation or interactions between the main factors (Table 6). The activity of maltase rose (P <0.01, R^2 = 0.27) in line with an increase in grain drying temperature but was not affected by the microbial enzyme supplements. There was an increase (P <0.01, R^2 = 0.18) in the activity of sucrase with an increase in drying temperature of grains but this activity was not affected in birds on diets supplemented with enzymes.

Table 6 Tissue protein content and activities of digestive enzymes in the pancreas and jejunum of broiler chickens at 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperatures with or without enzymes[1]

Treatment		Pancreas		Jejunum			
Drying temp.	Enzyme	Protein[2]	CA	Protein[2]	AP	Maltase (ηmol/mg protein)	Sucrase
Sun drying	–	249.9	1.6	266.1	6.5	219.1^c	15.2
	+	252.0	1.5	273.1	6.6	230.3^{bc}	18.4
80 °C	–	273.6	1.5	277.6	6.3	248.6^{abc}	17.2
	+	283.0	1.5	290.1	5.4	238.1^{abc}	18.6
90 °C	–	257.1	1.6	268.8	4.3	275.9^{ab}	19.9
	+	269.3	1.4	267.3	4.7	247.5^{abc}	20.6
100 °C	–	251.6	1.9	250.6	6.2	283.1^a	20.2
	+	280.0	1.5	279.7	5.6	279.6^{ab}	22.4
Pooled SEM		6.55	1.00	4.31	0.24	6.04	0.68
Source of variation							
Drying temp.		NS	NS	NS	NS	<0.01	<0.01
Enzyme		NS	NS	NS	NS	NS	NS

$^{a, b, c}$ Values with unlike superscripts within each column are significantly different (P <0.05);
[1] Each value represents the mean of six replicates;
[2] mg/g tissue; CA - Chymotripsin amidase (ηmol/mg protein); AP - Alkaline phosphatase (μmol/mg protein);
NS - Non-significant; SEM - Standard error of mean.

At 21 days of age the ileal digestibility of protein, gross energy and starch was not significantly changed by an increase in grain drying temperature or enzyme supplementation of diets (Table 7). However, the digestibility of protein was affected by the interaction (P <0.04) between drying temperature and enzyme, in which the digestibility of protein was higher on the diet containing grain dried at 100 °C with enzymes, than on diet containing grain dried at 90 °C without enzymes. In general, energy digestibility tended to increase in the diets containing the microbial enzyme supplements.

The concentrations of organic acids in the ileum of broiler chickens at day 21 are shown in Table 8. Formic acid content increased (P <0.01, R^2 = 0.25) with an increase in drying temperature of grains used in

Table 7 Ileal digestibility of protein, gross energy and starch of broiler chickens at 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperatures with or without enzymes[1]

Treatment		Protein	Gross energy	Starch
Drying temp.	Enzyme			
Sun drying	−	0.83[abc]	0.76	0.98
	+	0.85[ab]	0.78	0.98
80 °C	−	0.85[ab]	0.77	0.98
	+	0.81[bc]	0.73	0.96
90 °C	−	0.80[c]	0.75	0.96
	+	0.83[abc]	0.77	0.97
100 °C	−	0.83[abc]	0.75	0.97
	+	0.86[a]	0.78	0.98
Pooled SEM		0.01	0.01	0.01
Model P		0.08	NS	<0.05
Source of variation				
Drying temp.		NS	NS	NS
Enzyme		NS	NS	NS
Drying temp. × Enzyme		<0.04	NS	NS

[1] Each value represents the mean of six replicates; [a, b, c] Values with unlike superscripts within each column are significantly different (P <0.05); NS - Non-significant; SEM - Standard error of mean.

Table 8 Concentrations of organic acids (mg/g digesta) in the ileal content of broiler chickens at 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperatures with or without enzymes[1]

Treatment		Ileum		
Drying temp.	Enzyme	Formic acid	Acetic acid	Lactic acid
Sun drying	−	0.15	0.41	0.77
	+	0.23	0.43	0.79
80 °C	−	0.25	0.42	0.88
	+	0.23	0.44	0.83
90 °C	−	0.33	0.51	0.93
	+	0.30	0.51	0.92
100 °C	−	0.35	0.57	0.86
	+	0.32	0.60	0.59
Pooled SEM		0.02	0.02	0.05
Model P		<0.03	<0.01	NS
Source of variation				
Drying temp.		<0.01	<0.01	NS
Enzyme		NS	NS	NS

[1] Each value represents the mean of six replicates; Data were transformed ($\log_{10}$ + 1) prior to analysis; NS - Non-significant; SEM - Standard error of mean.

the diets, but the concentration of formic acid was not changed when diets were supplemented with microbial enzymes. There was a significant increase (P <0.01, R^2 = 0.34) in the concentration of acetic acid with increase in grain drying temperature but this was not affected by inclusion of microbial enzymes in the diets.

In the caecal content the concentrations of formic and acetic acids were not changed in response to an increase in drying temperature of grains used in the diets with or without enzyme supplementation (Table 9). The concentration of propionic acid increased (P <0.03, R^2 = 0.12) with increase in grain drying temperature but enzyme supplementation had no effect. The concentrations of isobutyric acid, butyric acid, and isovaleric acids were not affected by increase in drying temperature of grains, enzyme supplementation or interaction between the main factors. The concentration of valeric acid was increased (P <0.02, R^2 = 0.12) by an increase in grain drying temperature but the concentration of this acid was not changed on diets supplemented with microbial enzymes. There was a significant reduction in the concentration of lactic acid (P <0.02, R^2 = 0.14) and succinic acid (P <0.01, R^2 = 0.15) on diets with the enzyme supplements but the concentrations of these acids were not affected by grain drying temperature.

In the ileal content there was no significant effect of grain drying temperature or enzyme supplementation on total anaerobic bacterial count (Table 10). However, the interaction between drying temperature and enzyme supplementation on this bacterial count was significant (P <0.02). The populations of lactic acid bacteria (P <0.05, R^2 = 0.10) and lactobacilli bacteria (P <0.01, R^2 = 0.28) were decreased on diets supplemented with enzymes, but were not affected by increasing grain drying temperature. There were no significant changes in the populations of Enterobacteria and *C. perfringens* in the ileal content at 21 days of age as a result of variation in grain drying temperature, enzymes or their interactions.

In the caecal content the total anaerobic bacterial count was not significantly changed by increases in drying temperature of grains but the population of these bacteria was increased (P <0.01, R^2 = 0.17) on diets when supplemented with microbial enzymes (Table 10). The population of lactic acid bacteria was increased (P <0.01, R^2 = 0.18) as a result of an increase in grain drying temperature but this effect was absent on diets containing microbial enzymes. There were no significant changes in the populations of lactobacilli, Enterobacteria and *C. perfringens* in the caecal content at 21 days of age as a result of variation in grain drying temperature, enzyme supplementation or their interaction.

Table 9 Concentration of organic acids (mg/g digesta) in caecal content of broiler chickens at 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperatures with or without enzymes[1]

Treatment		Caecal content								
Drying temp.	Enzyme	Formic	Acetic	Propio nic	Iso-butyric	Butyric	Iso-valeric	Valeric	Lactic	Suc-cinic
Sun drying	–	0.16	1.7	0.73	0.28	0.81	0.12	0.26	0.48	1.04
	+	0.12	1.8	0.80	0.29	0.83	0.10	0.25	0.16	0.67
80 °C	–	0.13	1.8	0.78	0.30	0.78	0.13	0.27	0.09	0.72
	+	0.12	1.8	0.79	0.29	0.87	0.11	0.28	0.15	0.80
90 °C	–	0.13	1.8	0.85	0.31	0.87	0.12	0.31	0.38	0.87
	+	0.14	1.8	0.86	0.31	0.81	0.12	0.28	0.14	0.64
100 °C	–	0.16	1.8	0.82	0.26	0.83	0.09	0.29	0.19	1.02
	+	0.15	1.8	0.91	0.31	0.82	0.11	0.30	0.11	0.70
Pooled SEM		0.01	0.0	0.02	0.01	0.01	0.01	0.01	0.03	0.04
Model P		NS	NS	NS	NS	NS	NS	NS	<0.01	<0.05
Source of variation										
Drying temp		NS	NS	<0.03	NS	NS	NS	<0.02	NS	NS
Enzyme		NS	NS	NS	NS	NS	NS	NS	<0.02	<0.01

[1] Each value represents the mean of six replicates; Data were transformed ($\log_{10}$ +1) prior to analysis;
NS - Non-significant; SEM - Standard error of mean.

Table 10 Bacterial counts (log_{10} *cfu x*/g digesta*)* in ileum and caeca of broiler chickens at 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperatures with or without enzyme[1]

Treatment		Ileal content					Caecal content				
Drying temp.	Enzyme	Anae-robic	Lact. acid	Lacto-bacilli	Entero bact.	$C.p^2$	Anae-robic	Lact. acid	Lacto-bacilli	Entero bact.	$C.p^2$
Sun drying	−	7.4[a]	7.6	7.4	4.3	5.5	7.9	8.3	8.1	7.6	6.4[c]
	+	7.0[abc]	8.0	8.0	4.7	6.0	8.1	8.4	8.2	8.1	7.1[a]
80 °C	−	6.7[bc]	7.9	7.6	4.8	5.8	7.9	8.3	8.0	7.7	6.9[ab]
	+	6.5[c]	7.5	7.2	5.0	5.5	8.2	8.6	8.2	7.9	6.6[bc]
90 °C	−	7.3[a]	8.0	7.8	4.6	6.1	8.2	8.6	8.3	8.0	6.9[abc]
	+	6.9[abc]	7.9	7.4	5.2	6.4	7.7	8.6	8.2	7.7	7.0[ab]
100 °C	−	6.5[c]	7.9	7.5	4.7	5.9	7.8	8.7	8.2	7.7	7.1[a]
	+	7.1[bc]	7.7	7.2	4.6	5.9	8.1	8.7	8.0	8.0	6.8[abc]
Pooled SEM		0.07	0.07	0.07	0.08	0.09	0.06	0.05	0.04	0.06	0.06
Model P		NS	NS	NS	NS	NS	NS	<0.01	NS	NS	NS
Source of variation											
Drying temp.		NS	NS	NS	NS	NS	NS	<0.01	NS	NS	NS
Enzyme (Enz)		NS	0.05	<0.01	NS	NS	<0.01	NS	NS	0.09	NS
Drying temp. × Enz		<0.02	NS	NS	NS	NS	NS	NS	NS	NS	<0.01

[1] Each value represents the mean of six replicates; Data were transformed (log_{10} +1) prior to analysis;
[2] *C.p* - *C. perfringens*; [a, b, c] Values with unlike superscripts within each column are significantly different (P <0.05); NS - Non-significant; SEM - Standard error of mean.

Discussion

The present study demonstrated that the maize dried either naturally in the sun or forced-dried at 90 °C adequately supported chicken performance including FI, LW and FCR, up to 21 days of age. Enzyme supplementation also improved digestibility and bird performance on diets based on sun-dried and 90 °C–dried grains. Therefore, sun drying, as practised in many areas is a suitable method for processing of maize which will be used as broiler feed. However, where sun drying is not possible due to climatic or other factors, artificial drying at 90 °C is recommended, although enzyme supplementation may be needed to get the best results. This improvement is, perhaps, due to better chemical composition (Bhuiyan *et al.*, 2010) and ultra-structural properties found in grain dried at 90 °C and in sun-dried grain compared to grain dried at other high temperatures. The adverse effect of high temperature drying of maize have been reported previously (Kaczmarek *et al*., 2009) where authors also reported that exogenous enzymes were effective in partially ameliorating this inconsistency in energetic value if the grain is dried at more than 100 °C. In the current study, the worst performance results (LW and FCR) were found in the batch dried at 100 °C. This may be due to the decrease of its nutritional quality through high temperature drying, as reported previously (Brown, 1996; Iji *et al.*, 2003).

The improvement in body weight through microbial enzyme supplementation of the diet based on 90 °C-dried maize is consistent with our previous findings that maize dried at this temperature has a higher amylose concentration. In addition, Zanella *et al.* (1999) showed that enzyme supplementation improved BW and FCR ratio by 1.9 and 2.2%, respectively in broiler chickens.

In the present study, the relative weight of the small intestine varied according to the drying temperature of the grain in the diet and whether the diet was supplemented with enzymes or not. The relative weight of the small intestine of chickens fed diets containing sun-dried grain and without enzyme supplementation was found to be higher than that of birds on diets containing grain artificially dried at other temperatures with enzyme supplementation. These increases in the mass of the small intestine are approximately proportional to the increase in body weight, and might, therefore, be a general consequence of faster growth.

In this study, significant differences were observed in pancreatic protease (chymotrypsin amidase) activity due to interactions between drying temperature and enzymes, and higher pancreatic protease activity was found in birds on diets containing sun-dried grains with enzyme supplementation than in groups on unsupplemented diets. A dissimilar finding was reported by Mahagna *et al.* (1995), who observed a reduction in the activity of pancreatic enzymes *in situ* and in intestinal digesta as a result of supplementation with a microbial enzyme with proteolytic and amylolytic activities. The reasons for the discrepancy in these findings are unclear at this stage.

The activities of jejunal maltase and sucrase were significantly affected by drying temperature of grains but not by supplemental enzyme, and higher maltase and sucrase activities were found in birds on diets with grain dried at 80, 90 and 100 °C compared to sun-dried grain. The increase in intestinal enzymes is stimulated mechanically by chime passing through the digestive tract (Duke, 1986). This may be due to higher DM content of diets at 80, 90 and 100 °C than the sun-dried grain diet. The high activity of maltase and sucrase in the birds receiving these diets is probably related to substrate (maltose and sucrose) availability in the jejunum. The relationship between high-moisture grain dried at different temperatures and the activity of digestive enzymes has apparently not been examined previously. A reduction in jejunal tissue protein content was observed in this study at day 7, due to microbial enzyme application. Similar results have been reported by other workers (Danicke *et al.*, 2000a; Iji *et al.*, 2004), though grain with a lower moisture content was used.

There was a significant effect of drying temperature on starch digestibility, with higher starch digestibility found in sun-dried and 100 °C-dried grain than in grain dried at other temperatures. However, the enzyme supplementation also enhanced starch digestibility in each case. The starch component of maize is considered to be highly digestible, but Brown (1996) reported that such starch may be resistant to digestion, and such resistance increases when grains are dried artificially. Starch digestion was completed in the hindgut, suggesting that some of the starch was indeed resistant. The enzyme mixture may have improved digestion of this fraction. The above phenomenon could warrant the use of exogenous enzymes (xylanase, glucanase, pectinase, cellulase, mannanase, galactanase) to increase the digestibility of nutrients found in maize (Meng & Slominski, 2005).

In this study it was found that protein and starch digestibility were enhanced in diets containing grains dried at 90 and 100 °C. This may be due to the role that the addition of microbial phytase in poultry diets plays in increasing calcium and phosphorus availability, a finding that is well established in the literature. Phytic acid is a critical factor present in feedstuffs and it binds minerals, proteins, lipids and starch (Thompson & Yoon, 1984), reducing the digestibility of these nutrients for poultry (Sebastiana *et al.*, 1997). Recent studies have also shown a beneficial effect of phytase addition to broiler diet on metabolizable energy and total amino acid digestibility (Namkung & Leeson, 1999; Ravindran *et al.*, 1999). This also may be due to the activity of exogenous enzyme which improves the nutritive value of maize by hydrolyzing polysaccharides that are involved in the encapsulation of starch or protein, thereby rendering compounds available for digestion that were previously not accessible to endogenous enzyme (Bedford, 1996).

The changes in microbial populations may be due to a decrease in the quality of the grain. Although the ileal viscosity of birds was not measured in the present study, it is possible that there were major negative effects due to oven drying. However, *in vitro* viscosity has been measured using oven-dried grain in another study and no significant variation was found (Bhuiyan *et al.*, 2010). There was a significant effect on the number of lactobacilli with a higher lactobacilli population found in the diet without enzyme supplementation but only to a lesser extent. Carbohydrates that remain undigested in diets without microbial enzymes are fermented in the hind gut, leading to an increase in microbial populations. The populations of other species such as lactic acid bacteria, enterobacteria and *C. perfringens* were not affected by grain quality or enzyme supplementation, although the count of *C. perfringens*, the causative agent of necrotic enteritis, was slightly increased.

In caecal content there was a significant variation on total anaerobic count with higher total anaerobic bacteria found in chickens on enzyme-supplemented diets than those on the unsupplemented diets. This is an unusual occurrence as microbial enzymes tend to increase ileal digestion and reduce caecal fermentation (Apajalahti & Bedford, 1999; Fernandez *et al.*, 2000; Apajalahti *et al.*, 2004).

In the current study there was a significant effect of drying temperature on the concentration of formic acid and acetic acid in the ileum of broiler chickens, and the higher concentration of formic acid was

associated with grain dried at 100 °C rather than sun-dried grain. This result suggests that the grains dried at 80 and 100 °C were fermented more by the ileal microflora. Therefore, the growth and activity of formic acid- and acetic acid-producing bacteria in the ileum are likely promoted by the grain dried at 80 and 100 °C. The composition of microbiota or increase in their digestive activity can have positive as well as negative effects on the host (Gabriel *et al.*, 2006). The positive effects are competitive exclusion, SCFAs production and release of nutrients that can be absorbed in the intestine. Similar significant changes in the concentration of lactic acid were found due to enzyme supplementation, with a higher lactic acid content found in birds on diets without enzyme supplementation than on supplemented diets. Lactate was the most predominant organic acid in the ileal content, while acetic acid was predominant in the caecal digesta. Lactic acid is an electron sink, further oxidized to other SCFAs such as acetic, propionic and butyric acids in the caeca due to longer retention time of the caecal digesta (Vidanarachchi, 2006). An increased SCFA production through bacterial fermentation of undigested carbohydrates such as resistant starch also results in lower pH, which is an accepted mechanism for the inhibition of acidophilic bacteria such as enterobacteria and *C. perfringens* in broiler chickens (Terada *et al.*, 1994; Orban *et al.*, 1997). In the current study, however, no reductions in the populations of enterobacteria or *C. perfringens* were found due to grain drying or enzyme supplementation.

Conclusions

It may be concluded from the present study that diets based on sun-dried maize or maize dried at 90 °C gave comparatively better performance in terms of feed intake, live weight and FCR of broilers than diets based on maize dried at other temperatures. Moreover, it may be inferred that there was a significant positive response to supplementation with microbial enzymes. The relative weight of the small intestine and liver, the bacterial populations and starch digestibility were also higher in birds on diets based on sun-dried maize compared to those based on artificially dried grain. Considering all of the above points in this experiment, it may be assumed that, for broiler chickens, there is little or no difference in the nutritive value of sun-dried grain compared to grain artificially dried at 90 °C. Supplementation with a suitable enzyme may also be of some benefit in terms of visceral organ development, performance, protein digestibility and gut microflora.

Acknowledgement

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