[Sorghum TechNote PRF 8-15]

Additions of the reducing agent sodium metabisulphite in sorghum-based broiler diets based on eight different varieties

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1 Background

One of the unique features of sorghum is its protein characteristics which are mainly comprised of kafirin and glutelin. Kafirin is the dominant fraction as it classically makes up 55% of sorghum protein. Kafirin is found in discrete protein bodies located in the sorghum endosperm with a central core of α-kafirin enveloped by peripheral layers of β- and γ-kafirin. Kafirin protein bodies and starch granules are embedded in the glutelin protein matrix of sorghum endosperm. Both β- and γ-kafirin are relatively rich in cystine and disulphide cross-linkages. Kafirins can form resilient sheet-like structures within and between protein bodies due to the presence of disulphide cross-linkages which could interfere with digestive enzyme accessibility (De Mesa-Stonestreet *et al.*, 2010)

Sulphite reducing agents, including sodium sulphite (Na_2SO_3), sodium bisulphite ($NaHSO_3$) and sodium metabisulphite ($Na_2S_2O_5$), have the capacity to cleave disulphide cross-linkages. The Poultry Research Foundation has been interested in sodium metabisulphite (SMBS) addition to sorghum-based diets since 2011 when an initial investigation was completed with eight graded SMBS inclusion levels in sorghum-casein diets (Selle *et al.*, 2013a). *In vitro*, SMBS significantly decreased disulphide cross-linkages and increased free sulphydryl groups thus confirming its efficacy as a reducing agent. *In vivo*, SMBS significantly enhanced energy utilisation as it increased AME by 0.39 MJ, 0.53 MJ, 0.56 MJ and 0.32 MJ at inclusion levels of 1.25 g/kg, 5.00 g/kg, 10.0 g/kg and 12.5 g/kg, respectively. On this basis, 10.0 g/kg SMBS improved energy utilisation by 3.74% and numerically improved FCR at inclusion levels of 1.25, 2.50 and 5.00 g/kg by an average of 14.1% (1.251 versus 1.456) from 14 to 21 days post-hatch.

In a subsequent study SMBS was included in conventional sorghum-based broiler diets at 0, 1.5, 2.25, 3, 375, 4.5 and 5.25 g/kg. However, Nottingham University had generated a quite considerable amount of data to indicate that sulphite reducing agents also have the capacity to depolymerise starch polysaccharides via oxidative-reductive reactions (Paterson *et al.*, 1996, 1997). Therefore, the impact of SMBS on RVA starch pasting properties of the sorghum-based diets was determined by rapid visco-analysis (RVA). SMBS linearly decreased final RVA viscosities (r = -0.925; P < 0.001) culminating in a 24.6% (1935 versus 2567 cP) decrease at 5.25 g/kg. However, the impact of SMBS on RVA profiles of starch extracted from the diets was even more profound. Here, SMBS linearly decreased final RVA viscosities (r = -0.986; P < 0.001) with a 57.4% (1035 versus 441 cP) decrease at 5.25 g/kg as illustrated in Figure 1 (Liu *et al.*, 2014). In addition, graded dietary SMBS inclusion levels linearly decreased disulphide cross-linkages (r = - 0.775; P < 0.001), linearly increased free sulphydryl groups (r = 0.890; P < 0.001) and linearly increased protein solubility (r = 0 943; P < 0.001). Indeed, SMBS increased Promatest protein solubility of the diets from 34.8% to > 60.0% at both 4.50 and 5.25 g/kg (Selle *et al.*, 2014a).

In this study SMBS numerically improved FCR by up to 3.02% (1.414 versus 1.458 at 3.75 g/kg) and significantly improved AME by up to 0.40 MJ (13.85 versus 13.45 MJ/kg at 4.75 g/kg) and AMEn by up to 0.51 MJ (12.36 versus 11.85 MJ/kg at 4.75 g/kg). Clearly, SMBS inclusions in sorghum-based broiler diets displayed promise as presented in two conferences (Selle *et al.*, 2013b; 2014b). However, the genesis of these responses is not straightforward and could stem from the capacity of SMBS to reduce disulphide cross-linkages or its capacity to depolymerise starch polysaccharides. Therefore, the Poultry Research Foundation completed a further three feeding studies involving an additional six grain sorghum varieties for purposes of clarification and confirmation. The purpose of this TechNote is to provide a summary of these SMBS evaluations in sorghum-based broiler diets.

2 Responses of broilers offered diets based on eight sorghum varieties to Na metabisulphite

The basic data for the eight grain sorghum varieties that have been evaluated so far are shown in Table 1. Sorghums #12 and #13 were both from the 2011 Liverpool Plain harvest and had the same quite low protein content and were the subject of the studies considered in the previous section. Sorghums MP and HP had the highest protein contents and were also harvested on the Liverpool Plains but in 2013. HFQ was the only white sorghum investigated with SMBS and this sorghum was specially imported from Kansas, USA, which required prior hammer-milling to satisfy AQIS.

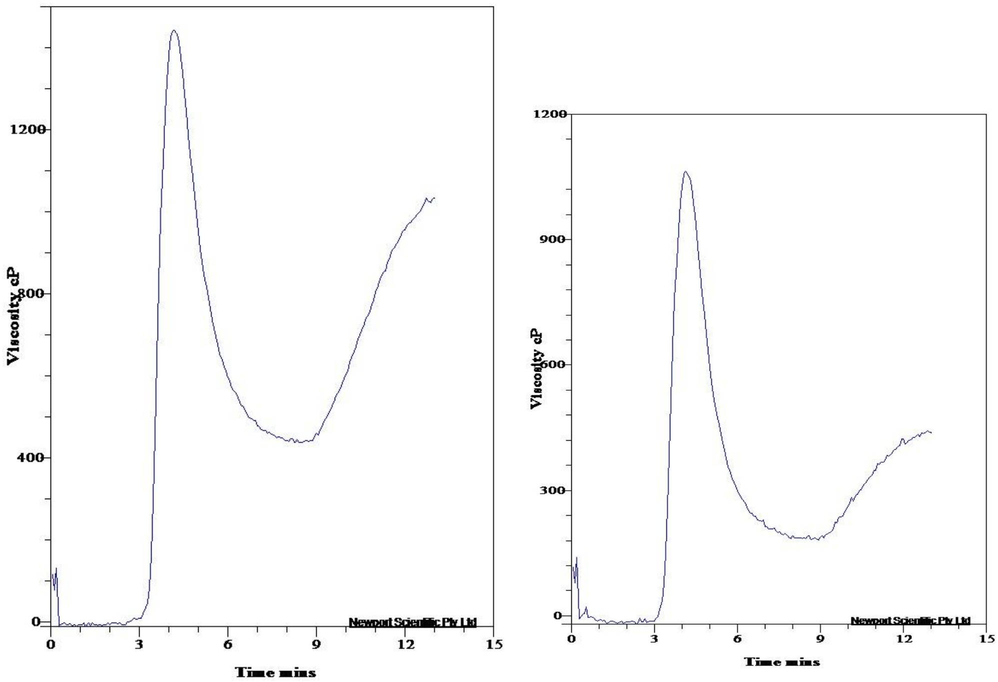

Figure 1 RVA starch pasting profiles of starch extracted from sorghum-based diets without and with 5.25 g/kg sodium metabisulphite
Peak viscosity: 1444 ⇒ 1065 cP [-26.2%] Final viscosity: 1035 ⇒ 441 cP [-57.4%]

From the preliminary study (Selle *et al*., 2013) it appears broilers chickens are able to tolerate dietary SMBS inclusions approaching 5.00 g/kg before feed intakes are depressed (Figure 2) and similar findings have been reported in pigs and poultry. However, inclusion levels under 5.00 g/kg have the capacity to enhance nutrient utilisation of sorghum-based diets.

Table 1 Background information and crude protein content for the eight sorghum varieties

Sorghum	Harvest	Growing location	Description	Protein (g/kg)
Sorghum #12	2011	Liverpool plains, NSW	Red	78.6
Sorghum #13	2011	Liverpool plains, NSW	Red	78.6
Tiger	2013	MIA, NSW	Red	99.9
MP	2013	Liverpool Plains, NSW	Red	100.2
HP	2013	Liverpool Plains, NSW	Red	109.1
JM	2013	na	Red	97.7
FW	2013	na	Red	92.5
HFQ	2013	Kansas, USA	White	98.0

With the exception of one sorghum [FW, a red sorghum evaluated in the study Truong *et al*. (2015a) study], responses in energy utilisation (AME, ME:GE ratio and AMEn) to SMBS inclusions up to 5.25 g/kg were all positive across five studies as shown in Table 2. In studies 2 to 5 inclusive (complete sorghum-based diets as opposed to sorghum-cased diets used in study 1), the average SMBS inclusion of 2.84 g/kg increased AME by 0.27 MJ, AMEn by 0.32 MJ, and ME:GE ratios by 1.90%. Median responses to SMBS were improvements of 0.34 MJ in AME, 0.38 MJ in AMEn and 2.22% in ME:GE ratios. SMBS also improved FCR in broilers offered complete sorghum-based diets by an average of 1.37% or a median response of 1.47%. Across all five studies, SMBS linearly increased AMEn ($r = 0.445$; $P < 0.05$) as illustrated in Figure 3.

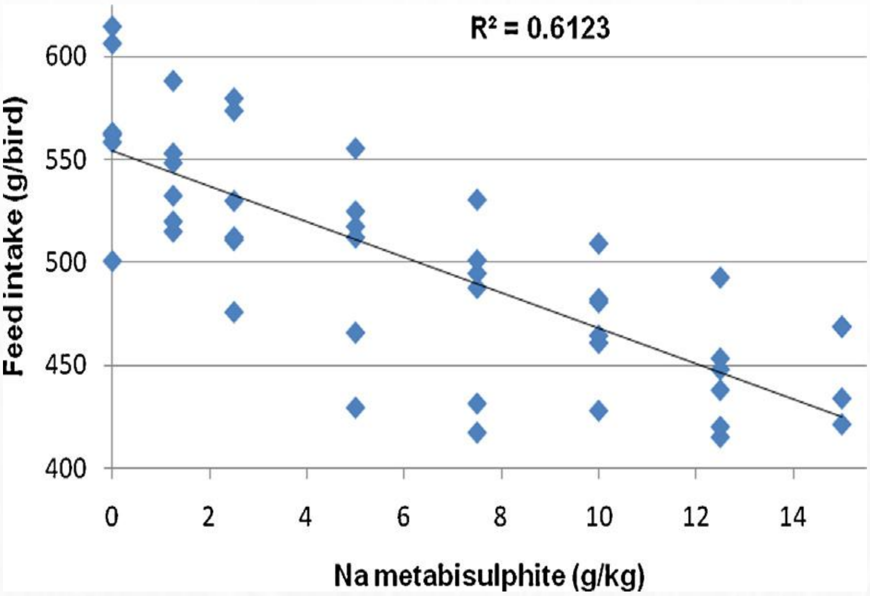

Figure 2 Linear relationship ($r = -0.783$; $P<0.001$) between dietary concentrations (from 0 to 15 g/kg) of sodium metabisulphite and voluntary feed intakes (g/bird) of broiler chickens from 14 to 21 days post-hatch (adapted from Selle *et al*., 2013a).

3 Mechanisms of SMBS energy utilisation and FCR responses

Across the five SMBS investigations, it is evident that Na metabisulphite has a positive "energy effect". In the study involving sorghum #13, the unequivocal increases in energy utilisation (AME and AMEn) appeared to be linked to the slowly digestible starch generated by SMBS. Sorghum #13 had a relatively low protein content of 78.6 g/kg which suggests that it also contained relatively little kafirin (Selle et al., 2010) so it seems possible that the capacity of SMBS to depolymerise starch polysaccharides was more integral to the unequivocal energy utilisation increases than its capacity to reduce disulphide cross-linkages in kafirin in particular. It is generally held that kafirin negatively influences sorghum starch utilisation via biophysical and biochemical protein-starch interactions involving kafirin protein bodies and starch granules in sorghum endosperm (Taylor, 2005).

Table 2 Responses to SMBS inclusions of up to 5.25 g/kg on energy utilisation parameters and FCR in five studies involving a total of eight sorghum varieties [negative responses]

SMBS inclusion	Study/ Sorghum	AME response (MJ)	ME:GE response (%)	AMEn response (MJ)	FCR response (%)
1.25	1/#12[1]	0.39	1.82	0.29	17.4
2.50	1/#12[1]	0.17	0.54	0.08	5.67
5.00	1/#12[1]	0.53	2.58	0.51	20.7
1.50	2/#13	0.36	2.22	0.45	2.47
2.25	2/#13	0.35	2.47	0.38	1.65
3.00	2/#13	0.29	1.73	0.31	2.26
3.75	2/#13	0.36	1.73	0.30	3.02
4.50	2/#13	0.40	2.47	0.51	2.33
5.25	2/#13	0.34	2.35	0.43	2.47
1.75	3/FW	[0.42]	[1.34]	[0.37]	3.13
1.75	4/MP	0.40	2.61	0.45	0.39
3.50	4/MP	0.25	1.65	0.38	[1.64]
1.75	4/JM	0.41	3.27	0.47	[1.82]
3.50	4/JM	0.31	2.32	0.48	[1.63]
1.75	5/HP	0.22	1.45	0.22	0.53
3.50	5/HP	0.34	2.46	0.35	8.57
1.75	5/Tiger	0.09	1.25	0.06	[0.60]
3.50	5/Tiger	0.29	1.94	0.29	1.47
1.75	5/HFQ	0.06	0.42	0.17	0.13
3.50	5/HFQ	0.50	3.38	0.59	0.54
(studies 2-5)	g/kg				
Mean	2.84	0.27 MJ	1.90%	0.32 MJ	1.37%
Median	3.00	0.34 MJ	2.22%	0.38 MJ	1.47%

[1]sorghum-casein diets

Disruption of disulphide cross-linkages in kafirin by SMBS may also facilitate starch digestion and promoted more rapidly digestible starch as observed in the sorghum FW study. The generation of slowly digestible starch was certainly not evident in the sorghum FW study and this may have been a consequence of SMBS reducing disulphide cross-linkages in kafirin, thereby diminishing starch-protein interactions and accelerating starch digestion.

Given the unexpected energy utilisation responses in the sorghum FW study, the influence of SMBS may depend on the properties of the specific sorghum grain where kafirin content could be a critical factor. However, overall it is evident that the influence of SMBS in sorghum-based diets is positive in seven out of eight sorghums and sorghum FW may have been an outlier. Inclusions of SMBS displays promise in commercial practice as it has positive effects on energy utilisation and efficiency of feed conversion. Its dietary inclusion cost is largely negated by its replacement of sodium bicarbonate to maintain diary sodium levels at intended levels.

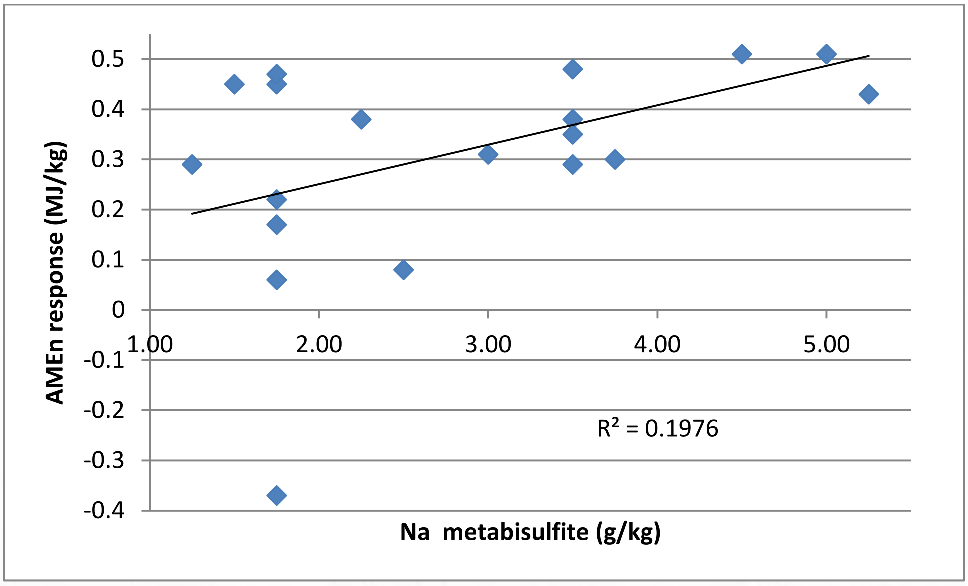

Figure 3 Linear relationship (r = 0.445; P< 0.05) between dietary concentrations (from 0 to 5.25 g/kg) of sodium metabisulphite and N-corrected AME (MJ/kg) of broiler chickens (adapted from Table 2)

4 Future Na metabisulphite R&D directions

The addition of SMBS at inclusion levels in the order of 2.0 g/kg to sorghum-based broiler diets enhances energy utilisation. It would be advantageous to ascertain if these improvements stem mainly from reductions of disulphide cross-linkages and increased solubility of proteins, perhaps especially kafirin, or from starch depolymerisation. This is only complicated by the likelihood that these two impacts are interdependent given the importance placed on starch-protein interactions in sorghum specifically (Rooney and Pflugfelder, 1986), and in general (Truong et al., 2015b). There is the real possibility that SMBS is modifying the digestive dynamics of both starch and protein to benefit energy utilisation in broiler chickens (Liu and Selle, 2015).

It seems likely that SMBS responses with certain grain sorghum varieties will be more robust than others. One avenue under investigation is to determine if the RVA starch pasting profile of sorghums, or the impact of SMBS on those RVA profiles, are in any way predictive of the responses that would be observed in broiler chickens.

An obvious question is whether or not SMBS inclusions in maize- and wheat-based broiler diets are advantageous. The results of an initial study completed by the PRF in wheat-based diets were equivocal in this regard and a further study is planned. Clearly, both wheat and sorghum contain starch and their protein fractions contain disulphide cross-linkages. If the advantages of SMBS do not extend to wheat it may be that the unique structure of kafirin protein bodies, with their peripheral layers of β- and γ-kafirin, and their close proximity to starch granules in sorghum endosperm is the pivotal difference. It also may be possible that protein sources including soybean, canola and meat-and-bone meals, perhaps the last one particularly, would benefit from dietary inclusions of reducing agents. If nothing else, future feeding studies involving sodium metabisulphite should prove interesting and instructive.

Acknowledgement

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