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Concentrations of specific phenolic compounds in six red sorghums influence nutrient utilisation in broiler chickens

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ABSTRACT

Concentrations of polyphenolic compounds and free, conjugated and bound phenolic acids were determined in six red grain sorghum varieties harvested on the Liverpool Pains in 2009. Categories of polyphenolic compounds quantified included total phenolics, anthocyanins, flavan-4ols, luteolinidin, apigeninidin, 5-methoxy-luteolinidin, 7-methoxy-apigeninidin, apigenin, luteolin, eriodictyol and naringenin. Phenolic acids that were quantified included *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, ferulic, syringic and sinapic acids. These sorghums were also extensively characterised for additional properties including rapid visco-analysis (RVA) of starch pasting profiles, protein solubility and quantification of kafirin and phytate. A pigmented testa was not detected in the six varieties by the quantal Clorox bleach test, which indicated that they were Type I sorghums that do not possess condensed tannin. This was confirmed by vanillin assays. The six varieties were incorporated into unprocessed sorghum–casein mash diets and offered to broiler chickens to compare nutrient utilisation parameters and protein (N) digestibility coefficients. One objective of this study was to assess if non-tannin phenolic compounds influence energy utilisation in broiler chickens. Negative correlations between certain conjugated phenolic acids in sorghum and parameters of energy utilisation (AME, ME:GE ratios, AMEn) in birds were observed. For example, *p*-coumaric ($r = -0.826$), ferulic ($r = -0.831$) and total conjugated phenolic acids ($r = -0.832$) were negatively correlated with ME:GE ratios to significant ($P < 0.05$) extents. Interestingly, protein solubility was positively correlated to AME ($r = 0.874$), ME:GE ratio ($r = 0.862$) and AMEn ($r = 0.837$) to significant ($P < 0.05$) extents. This study suggests that specific phenolic compounds, namely conjugated phenolic acids of which ferulic and *p*-coumaric acids were the most abundant, negatively influence energy utilisation in sorghum-based broiler diets. Therefore, our tentative conclusion is that specific non-tannin phenolics are deleterious rather than innocuous components of grain sorghum in the context of chicken-meat production.

Abbreviations: Abs, absorbance; AME, apparent metabolisable energy; AMEn, N-corrected apparent metabolisable energy; GAE, gallic acid equivalents; GE, gross energy; ME, metabolisable energy; PSI, particle size index.

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1. Introduction

The performance of broiler chickens offered sorghum-based diets is considered to be sub-optimal under Australian conditions (Bryden et al., 2009). The effective comparison is with their counterparts offered wheat-based diets containing an exogenous xylanase. However, our impression is that while bird performance may be inconsistent, sorghum is a better feedstuff for chicken-meat production than is generally believed. The reluctance of monogastric nutritionists to include sorghum in their diets partially stems from the stigma associated with 'bird-proof' sorghums that contain condensed tannin. Condensed tannin possesses potent anti-nutritive properties and has even been described as a toxic factor in poultry (McClymont and Duncan, 1952). In an informal survey of practical Australian nutritionists, 19% of respondents believed that contemporary sorghum crops contain sufficient condensed tannin to compromise animal performance. It is highly unlikely that this position is accurate (Walker, 1999; Hughes and Choct, 1999) and Henzell (1992) argued that Australian breeding programmes strongly select against condensed tannin in sorghum. Nevertheless, the stigma of 'bird-proof' sorghum appears to persist.

The Clorox bleach test for a pigmented testa is a straightforward, quantal test for the presence of condensed tannin in sorghum; sorghums lacking a pigment testa are considered to be 'tannin-free' (Waniska et al., 1992). As this research group has tested over forty sorghum samples, including those in the present study, without detecting one pigmented testa, the conclusion has been drawn that contemporary Australian sorghum crops do not contain condensed tannin. The anti-nutritive properties of condensed tannin are irrefutable (Gualtieri and Rapaccini, 1990; Nyachoti et al., 1997); however, there is the perception that anti-nutritive properties of phenolic compounds are entirely the province of condensed tannin and that the balance of polyphenolic compounds and phenolic acids present in sorghum is innocuous. Nevertheless, red non-tannin sorghums are highly pigmented with anthocyanins and anthocyanidins and Taylor (2005) did allow that that these phenolic compounds may be slightly anti-nutritional.

Anecdotally, white sorghums are considered to be better feedstuffs than red sorghums although the vast majority of Australian sorghums have red pericarps. It is axiomatic that red sorghums contain more phenolic compounds than white sorghum because they contain polyphenolic pigments. For example, Brohan et al. (2011) recorded an average of 12.9 mg GAE/g total polyphenols in seven red sorghums as opposed to 2.0 mg GAE/g in two white sorghums. The red-white colour differential is illustrated by Cielab L^*, a^* and b^* values. Three 'chalky red' sorghums had L^*, a^* and b^* values of 40.4, 17.4 and 12.9, respectively. In contrast, one 'pearly white' sorghum had corresponding values of 62.2, 3.8 and 19.2, as reported by Dykes et al. (2005). The higher L^* value in the white sorghum is indicative of its lighter coloured pericarp and the higher a^* value in the red sorghum is indicative of its 'redness'. While simplistic, there is the possibility that the intrinsically lower phenolic compound concentrations in white sorghums may be associated with their perceived superiority over red sorghums amongst non-tannin varieties.

This paper is an investigation into concentrations of polyphenolic compounds and phenolic acids in six characterised red sorghum varieties that were harvested on the Liverpool Plains of New South Wales in 2009. These six sorghums were offered to broiler chickens as unprocessed sorghum–casein mash diets to determine their influence on nutrient utilisation. Energy utilisation in sorghum-based broiler diets is considered to be sub-standard (Black et al., 2005) and for this reason metabolisable energy to gross energy (ME:GE) ratios were calculated as an indicator of the efficiency of energy utilisation. The prime objective of the present study is to report the concentrations of phenolic compounds in six 'tannin-free' red sorghum varieties and to consider if these findings have a bearing on broiler performance.

2. Materials and methods

The six Liverpool Plains sorghums were analysed extensively by standard procedures and the more relevant characteristics are tabulated (Table 1). These include crude protein (N), Promatest protein solubility, starch, amylopectin, amylose, total phosphorus, grain texture (Symes PSI, percentage vitreousness) and amino acid profiles determined by NIR. Because of their likely relevance to efficiency of energy utilisation, concentrations of kafirin and IP_6 phytate were determined. Kafirin was quantified by methodology adapted from Wallace et al. (1990) and IP_6 phytate was quantified via HPLC. Promatest protein solubilities of the sorghums were determined according to procedures described by Odjo et al. (2012)

The presence or absence of a pigmented testa was determined by the Clorox bleach test (Waniska et al., 1992). Sorghum grain (15 g) was mixed with 7.5 g KOH and 70 ml NaOCl solution (bleach) with constant stirring at 60 °C for 7 min, rinsed and washed with cold water. The grains that turned white or yellow were classified as Type I or tannin-free sorghums, while a black colour indicated the presence of condensed tannin (Type II or III). For confirmation of this quantal test, condensed tannin was determined by the modified vanillin/HCl assay described by Price et al. (1978). A milled sample (0.2 g) was mixed with 10 ml of 1% HCl in MeOH (v/v) for 20 min while shaking at low speed (KS501 D shaker, IKA Labortechnik, Germany). The mixture was then centrifuged at 3000 × g for 10 min. Both the supernatant and reagents were at 30 °C at the start of the assays. An aliquot (1 ml) of the extract was transferred to a capped test tube and within 1 min 5 ml of vanillin reagent was added and the mixture kept in a water bath at 30 °C for 20 min. The absorbance was read at 500 nm. A blank was prepared by replacing the vanillin reagent with 4% HCl in MeOH. Catechin was applied as a standard for the condensed tannin assay.

In addition to total phenols, the concentrations of the following phenolic compounds were determined: anthocyanin, flavan-4ols, luteolinidin, apigeninidin, 5-methoxy-luteolinidin, 7-methoxy-apigeninidin, apigenin, luteolin, eriodictyol and