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Graded inclusions of sodium metabisulphite in sorghum-based diets: I. Reduction of disulphide cross-linkages *in vitro* and enhancement of energy utilisation and feed conversion efficiency in broiler chickens

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ABSTRACT

Seven sorghum-based diets containing 0, 1.50, 2.25, 3.00, 3.75, 4.50 and 5.25 g/kg of the reducing agent sodium metabisulphite (SMBS) were prepared in which sodium levels were balanced with sodium bicarbonate. A red sorghum (766.4 g/kg starch, 78.6 g/kg protein) was hammer-milled through a 3.2 mm screen prior to being mixed into complete broiler diets containing 195 g/kg protein with an energy density12.97 MJ/kg, which were steam-pelleted at an 84 °C conditioning temperature. Each of the seven dietary treatments was offered to a total of 294 male Ross 308 chicks in seven replicate cages (6 birds per cage) from 10 to 24 days post-hatch to determine the effects of SMBS on growth performance, nutrient utilisation and nitrogen (N) digestibility coefficients in four small intestinal segments. SMBS did not linearly influence ($P > 0.50$) weight gains and feed intakes. However, SMBS quadratically improved ($r = 0.416$; $P < 0.05$) feed conversion ratios and it may be deduced from the quadratic equation that 4.75 g/kg SMBS improved FCR by 3.06% (1.424 versus 1.469). At all positive inclusions, SMBS significantly enhanced apparent metabolisable energy (AME) and N-corrected AME (AMEn); at the lowest inclusion, 1.50 g/kg SMBS significantly improved AME by 0.36 MJ (13.81 versus 13.45 MJ/kg; $P = 0.005$), AMEn by 0.45 MJ (12.30 versus 11.85 MJ/kg; $P = 0.001$) and FCR by 2.47% (1.422 versus 1.458; $P = 0.025$) on the basis of pair-wise comparisons. SMBS linearly decreased concentrations of disulphide bonds ($r = -0.775$; $P < 0.001$) and linearly increased free sulphydryl groups ($r = 0.890$; $P < 0.001$) and protein solubility of the diets ($r = 0.943$; $P < 0.001$). However, SMBS had little influence on N digestibility coefficients as the sole significant response was a 6.41% increase (0.797 versus 0.749; $P < 0.01$) in the distal ileum at 3.75 g/kg and SMBS did not influence small intestinal sites of protein disappearance. Consideration is given to the possibility that the sulphite reducing agent, SMBS, caused oxidative-reductive depolymerisation of starch polysaccharides which may have been responsible for the improvements in energy utilisation and feed conversion efficiency.

Abbreviations: AIA, acid insoluble ash; AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy; DI, distal ileum; DJ, distal jejunum; FCR, feed conversion ratio; GE, gross energy; NBD-Cl, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; NIRS, near infra red spectroscopy; NSP, non-starch polysaccharides; ORD, oxidative–reductive depolymerisation; PI, proximal ileum; PJ, proximal jejunum; –SH, free sulphydryl group; –S–S–, disulphide bond; SMBS, sodium metabisulphite.

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1. Introduction

Under Australian conditions, the performance of broiler chickens offered sorghum-based diets is considered to be inferior to wheat-based diets; however, wheat-based diets are usually advantaged by inclusions of NSP-degrading enzymes and better pellet quality because wheat has a lower starch gelatinisation temperature than sorghum (Taylor and Dewar, 2001). Real efforts have been made to identify the contributing factors for the relative inferiority of sorghum as a feedstuff for chicken-meat production (Bryden et al., 2009; Selle et al., 2010). Kafirin, the dominant protein fraction in grain sorghum, is often held responsible for poor sorghum protein quality due to its low lysine content, hydrophobicity and the structure of protein bodies (Selle, 2011).

Taylor et al. (1984) reported average proportions of 48.0% kafirin and 27.7% glutelin of total protein in 41 sorghum samples with a mean protein content of 111 g/kg. However, as is evident in this data, the relative proportions are influenced by sorghum protein content; as protein levels increase so does the proportion of kafirin at the expense of glutelin (Selle et al., 2010). Kafirin is located in protein bodies which, together with starch granules, are embedded in the glutelin protein matrix of sorghum endosperm (Seckinger and Wolf, 1973). The central core of the protein body consists of α-kafirin and the peripheral layers are composed of β-kafirin and γ-kafirin. There has been a considerable focus on the presence of disulphide cross-linkages, especially in the relatively cystine-rich β- and γ-kafirin fractions, limiting the nutritive value of sorghum (Bryden et al., 2009; Selle et al., 2010; Selle, 2011). However, disulphide cross-linkages are also present in glutelin (Beckwith, 1972) and there is evidence that the balance of sorghum protein, which is predominantly glutelin, is equally susceptible to the induction of disulphide cross-linkages by wet-cooking (Emmambux and Taylor, 2009).

Inclusions of the sulphite reducing agent sodium metabisulphite (SMBS) in 'all-sorghum', mash diets were investigated in a preliminary assessment (Selle et al., 2013b). At eight inclusions ranging from 0 to 15.0 g/kg SMBS significantly decreased concentrations of disulphide bonds. Nevertheless, the outstanding finding was that SMBS significantly ($P < 0.05$ to <0.001) improved apparent metabolisable energy (AME) at 1.25, 5.0 10.0 and 12.5 g/kg and numerically increased AME (MJ/kg DM) at all inclusion levels. The improvement of 0.39 MJ generated by 1.25 g/kg SMBS (14.80 versus 14.41; $P < 0.05$) is promising because the low inclusion may be economically feasible. However, SMBS linearly depressed voluntary feed intakes ($r = -0.783$; $P < 0.001$) and it appeared that the maximum SMBS inclusion that can be tolerated by broiler chickens was in the order of 5.0 g/kg.

SMBS significantly increased starch digestibility coefficients in the proximal (0.966 versus 0.931; $P < 0.01$) and distal ileum (0.980 versus 0.949; $P < 0.001$) where 5.0 g/kg was the only level assessed for starch digestibility. This starch digestibility response was associated with an increase of 0.53 MJ (14.94 versus 14.41 MJ/kg; $P < 0.01$) in AME. Sulphite reducing agents are also antioxidants (Elmas et al., 2005) and it has been found that sulphite reducing agents have the capacity to induce oxidative-reductive depolymerisation of starch polysaccharides (Paterson et al., 1996, 1997). Therefore, Selle et al. (2013b) suggested that starch digestibility and AME responses to 5.0 g/kg SMBS may have been more related to SMBS inducing depolymerisation of starch, that is the conversion of starch polymers into component monomers, than the reduction of disulphide cross-linkages in proteins.

The present study investigates SMBS inclusions of 0.0, 1.50, 2.25, 3.00, 3.75, 4.50 and 5.25 g/kg in complete, steam-pelleted, sorghum-based broiler diets in which sodium levels and dietary electrolyte balances were maintained constant. The maximum inclusion level was restricted to 5.25 g/kg SMBS, which was anticipated to have a negative impact on feed intakes. The study will be presented as two papers. In this first paper the effects of SMBS on *in vitro* reduction of disulphide cross-linkages in sorghum-based diets will be determined. In broiler chickens, the effects of SMBS on growth performance, nutrient utilisation and N digestibility coefficients in four segments of the small intestine will be investigated. In the second paper, the effects of SMBS on starch pasting properties *in vitro*, starch digestibility in four small intestinal segments, starch and nitrogen digestion dynamics in broiler chickens will be investigated.

2. Materials and methods

2.1. Outline and in vitro determinations

The red sorghum used in this feeding study contained 766.4 g/kg starch and 78.6 g/kg protein on a dry matter basis. Grain sorghum was incorporated into seven dietary treatments at a concentration of 622.7 g/kg, which were formulated to contain 195 g/kg protein with an energy density of 12.97 MJ/kg as shown in Table 1. Sorghum has hammer-milled through a 3.2 mm screen prior to being mixed into complete diets which were steam-pelleted at a conditioning temperature of 84 °C. The sorghum-based broiler diets contained meat-and-bone meal and soybean meal and were formulated to contain 1.86 g/kg sodium (Na) with and a dietary electrolyte balance ($DEB = Na^+ + K^+ - Cl^-$ as meq/kg) of 237 meq/kg. SMBS was added to the basal diets at inclusions of 0, 1.50, 2.25, 3.00, 3.75, 4.50 and 5.25 g/kg to provide seven experimental diets. In order to maintain constant dietary Na levels, sodium bicarbonate was included at from 0 to 4.234 g/kg and differences in dietary concentrations between the two Na sources were balanced by varying Celite inclusion levels from 14.850 to 15.866 g/kg.

To determine concentrations of disulphide bonds and free sulphydryl groups milled samples of the diets were analysed for free sulphydryl (-SH) concentrations using 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) according to the proteolytic extraction method of Andrews et al. (1995). Total sulfhydryl concentrations were determined after reduction of the extract