



## Original research article

# Performance of broiler chickens offered nutritionally-equivalent diets based on two red grain sorghums with quantified kafirin concentrations as intact pellets or re-ground mash following steam-pelleting at 65 or 97°C conditioning temperatures



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## ABSTRACT

The Liverpool Plains is a fertile agricultural region in New South Wales, Australia. Two sorghums from the 2009 Liverpool Plains harvest, sorghums #3 and #5, were extensively characterised which included concentrations of kafirin and phenolic compounds plus rapid visco-analysis (RVA) starch pasting profiles. Diets based on these two sorghums were formulated to be iso-nitrogenous and iso-energetic and were offered to male Ross 308 broiler chicks from 7 to 28 days post-hatch as either intact pellets or reground mash following steam-pelleting at conditioning temperatures of either 65 or 97°C. Thus the feeding study consisted of a 2 × 2 × 2 factorial array of dietary treatments: two sorghum varieties, two feed forms and two conditioning temperatures. Each of the eight treatments was replicated six times with six birds per replicate cage. Assessed parameters included growth performance, nutrient utilisation, apparent starch and protein (N) digestibility coefficients and disappearance rates from the distal jejunum and distal ileum. Intact pellets supported higher ( $P < 0.001$ ) feed intakes and weight gains by 9.83 and 9.08%, respectively, than reground mash diets. Feed conversion ratios of broilers offered diets steam-conditioned at 97°C were 2.46% inferior ( $P < 0.001$ ) in comparison to 65°C diets and both apparent metabolizable energy (AME) and N-corrected AME (AMEn) were compromised. Broilers offered sorghum #3-based diets significantly ( $P < 0.001$ ) outperformed their sorghum #5 counterparts in terms of weight gain by 3.75% (1,334 versus 1,223 g/bird), FCR by 4.81% (1.524 versus 1.601), AME by 1.06 MJ (13.61 versus 12.55 MJ/kg), ME:GE ratio (ME:GE) by 4.81% (0.806 versus 0.769) and AMEn by 1.03 MJ (12.38 versus 11.35 MJ/kg). The inferiority of sorghum #5 appeared to be associated with higher concentrations of kafirin (61.5 versus 50.7 g/kg) and conjugated phenolic acids, including ferulic acid (31.1 versus 25.6 µg/g). There were no significant differences in jejunal and ileal starch and protein (N) digestibility coefficients between the two sorghums. However, starch to protein (N) disappearance rate ratios from the distal jejunum were significantly ( $P < 0.001$ ) correlated with ME:GE and AME. The multiple linear regression equations indicated that energy utilisation was enhanced by coupling rapidly digestible protein with slowly digestible starch, which suggests that bilateral bioavailability of starch and protein is pivotal to efficient energy utilisation.

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## 1. Introduction

Sorghum has been described as an enigmatic grain for chicken-meat production because sorghum-based broiler diets have been associated with sub-optimal performance of chickens under Australian conditions (Selle et al., 2013). Six red 'tannin-free' grain sorghum varieties harvested on the Liverpool Plains of New South Wales in 2009 were extensively characterised and compared in broilers offered sorghum-casein diets (Khoddami et al., submitted for publication). On the basis of this comparison, two sorghums (sorghums #3 and #5) were selected and incorporated into conventional, iso-nitrogenous and iso-energetic diets that were steam-pelleted at conditioning temperatures of 65 or 97°C and were offered to broilers as either intact pellets or reground mash from 7 to 28 days post-hatch as a 2 × 2 × 2 factorial array of dietary treatments. It was anticipated from the initial comparison that broiler chickens offered diets based on sorghum #3 would outperform their sorghum #5 counterparts.

It is often considered that kafirin, the dominant sorghum protein fraction, is a poor source of protein and also negatively impacts on starch utilisation (Taylor, 2005). Both kafirin protein bodies and starch granules are embedded in close proximity in the glutelin protein matrix of sorghum endosperm (Selle et al., 2010). This close proximity would facilitate any biophysical and biochemical interactions between kafirin and starch in grain sorghum (Wong et al., 2010). A novel method to quantify kafirin in sorghum was developed during this investigation; therefore, the methodology is described in detail. Also, it has been suggested that 'non-tannin' phenolic compounds in sorghum may have negative effects on starch utilisation (Khoddami et al., submitted for publication). This infers that the deleterious effects of phenolic compounds are not solely the province of condensed tannin. Thus the objectives of this paper include assessments of the influence of kafirin and 'non-tannin' phenolic compounds on the performance of broiler chickens offered nutritionally equivalent diets based on sorghums #3 and #5. Another objective is to appraise starch and protein (N) digestive dynamics in tandem by coupling their digestibility coefficients and disappearance rates in the distal jejunum and distal ileum.

## 2. Materials and methods

The six red sorghums from the 2009 Liverpool Plains harvest were extensively characterised. The more relevant data specific to sorghums #3 and #5 are shown in Table 1. Neither sorghum contained a pigmented testa and, therefore do not contained condensed tannin which was confirmed by a vanillin assay (Khoddami et al., submitted for publication).

### 2.1. Kafirin quantification

The kafirin concentrations were quantified by the following procedures. Kafirin was extracted from sorghum meal according to Wallace et al. (1990) and Hamaker et al. (1995). Sorghum meal (100 mg) was incubated with 1 mL total protein extraction buffer (12.5 mM sodium borate, pH 10.0, 1% SDS, 2% 2-mercaptoethanol) for 1 h with agitation at room temperature. Each sample was centrifuged for 15 min at 14,000 rpm at room temperature. Total protein was extracted twice more as above and the supernatants pooled. To isolate kafirin from the total protein extract, 100% *t*-butanol was added to the pooled supernatant at a final concentration of 60%, and incubated for 30 min at room temperature with occasional mixing. The extract was then centrifuged for 15 min at 9,500 rpm at room temperature, which resulted in the formation of a pellet (non-kafirin) and supernatant (alcohol-soluble kafirin). Kafirin was extracted from each sorghum sample in duplicate.

Both kafirin and total protein content were quantified by amino acid analysis. The amino acid analysis procedure employed pre-column derivatisation with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) and ultra-performance liquid chromatography (UPLC) analysis after first performing acid hydrolysis on the kafirin extract and whole sorghum grain samples to release the protein-bound amino acids. Each sample was quantified in duplicate. The method is based on Cohen (2001) but the amino acids were analysed on a UPLC system (Boogers et al., 2008). For quantification of total sorghum protein, 200 to 250 mg of sorghum meal was hydrolysed in duplicate by adding 5.0 mL of 20% HCl to the sorghum meal in a 10 mL hydrolysis vial, flushing with nitrogen for 1 min, followed by incubation at 110°C for 24 h. An internal standard (norvaline) was then added to the

**Table 1**  
Selected characteristics of sorghum 3# and sorghum #5.

Item	Sorghum #3	Sorghum #5	Item, µg/g	Sorghum #3	Sorghum #5
Kafirin	50.7	61.5	Total anthocyanin, Abs/(mL · g)	11.55	7.27
Protein	99.4	116.3	Flavan-4ols, Abs/(mL · g)	4.05	4.12
Kafirin proportion, %	51.0	52.9	Luteolinidin	5.58	1.88
Protein solubility, %	49.5	41.2	Apigeninidin	16.84	6.06
			5-methoxy-luteolinidin	5.80	2.41
Starch	624	620	7-methoxy-apigeninidin	25.60	11.45
Amylose, %	26.4	27.2	Apigenin	9.25	5.62
Amylopectin, %	73.6	72.8	Luteolin	8.30	4.75
Peak RVA viscosity, cP	4,202	3,750	Eriodictyol	57.29	62.27
Holding RVA viscosity, cP	3,088	3,086	Naringenin	86.51	85.68
Final RVA viscosity, cP	6,644	7,132			
			Total phenolic acids	545.4	538.1
Total phosphorus	2.95	3.35	Bound phenolic acids	458.0	448.0
Phytate-P	2.35	2.40	Conjugated phenolic acids	53.1	76.0
Phytate-P proportion, %	79.7	71.6	Free conjugated acids	34.3	14.1
Phytate	8.33	8.51			
			Total ferulic acid	421.7	407.9
Symes PSI texture	10	9	Bound ferulic acid	393.1	374.9
			Conjugated ferulic acid	25.6	31.1
Total phenolics, mg GAE/g	3.52	3.59	Free ferulic acid	3.0	1.9
Pigmented testa	0	0			

RVA = rapid visco-analysis; GAE = gallic acid equivalents.

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