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# Phytase supplementation of maize-, sorghum- and wheat-based broiler diets with identified starch pasting properties influences phytate (IP<sub>6</sub>) and sodium jejunal and ileal digestibility



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#### ABSTRACT

The effects of phytase supplementation on growth performance, nutrient utilisation, starch and protein digestive dynamics in broiler chickens offered maize-, sorghum- and wheat-based diets were determined in a previous study (Liu et al., 2014). Responses to phytase were most pronounced in maize-based diets, which suggest that more phytate was degraded in these diets. Relevant retained samples of grain, diets and digesta from four small intestinal segments were retrospectively analysed for concentrations of phytate, sodium and starch pasting properties to investigate the hypothesis that phytate in maize-based diets was more completely degraded by exogenous phytase. Exogenous phytase significantly (P<0.001) degraded dietary phytate in the proximal jejunum, distal jejunum, proximal ileum and distal ileum and increased distal ileal phytate digestibility coefficients from 0.238 to 0.631. There were significant differences (P<0.001) between diets based on maize (0.515), wheat (0.449) and sorghum (0.340) for distal ileal phytate digestibility coefficients. Phytase accelerated phytate disappearance rates from all four segments and increased distal ileal phytate disappearance rates from 201 to 535 mg/bird/day. This was significantly more pronounced in maize (459 mg/bird/day) than in diets based on sorghum (301 mg/bird/day) and wheat (343 mg/bird/day). Sodium digestibility coefficients were significantly improved (P<0.01) by exogenous phytase in proximal jejunum, distal jejunum and proximal ileum. Exogenous phytase significantly influenced starch properties of experimental diets determined by rapid visco-analysis (RVA). There were significant negative correlations between RVA setback viscosity of starch in experimental diets and starch digestibility coefficients at the distal jejunum (r = -0.438; P < 0.01) and proximal ileum (r = -0.591; P < 0.001) determined

Abbreviations: AIA, acid insoluble ash; AID, apparent ileal digestibility; AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy; cP, centipoise; DJ, distal jejunum; DI, distal ileum; FCR, feed conversion ratio; IP<sub>6</sub>, *myo*-inositol hexaphosphate; Na<sup>+</sup>,K<sup>+</sup>-ATPase, sodium-potassium adenosine triphosphate or 'sodium pump'; pI, isoelectric point; PJ, proximal jejunum; PI, proximal ileum; RVA, rapid visco-analysis; SGLT-1, sodium-glucose linked transporter-1.

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in the Liu et al. (2014) study. Distal ileal phytate digestibility coefficients appeared to be higher in non-supplemented, maize-based diets (0.349) than in diets based sorghum (0.128) and wheat (0.239) thus the likelihood is that phytate in maize-based diets was more readily degraded by endogenous, mucosal phytase in the small intestine. Consideration is given to the possibilities that location of phytate within grains influences phytate degradation and that the relatively low sodium concentrations in maize-based diets may have contributed to the more robust responses to exogenous phytase observed.

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#### 1. Introduction

The effects of phytase supplementation of maize-, sorghum- and wheat-based broiler diets were previously investigated (Liu et al., 2014). A notable outcome was that phytase generated more robust responses in maize-based diets in comparison to those based on sorghum and wheat, especially for parameters of nutrient utilisation. Significant interactions were observed for apparent metabolisable energy (AME), nitrogen (N) retention and N-corrected AME (AMEn). For example, 1000 FTU/kg phytase significantly increased AMEn by 0.44 MJ/kg in maize-based diets but numerically decreased AMEn by 0.16 and 0.05 MJ/kg, in sorghum- and wheat-based diets, respectively. Leske and Coon (1999) had reported that phytate in maize was degraded to a greater extent than in wheat by an exogenous phytase in poultry. Therefore, one possible reason for the robust phytase responses observed in maize-based diets is that phytate was more readily degraded by exogenous phytase. Consequently, diets and digesta samples were retrospectively analysed for phytate (myo-inositol hexaphosphate; IP<sub>6</sub>) to determine phytate degradation in four small intestinal sites. Sodium (Na) concentrations in the retained samples were also analysed because phytate and phytase have been shown to have tangible effects on Na digestibility on a total tract basis (Cowieson et al., 2004) and in the terminal ileum (Ravindran et al., 2006, 2008; Selle et al., 2009b) in broiler chickens. The four reports cited indicate that phytate triggers an egress of Na into the small intestinal lumen, which is counteracted by phytase. Consequently, it has been suggested that this transition of Na may influence absorption of nutrients, including glucose and amino acids, because increasing Na concentrations in the gut lumen may compromise 'sodium pump' (Na+,K+-ATPase) activity and Na<sup>+</sup>-dependent transport systems (Selle et al., 2012).

The impact of phytate and phytase on starch digestibility is an elusive topic. In the Liu et al. (2014) study, significant phytase effects on starch digestibility were confined to an increase of 2.56% (0.920 *versus* 0.897) for all three grains in the proximal ileum and an increase of 19.9% (0.804 *versus* 0.704) for wheat-based diets in the proximal jejunum. Alternatively, starch in maize-based diets was significantly more digestible in the three posterior small intestinal segments than in diets based on sorghum and wheat. For possible clarification, starch pasting properties of unprocessed grains and experimental diets were identified by rapid visco-analysis (RVA). The main objective of these retrospective analyses was to investigate the genesis of the more robust phytase responses observed in maize compared to sorghum- and wheat-based diets with a focus on phytate degradation and the digestibilities of Na and starch.

#### 2. Materials and methods

The methodologies adopted for the underlying study have been outlined (Liu et al., 2014). This study investigated the effects of phytase supplementation on growth performance, nutrient utilisation and digestive dynamics of starch and protein. Ross 308 chicks were offered nutritionally equivalent, P-adequate, steam-pelleted diets based on 560 g/kg maize, sorghum or wheat, without or with 1000 FTU/kg phytase. The feed enzyme used was a *Buttiauxella* phytase produced in *Trichoderma reesei* (Axtra® PHY; Danisco Animal Nutrition, Marlborough, UK). The six dietary treatments were offered to 8 replicates (6 birds per cage) from 7 to 27 days post-hatch. More specifically, starch pasting properties of maize, sorghum and wheat and the diets based on these grains were determined with a Rapid-Visco-Analyzer (RVA-4. Newport Scientific Pty Ltd. Warriewood NSW, Australia) following procedures outlined by Hernandez et al. (2008). Within 13 min intervals, 28 g mixtures of diets and water (15:85 w/w on a dry basis) were prepared and held at a temperature of 50 °C for 1 min and then heated from 50 °C to 95 °C. After holding the hot paste at 95 °C for 2.5 min, the slurry was again cooled to 50 °C, and then held at that temperature for 2 min.

The phytate analysis methodology followed in the present study was similar to that reported by Carlsson et al. (2001) with minor revisions (Yu et al., 2012). For elemental analysis, sodium ions were analysed on an ICP Emission Spectrometer (iCAP 6000 Series) according to manufacturer's instructions (Thermo Electron Corporation. Waltham, M.A.). Feed and digesta samples (1 g) were ground and extracted with 20 ml HCl (0.5 N). The filtered 40  $\mu$ l extract was directly injected on HPIC for IP<sub>6</sub> analysis while for Na analysis the extract was diluted 100 to 200 fold.5 N). Acid insoluble ash (AIA) was used as the inert dietary marker and AIA concentrations were determined by the method of Siriwan et al. (1993). The apparent digestibility coefficients of IP<sub>6</sub> phytate and Na at four small intestinal sites were calculated from the following equation:

$$apparent \ digestibility \ coefficient = \frac{(IP_6/Na:AIA)_{diet} - (IP_6/Na:AIA)_{digesta}}{(IP_6/Na:AIA)_{diet}}$$

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