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Sodium metabisulphite enhances energy utilisation in broiler chickens offered sorghum-based diets with five different grain varieties

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ABSTRACT

The reducing agent, sodium metabisulphite, was included in sorghum-based broiler diets at 0.00, 1.75 and 3.50 g/kg from 7 to 28 days post-hatch. In Experiment 1 two sorghum varieties (MP, JM) were evaluated and three sorghums (HP, Tiger, HFQ) in Experiment 2; the two experiments were separate but very similar. The five sorghums were extensively characterised and all five diets contained 620 g/kg sorghum and were formulated to be nutritionally equivalent. In both studies, each dietary treatment was offered to six replicate cages (6 birds per cage) for a combined total of 540 male Ross 308 chicks. The effects of sodium metabisulphite on growth performance, nutrient utilisation, apparent digestibility coefficients of starch and protein (N) and starch and protein digestive dynamics were determined. Sodium metabisulphite significantly enhanced energy utilisation. Across both experiments, 3.50 g/kg sodium metabisulphite increased AME on a dry matter basis by 0.34 MJ from an average of 11.97–12.31 MJ/kg and similarly increased N-corrected AME by 0.42 MJ from 11.43 to 11.85 MJ/kg. Sorghum variety by sodium metabisulphite interactions were not observed for energy utilisation (AME, ME:GE ratios, AMEn) parameters but this was not always the case for other parameters, which indicates that diverse sorghums respond differently to sodium metabisulphite. Consideration is given to the basis for these variable responses to sodium metabisulphite because of its dual modes of action (reducing disulphide cross-linkages, depolymerising starch polysaccharides) and the possible relevance of RVA starch pasting profiles of grain sorghum to feed grain quality.

Abbreviations: AIA, acid insoluble ash; AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy; cP, centipoise; DJ, distal jejunum; DI, distal ileum; FCR, feed conversion ratio; ME:GE, metabolisable: gross energy ratios; N, nitrogen; PJ, proximal jejunum; PI, proximal ileum; RVA, rapid visco analysis.

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1. Introduction

Sorghum is a problematic feed grain for chicken-meat production which has been considered in two reviews (Selle et al., 2010; Liu et al., 2015). A distinctive feature of sorghum is kafirin, the dominant protein fraction. Kafirin classically makes up 55% of sorghum protein and is present as discrete protein bodies located in the sorghum endosperm with a central core of a-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. As a consequence of disulphide cross-linkages and protein polymerisation, kafirin can form resilient sheet-like structures within sorghum endosperm with the potential to impede starch gelatinisation and to deny amylase access to its substrate (De Mesa-Stonestreet et al., 2010).

A range of sulphite reducing agents, including sodium metabisulphite ($Na_2S_2O_5$), have the capacity to cleave disulphide cross-linkages according to the following Cecil and Wake (1962) equation: $RS^*SR + SO_3{}^{2-} \Leftrightarrow RS^{1-} + RS^*SO_3{}^{1-}$. On the basis of *in vitro* data, reducing agents have the capacity to enhance both protein (Hamaker et al., 1987; Rom et al., 1992; Oria et al., 1995) and starch (Zhang and Hamaker, 1998) digestibility of grain sorghum. Given this potential, eight sodium metabisulphite inclusion levels, ranging from 0.0 to 15.0 g/kg, were previously assessed in 'all-sorghum' diets (Selle et al., 2013). Inclusions of 1.25, 5.0, 10.0 and 12.5 g/kg sodium metabisulphite significantly increased AME by 0.39, 0.53, 0.56 and 0.32 MJ, respectively, in a quadratic manner and 5.0 g/kg sodium metabisulphite (the only level assessed) significantly increased proximal jejunal and distal ileal starch digestibility coefficients. Finally, sodium metabisulphite linearly increased free sulphydryl groups and linearly decreased disulphide bonds in the 'all-sorghum' diets.

Sodium metabisulphite (six inclusions from 1.50 to 5.25 g/kg) was subsequently evaluated in conventional, steam-pelleted (84 °C) sorghum-based broiler diets. As reported by Selle et al. (2014), sodium metabisulphite again linearly increased free sulphydryl groups and decreased disulphide bonds to significant extents and, notably, linearly increased protein solubility of the diets from 34.8% to >60.0%. Sodium metabisulphite improved FCR in a linear manner and every positive inclusion level significantly increased energy utilisation (AME and AMEn).

Clearly, the status of sodium metabisulphite as a reducing agent was confirmed by these stiudies. However, sulphite reducing agents also have the capacity to depolymerise starch polysaccharides via oxidative-reductive reactions (Paterson et al., 1996, 1997). Therefore, the impact of sodium metabisulphite on starch pasting properties of sorghum-based diets was determined by rapid visco-analysis (RVA). Liu et al. (2014) reported that sodium metabisulphite linearly reduced peak, holding, final and setback RVA viscosities to significant extents, which was attributed to starch depolymerisation. Interestingly, in the Liu et al. (2014) study, sodium metabisulphite reduced rapidly digestible starch but increased slowly digestible starch and it was thought that this shift in starch digestive dynamics in favour of slowly digestible starch may have contributed to the FCR improvements.

A further evaluation of sodium metabisulphite in conventional sorghum–based broiler diets was completed without and with exogenous phytase and it was anticipated that this study would confirm the previous findings. However, Truong et al. (2015a) reported that 1.75 g/kg sodium metabisulphite numerically reduced AME by 0.42 MJ and AMEn by 0.37 MJ and tended to increase rapidly digestible starch. Predictably, these contradictory outcomes prompted additional evaluations of sodium metabisulphite in sorghum–based broiler diets in an attempt to appreciate the potential reducing agents may have in this and other contexts. Thus this paper investigates the impacts of 1.75 and 3.50 g/kg sodium metabisulphite inclusions in broiler diets based on five sorghum grain varieties on bird performance in two separate, but similar, feeding studies.

2. Materials and methods

Five grain sorghum varieties were used in the two experiments to evaluate the impacts of sodium metabisulphite on broiler growth performance and nutrient utilisation. As outlined in Table 1, MP and JM sorghums were used in Experiment 1 and HP, Tiger and HFQ sorghums were used in Experiment 2. White HFQ sorghum was imported from Kansas, USA which meant that HFQ had to be ground (4.0 mm hammer-mill screen) prior to being shipped to Australia to meet quarantine requirements. The five sorghums were extensively characterised and concentrations of protein, kafirin, total P, phytate-P, grain texture, AusScan data and RVA starch pasting profiles are shown in Table 2. Kafirin was quantified by analytical procedures adapted from Wallace et al. (1990) and Hamaker et al. (1995) and the amino acid profiles of MP and HP sorghums appear in Table 3. Concentrations of total phenolic compounds, polyphenols, free, conjugated and bound phenolic acids in

Table 1
Background information for the five grain sorghum varieties used in Experiment 1 (MP, JM) and Experiment 2 (HP, Tiger, HFQ).

Sorghum	Harvest	Growing location	Colour	Pigmented testa[a]
MP	2013	Liverpool Plains, NSW	Red	Negative
JM	2013	Not known	Red	Negative
HP	2013	Liverpool Plains, NSW	Red	Negative
Tiger	2013	Murrumbidgee Irrigation Area, NSW	Red	Negative
HFQ	2013	Kansas, USA	White	Negative

[a] Pigmented testas were not detected by the Clorox bleach test, which indicates that the sorghums are Type I and do not contain condensed tannin.

Table 2
Characteristics of grain sorghums including concentrations of protein, kafirin, total P, phytate-P, texture, AusScan data and RVA starch pasting profiles.

Item (g/kg)	MP	JM	HP	Tiger	HFQ
Crude protein (g/kg)	100.2	97.7	109.1	99.9	98.1
Kafirin (g/kg)	51.1	50.1	50.5	51.3	48.0
Percentage of total protein (%)	51.0	51.3	46.2	56.4	48.9
Phytate (g/kg)	8.30	8.94	7.77	8.40	6.31
Phytate-P (g/kg)	2.34	2.52	2.19	2.37	1.78
Total P (g/kg)	3.22	3.24	3.12	3.04	2.39
Phytate-P/total P (%)	72.7	77.8	70.2	78.0	74.4
Symes PSI texture (%)	9	10	8	9	na
NIR AusScan					
AME broilers (MJ/kg as-fed)	14.0	14.1	14.2	14.6	14.0
NIR Protein (g/kg)	107	109	113	102	98
Total starch (g/kg)	797	772	818	830	881
Crude fibre (g/kg)	33	37	30	25	24
Acid detergent fibre (g/kg)	95	97	97	76	91
Neutral detergent fibre (g/kg)	101	101	104	93	78
Total soluble NSP (g/kg)	<0.1	<0.1	<0.1	<0.1	<0.1
Total insoluble NSP (g/kg)	69	70	57	30	63
Insoluble arabinoxylans (g/kg)	45	50	43	36	52
Hydration capacity (%)	8.9	9.9	8.6	9.9	25.1
RVA starch pasting profiles					
Peak viscosity (cP)	3619	5559	2591	4771	4454
Holding viscosity (cP)	3022	3202	2517	2904	3695
Breakdown viscosity (cP)	597	2357	74	1867	760
Final viscosity (cP)	6347	5726	5554	5746	8115
Setback viscosity (cP)	3325	2524	3037	2846	4421
Peak time (minutes)	5.64	5.00	6.20	5.13	5.53
Pasting temperature (°C)	77.9	73.2	82.6	75.1	80.3

Table 3
Amino acid profile of kafirin in MP sorghum and HP sorghum.

Amino acid	MP sorghum (g/100 g protein)	HP sorghum (g/100 g protein)
Arginine	2.19	2.20
Histidine	1.95	1.84
Isoleucine	4.13	4.14
Leucine	15.70	15.83
Lysine	0.58	0.46
Methionine	1.17	1.18
Phenylalanine	5.59	5.72
Threonine	2.72	2.72
Valine	4.79	4.80
Alanine	10.10	10.19
Aspartic acid	6.18	6.09
Glutamic acid	24.32	24.24
Glycine	2.13	2.13
Proline	9.51	9.53
Serine	4.22	4.23
Tyrosine	4.71	4.71

the five sorghums are shown in Table 4. The complex methodologies used in the quantification of phenolic compounds have been described in detail by Khoddami et al. (2015).

The composition and nutrient specifications of the five basal experimental diets are shown in Table 5 and each diet contained 620 g/kg of the relevant grain sorghum. The diets were nutritionally equivalent in respect of energy density (12.95 MJ/kg ME), concentrations of key amino acids and Ca and P levels. Sodium metabisulphite inclusions of 1.75 and 3.50 g/kg were accommodated by adjustments to sodium bicarbonate and Celite levels to ensure constant Na contents and dietary electrolyte balances. The diets were steam-pelleted at a conditioning temperature of 84 °C and crumbled after sorghum grain (and soybean meal) had been ground through a 3.2 mm hammer-mill screen.

The two studies were completed six weeks apart in the same facility and the methodologies employed were identical. Feather-sexed, male Ross 308 chicks were housed in an environmentally-controlled facility and were initially offered a proprietary starter ration prior to being offered dietary treatments from 7 to 28 days post-hatch. Birds had unlimited access to feed and water under a “23 h on–1 h off” lighting schedule. There were six replicate cages (6 birds per cage) per treatment in both studies for total bird numbers of 216 and 324 in Experiments 1 and 2, respectively. Body weights were determined on days 7 and 28 and feed intakes recorded over the entire period to calculate FCRs with adjustments made from the weight of any dead or culled birds, which were monitored on a daily basis. On day 28 the birds were euthanised (intravenous injection

Table 4
Characteristics of grain sorghums including concentrations of total phenolic compounds, polyphenols, free conjugated and bound phenolic acids bound.

Item	MP	JM	HP	Tiger	HFQ
Polyphenols					
Total phenolics (mg GAE/g)	3.21	3.90	3.52	4.12	1.94
Total flavonoids (μg/g)	1040	880	1100	1037	693
Anthocyanin (ABS/ml/g)	5.65	4.05	5.09	10.86	0.68
Flavan-4-ol (ABS/ml/g)	5.36	3.63	4.82	5.04	0.42
Luteolinidin (μg/g)	3.53	2.24	1.92	7.83	nd
Apigeninidin (μg/g)	5.12	4.04	5.79	7.25	nd
5-methoxy-luteolinidin (μg/g)	4.52	3.99	4.19	6.40	nd
7-methoxy-apigeninidin (μg/g)	2.98	5.82	3.88	11.58	nd
Free phenolic acids (μg/g)					
Benzoic	3.79	2.53	3.75	4.32	7.32
Vanillic	1.82	4.55	1.72	2.11	3.06
Caffeic	8.66	10.43	9.91	18.35	8.80
p-Coumaric	1.15	1.23	0.82	2.25	1.64
Ferulic	2.44	2.36	2.07	1.88	2.69
Syringic	1.57	1.24	1.27	1.69	nd
Conjugated phenolic acids (μg/g)					
Benzoic	7.32	5.60	8.08	10.51	15.71
Vanillic	5.54	5.39	7.61	4.92	14.05
p-Coumaric	14.55	12.71	13.62	16.42	24.71
Ferulic	30.07	27.39	33.57	33.20	46.32
Syringic	10.40	8.01	11.25	13.24	14.81
Sinapic	2.62	0.93	2.13	5.62	2.07
Bound phenolic acids (μg/g)					
Benzoic	6.05	7.73	5.78	11.47	9.11
Vanillic	3.67	5.40	3.98	3.17	14.44
p-Coumaric	19.48	28.43	24.64	19.40	44.22
Ferulic	342.5	316.4	290.0	246.1	789.3
Syringic	6.30	8.74	6.73	7.36	10.19
Sinapic	nd	0.99	nd	1.44	nd
Total phenolic acids (μg/g)	468	450	427	403	1008

of Na pentobarbitone) and digesta samples were collected in their entirety from the proximal jejunum, distal jejunum, proximal ileum and distal ileum and pooled for each cage. This was in order to determine nutrient (starch and crude protein) digestibility coefficients and nutrient disappearance rates (g/bird/day).

Total excreta collection over a 48 h period at 25 days post-hatch was used to determine AME on a dry matter basis, ME:GE ratios, N retention and AMEn. Total excreta were quantitatively collected from each cage and feed intakes recorded for the 72 h collection period. Excreta were dried in a forced-air oven at 80 °C for 24 h and the gross energy (GE) of excreta and diets were determined using an adiabatic bomb calorimeter. The AME values of the diets on a dry matter basis were calculated from the following equation:

$$\mathrm{AME_{diet}}\left(\mathrm{MJ/kgDM}\right) = \frac{(\text{food intake} \times \mathrm{GE_{diet}}) - (\text{excreta output} \times \mathrm{GE_{excreta}})}{\text{feed intake}}$$

ME:GE ratios were calculated by dividing AME by the GE of the appropriate diets. N contents of diets and excreta were determined using a nitrogen determinator (Leco Corporation, St Joseph, MI) and N retentions calculated from the following equation:

$$\text{N retention}\,(\%) = \frac{(\text{feed intake} \times \mathrm{N_{diet}}) - (\text{excreta output} \times \mathrm{N_{excreta}})}{\text{feed intake} \times \mathrm{N_{diet}}}$$

N-corrected AME (AMEn MJ/kg DM) values were calculated by correcting N retention to zero using the factor of 36.54 kJ/g N retained in the body (Hill and Anderson, 1958). Digesta samples were freeze-dried to determine apparent digestibilities of starch and crude protein (N) using acid insoluble ash (AIA) as the inert dietary marker. Starch concentrations in diets and digesta were determined by a procedure based on dimethyl sulphoxide, α-amylase and amyloglucosidase as described by Mahasukhonthachat et al. (2010). N concentrations were determined as already stated and AIA concentrations were determined by the method of Siriwan et al. (1993). The apparent digestibility coefficients for starch and protein (N) at four small intestinal sites were calculated from the following equation:

$$\text{Apparently digestibility coefficient}=\frac{\left(\mathrm{nutrient/AIA}\right)_{\mathrm{diet}} - \left(\mathrm{nutrient/AIA}\right)_{\mathrm{digesta}}}{\left(\mathrm{nutrient/AIA}\right)_{\mathrm{diet}}}$$

Table 5
Composition and nutrient specifications of basal broiler diets based on five grain sorghum varieties.

Item (g/kg)	Experiment 1		Experiment 2		
	MP	JM	HP	Tiger	HFQ
Sorghum	620.0	620.0	620.0	620.0	620.0
Soybean meal	226.3	226.4	230	226.4	209.5
Canola meal	75.0	75.0	75.0	75.0	75.0
Sunflower oil	25.0	25.0	20.0	25.0	39.7
Dicalcium phosphate	17.2	17.2	16.1	17.2	17.3
Limestone	6.2	6.2	6.9	6.2	6.2
Lysine HCl	3.1	3.1	3.3	3.1	3.6
Methionine	2.5	2.5	2.9	2.5	2.7
Threonine	1.0	1.0	1.2	1.0	1.3
Arginine	1.1	1.0	1.1	1.0	1.5
Isoleucine	0.0	0.0	0.3	0.0	0.2
Valine	0.0	0.0	0.6	0.0	0.2
Sodium chloride	0.8	0.8	0.7	0.8	0.6
Vitamin-mineral premix[a]	2.0	2.0	2.0	2.0	2.0
Sodium bicarbonate	4.8	4.8	4.9	4.8	5.1
Celite	15.0	15.0	15.0	15.0	15.0
Nutrient specifications					
ME (MJ/kg)	12.95	12.95	12.95	12.95	12.98
Protein	190.9	190.8	187.1	190.9	184.9
Starch	387.8	387.8	387.9	387.8	387.8
Fat	51.2	51.2	52.6	51.2	65.5
Calcium	7.5	7.5	7.5	7.5	7.5
Total phosphorus	6.6	6.6	6.9	6.6	6.6
Available phosphorus	3.8	3.8	3.8	3.8	3.8
Lysine	11.8	11.8	11.9	11.9	11.8
Methionine	5.5	5.5	5.7	5.5	5.6
Threonine	8.4	8.4	8.4	8.4	8.3
Isoleucine	8.5	8.5	8.2	8.5	8.4
Tryptophan	2.6	2.6	2.5	2.6	2.5
Sodium	1.8	1.8	1.8	1.8	1.8
Potassium	7.5	7.5	7.6	7.5	7.2
Chloride	2.2	2.2	2.2	2.2	2.2

[a] The vitamin-mineral premix supplied per tonne of feed: [MIU] retinol 12, cholecalciferol 5, [g] tocopherol 50, menadione 3, thiamine 3, riboflavin 9, pyridoxine 5, cobalamin 0.025, niacin 50, pantothenate 18, folate 2, biotin 0.2, copper 20, iron 40, manganese 110, cobalt 0.25, iodine 1, molybdenum 2, zinc 90, selenium 0.3.

Starch and protein (N) disappearance rates (g/bird/day) were deduced from feed intakes over the final phase of the feeding period from the following equation:

$$\text{Nutrient disappearance rate}_{(\text{g/bird/day})} = \text{feed intake}_{(\text{g/bird})} \times \text{dietary nutrient}_{(\text{g/kg})}$$
$$\times\ \text{nutrient digestibility}_{(\text{apparent digestibility coefficient})}$$

Ratios of starch to protein disappearance rates in the intestinal segments were calculated as this effectively cancels the potential confounding influence of feed intake.

Experimental data were analysed using the IBM® SPSS® Statistics 20 program (IBM Corporation. Somers, NY) and Experiments 1 and 2 were analysed individually. The experimental units were cage means and statistical procedures included univariate analyses of variance using the general linear models procedure, Pearson correlations and linear and quadratic regressions. A probability level of less than 5% was considered to be statistically significant. The feeding studies complied with specific guidelines approved by the Animal Ethics Committee of the University of Sydney.

3. Results

The effect of three sodium metabisulphite inclusions in nutritionally-equivalent diets based on either two sorghum varieties (Experiment 1) or three varieties (Experiment 2) in growth performance of broilers from 7 to 28 days post-hatch are shown in Table 6. The acceptably low mortality rates of 0.93% and 1.55% in Experiments 1 and 2, respectively, were not related to treatment. In Experiment 1, sodium metabisulphite significantly ($P < 0.03$) increased feed intake by up to 4.47% (2366 versus 2267 g/bird) at 3.50 g/kg and sodium metabisulphite linearly increased feed intake ($r = 0.417$; $P < 0.015$). While not significant, it is noteworthy that 3.50 g/kg Na metabisulphite tended to increase weight gain by 6.24% (1567 versus 1475 g/bird; $P = 0.086$) of birds offered MP sorghum-based diets. In contrast, the effect of sodium metabisulphite in JM sorghum-based diets on weight gain was negligible.

Table 6
Effects of sodium metabisulphite (SMBS) inclusions on the growth performance of broilers offered sorghum-based diets from 7 to 28 days post-hatch.

Experiment 1					
Treatment		Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Mortality rate (%)
Sorghum	SMBS (g/kg)				
MP	0.00	1475	2239	1.520	2.78
	1.75	1544	2238	1.514	2.78
	3.50	1567	2420	1.545	0.00
JM	0.00	1496	2295	1.535	0.00
	1.75	1495	2336	1.563	0.00
	3.50	1483	2311	1.560	0.00
SEM		27.548	35.700	0.0224	1.6041
Sorghum					
MP		1529	2332	1.526	1.85
JM		1492	2314	1.553	0.00
SMBS					
0.00		1486	2267a	1.527	1.39
1.75		1520	2337ab	1.539	1.39
3.50		1525	2366b	1.552	0.00
Significance (P=)					
Sorghum		0.106	0.537	0.142	0.168
SMBS		0.311	0.028	0.524	0.611
Interaction		0.170	0.080	0.686	0.611

Experiment 2					
Treatment		Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Mortality rate (%)
Sorghum	SMBS (g/kg)				
HP	0.00	1435	2159cd	1.505b	0.00
	1.75	1467	2196d	1.497b	0.00
	3.50	1444	1985a	1.376a	2.78
Tiger	0.00	1384	2076bc	1.501b	5.57
	1.75	1366	2059ab	1.510b	5.57
	3.50	1417	2095bc	1.479b	0.00
HFQ	0.00	1385	2062ab	1.489b	0.00
	1.75	1412	2101bc	1.487b	0.00
	3.50	1387	2054ab	1.481b	0.00
SEM		23.951	29.743	0.0129	1.901
Sorghum					
HP		1449b	2113	1.459	0.93
Tiger		1389a	2077	1.497	3.71
HFQ		1395a	2072	1.486	0.00
SMBS					
0.00		1401	2099	1.498	1.86
1.75		1415	2118	1.498	1.86
3.50		1416	2044	1.445	0.93
Significance (P=)					
Sorghum		0.007	0.189	0.001	0.055
SMBS		0.704	0.011	<0.001	0.789
Interaction		0.484	0.002	<0.001	0.174

ab Means within columns not sharing a common suffix are significantly different ($P < 0.05$).

Table 7
Effects of sodium metabisulphite (SMBS) inclusions on energy utilisation of broilers offered sorghum-based diets from 7 to 28 days post-hatch.

		Experiment 1						Experiment 2			
Treatment		AME (MJ/kg DM)	ME:GE ratio	N retention (%)	AMEn (MJ/kg DM)	Treatment		AME (MJ/kg DM)	ME:GE ratio	N retention (%)	AMEn (MJ/kg DM)
Sorghum	SMBS (g/kg)					Sorghum	SMBS (g/kg)				
MP	0.00	12.03	0.728	61.03	11.34	HP	0.00	11.50	0.690	59.86	11.05
	1.75	12.43	0.747	58.67	11.79		1.75	11.72	0.700	59.16	11.27
	3.50	12.28	0.740	56.85	11.72		3.50	11.84	0.707	58.62	11.40
JM	0.00	12.04	0.733	58.48	11.44	Tiger	0.00	12.17	0.720	62.06	11.75
	1.75	12.45	0.757	55.85	11.91		1.75	12.26	0.729	61.57	11.81
	3.50	12.35	0.750	50.16	11.92		3.50	12.46	0.734	59.99	12.04
						HFQ	0.00	12.12	0.710	64.50	11.56
							1.75	12.18	0.713	63.03	11.73
							3.50	12.62	0.734	64.38	12.15
SEM		0.1378	0.0083	1.5053	0.1252						
Sorghum						SEM		0.1597	0.0129	1.0935	0.1449
MP		12.25	0.738	58.85a	11.62	Sorghum					
JM		12.28	0.747	54.83b	11.76	HP		11.69a	0.699a	59.21a	11.24a
SMBS						Tiger		12.30b	0.727b	61.21b	11.86b
0.00		12.04a	0.731a	59.75b	11.39a	HFQ		12.31b	0.719b	63.97c	11.84b
1.75		12.44b	0.752b	57.26b	11.85b	SMBS					
3.50		12.32ab	0.745ab	53.51a	11.82b	0.00		11.93a	0.707	62.14	11.48a
Significance (P =)						1.75		12.05ab	0.714	61.26	11.60a
Sorghum		0.755	0.226	0.003	0.183	3.50		12.31b	0.725	61.00	11.86b
SMBS		0.020	0.04993	<0.001	<0.001	Significance (P=)					
Interaction		0.976	0.941	0.319	0.920	Sorghum		<0.001	0.002	<0.001	<0.001
						SMBS		0.019	0.068	0.414	0.008
						Interaction		0.864	0.925	0.750	0.865

ab Means within columns not sharing a common suffix are significantly different ($P < 0.05$).

In Experiment 2, HP sorghum supported significantly higher weight gains by an average of 4.09% (1449 versus 1392 g/bird) in comparison to broilers offered Tiger and HFQ sorghum-based diets. There were significant treatment interactions for feed intake ($P < 0.005$) as feed intakes of Tiger and HFQ sorghum-based diets did not differ with sodium metabisulphite inclusions; whereas 3.50 g/kg sodium metabisulphite significantly reduced feed intakes of birds offered sorghum HP-based diets by 8.06% (1985 versus 2159 g/bird) relative to the control diet. There was a significant treatment interaction ($P < 0.001$) for FCR where feed conversion efficiencies of birds offered Tiger and HFQ sorghum-based diets were not altered following sodium metabisulphite inclusions. Again, in contrast, 3.50 g/kg sodium metabisulphite significantly improved FCR of birds offered sorghum HP-based diets by 8.57% (1.367 versus 1.505; $P < 0.05$) relative to the control diet.

The effects of dietary treatments on parameters of nutrient utilisation of poultry are shown in Table 7 where there were no significant treatment interactions in both experiments. In Experiment 1, there were not any statistical differences between the two sorghum varieties for AME, ME:GE ratios and AMEn. Sodium metabisulphite significantly influenced AME ($P < 0.025$), ME:GE ratios ($P < 0.05$), N retention ($P < 0.001$) and AMEn ($P < 0.001$). More pronounced responses were observed with 1.75 g/kg sodium metabisulphite significantly increased AME by 0.40 MJ (12.44 versus 12.04 MJ/kg), ME:GE ratios by 2.87% (0.752 versus 0.731) and AMEn by 0.46 MJ (11.85 versus 11.39 MJ/kg). There were only numerical improvements in AME and ME:GE ratios at the higher 3.50 g/kg inclusion level but the 0.43 MJ increase in AMEn was significant. MP sorghum-based diets supported superior N retention by 4.02 percentage units or 7.33% (58.85 versus 54.83%; $P < 0.005$) in comparison to JM sorghum. Sodium metabisulphite significantly ($P < 0.001$) depressed N retention by 6.24 percentage units (53.51 versus 59.75%) at 3.50 g/kg.

In Experiment 2, the impact of sorghum variety on nutrient utilisation parameters was highly significant ($P < 0.005$). HP sorghum had inferior nutrient utilisation parameters in comparison to Tiger and HFQ sorghums by an average of 0.62 MJ (11.69 versus 12.31 MJ) in AME, by 3.32% (0.699 versus 0.723) in ME:GE ratios, by 3.38 percentage units (59.21 versus 62.59%) in N retention and by 0.61 MJ (11.24 versus 11.85 MJ/kg) in AMEn. HFQ sorghum-based diets (63.97%) supported significantly better N retentions than Tiger (61.21%) and HP (59.21%). Graded sodium metabisulphite inclusions linearly enhanced AME ($r = 0.314$; $P < 0.025$); ME:GE ratios ($r = 0.291$; $P < 0.04$) and AMEn ($r = 0.333$; $P < 0.02$). At 3.50 g/kg, sodium metabisulphite significantly increased AME by 1.38 MJ (13.31 versus 11.93 MJ/kg) and AMEn by 0.38 MJ (11.86 versus 11.48 MJ/kg).

The effects of dietary treatments on protein (N) digestibility coefficients in four small intestinal segments are shown in Table 8 where there were significant treatment interactions ($P < 0.005$) at all four sites in Experiment 1. In MP sorghum-based diets, graded sodium metabisulphite inclusions did not influence protein digestibility coefficients in the distal jejunum and proximal ileum. However, 1.75 g/kg sodium metabisulphite significantly depressed protein digestibility coefficients by 23.5% (0.349 versus 0.456) in the proximal jejunum (0.349 versus 0.456) and 3.50 g/kg sodium metabisulphite significantly depressed protein digestibility coefficients by 6.12% (0.690 versus 0.735) in the distal ileum. Conversely, sodium metabisulphite had positive impacts in JM sorghum-based diets where 1.75 g/kg sodium metabisulphite significantly enhanced protein

Table 8
Effects of sodium metabisulphite (SMBS) inclusions on apparent protein (N) digestibility coefficients in four small intestinal segments of broilers offered sorghum-based diets at 28 days post-hatch.

Treatment		Experiment 1				Treatment		Experiment 2			
		Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum			Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Sorghum	SMBS (g/kg)					Sorghum	SMBS (g/kg)				
MP	0.00	0.456cd	0.479b	0.659b	0.735cd	HP	0.00	0.286	0.480	0.654	0.711
	1.75	0.349ab	0.494b	0.642b	0.715bc		1.75	0.209	0.430	0.596	0.679
	3.50	0.404bc	0.547b	0.664b	0.690b		3.50	0.326	0.486	0.626	0.677
JM	0.00	0.262a	0.352a	0.563a	0.646a	Tiger	0.00	0.193	0.482	0.629	0.665
	1.75	0.346ab	0.493b	0.652b	0.692b		1.75	0.202	0.425	0.612	0.640
	3.50	0.549d	0.635c	0.741c	0.771d		3.50	0.240	0.480	0.630	0.685
						HFQ	0.00	0.336	0.555	0.682	0.710
							1.75	0.284	0.457	0.637	0.688
							3.50	0.324	0.508	0.656	0.691
SEM		0.0365	0.0289	0.0183	0.0129	SEM		0.0447	0.0289	0.0130	0.0190
Sorghum						Sorghum					
MP		0.403	0.507	0.655	0.713	HP		0.274ab	0.465	0.625a	0.689
JM		0.386	0.494	0.652	0.703	Tiger		0.212a	0.462	0.624a	0.663
SMBS						HFQ		0.315b	0.502	0.658b	0.696
0.00		0.359	0.416	0.611	0.690	SMBS					
1.75		0.348	0.494	0.647	0.703	0.00		0.272	0.506b	0.655b	0.695
3.50		0.476	0.591	0.702	0.730	1.75		0.232	0.437a	0.615a	0.669
Significance (P=)						3.50		0.296	0.491b	0.637b	0.684
Sorghum		0.573	0.565	0.844	0.251	Significance (P=)					
SMBS		0.002	<0.001	<0.001	0.003	Sorghum		0.021	0.106	0.003	0.094
Interaction		<0.001	0.002	<0.001	<0.001	SMBS		0.201	0.011	0.002	0.250
						S × SMBS interaction		0.786	0.866	0.596	0.613

ab Means within columns not sharing a common suffix are significantly different ($P < 0.05$).

digestibility coefficients in the distal jejunum, proximal ileum and distal ileum by 10.1%, 15.8% and 7.12% respectively. Again in JM sorghum-based diets, 3.50 g/kg sodium metabisulphite significantly enhanced protein digestibility by 110% in the proximal jejunum (0.549 versus 0.262), 80.4% in the distal jejunum (0.635 versus 0.352), by 31.6% in the proximal ileum (0.741 versus 0.563) and by 19.3% in the distal ileum (0.771 versus 0.646).

There were not any significant treatment interactions in Experiment 2. HFQ sorghum-based diets supported 48.6% higher (0.315 versus 0.212) digestibility coefficients than Tiger sorghum-based diets in the proximal jejunum. In the proximal ileum, HFQ supported significantly higher protein digestibility coefficients by an average of 10.5% (0.658 versus 0.6245) relative to diets based on HP and Tiger sorghums. Sodium metabisulphite significantly depressed distal jejunal protein digestibility coefficients by 13.6% (0.437 versus 0.506) at 1.75 g/kg; however, at the higher inclusion of 3.50 g/kg protein digestibility was comparable to the control. The 1.75 g/kg inclusion of sodium metabisulphite significantly depressed proximal ileal protein digestibility coefficients by 6.11% (0.615 versus 0.655) at 1.75 g/kg; but again at the higher inclusion level protein digestibility did not differ from the control. Significant differences between treatments were not observed in the distal ileum.

The effects of dietary treatments on protein disappearance rates in four small intestinal segments are shown in Table 9. In Experiment 1, a significant treatment interaction was not observed in the proximal ileum which was not the case in the other three sites. In the proximal ileum protein disappearance rates from MP sorghum-based diets exceeded those of JM sorghum by 12.2% (14.33 versus 12.77 g/bird/day; $P = 0.001$). Sodium metabisulphite significantly increased proximal ileal protein disappearance rates by 8.79% (13.49 versus 12.40 g/bird/day) at 1.75 g/kg and by 19.1% (14.77 versus 12.40 g/bird/day) at the 3.5 g/kg inclusion with a significant linear effect ($r = 0.555$; $P < 0.001$). However, significant treatment interactions were observed in the three remaining small intestinal segments. Generally, sodium metabisulphite responses in MP sorghum-based diets were modest with nearly identical protein disappearance rates in the distal ileum; whereas, in contrast, sodium metabisulphite responses in JM sorghum-based diets were robust. For example, 3.50 g/kg sodium metabisulphite generated increases in protein disappearance rates of 111% (10.73 versus 5.09 g/bird/day) in the proximal jejunum, 80.1% in the distal jejunum (12.41 versus 6.89 g/bird/day) and 20.1% in the distal ileum (15.06 versus 12.54 g/bird/day).

There were not any significant treatment interactions in Experiment 2. Tiger sorghum-based diets supported the slowest protein disappearance rates in the proximal jejunum by an average of 31.1% (4.17 versus 6.05 g/bird/day) and in the proximal ileum by 6.72% (12.43 versus 13.325 g/bird/day). In the distal ileum, HP sorghum supported the most rapid protein disappearance rates by an average of 9.16% (14.77 versus 13.53 g/bird/day). Sodium metabisulphite retarded protein disappearance rates in the three caudal segments of the small intestine. This effect was most pronounced in the proximal ileum ($P < 0.001$) where sodium metabisulphite retarded disappearance rates by an average of 6.81% (12.55 versus 13.58 g/bird/day). HFQ generated significantly faster protein disappearance than HP by 7.19% (6.26 versus 5.84 g/bird/day) and by 50.1% (6.26 versus 4.17 g/bird/day) relative to Tiger in the proximal jejunum. In the distal jejunum, sodium metabisulphite significantly retarded protein disappearance by 13.4% (9.08 versus 10.48 g/bird/day) at 1.75 g/kg and by 7.54% (9.69

Table 9

Effects of sodium metabisulphite (SMBS) inclusions on accumulative apparent protein (N) disappearance rates (g/bird/day) in four small intestinal segments of broilers offered sorghum-based diets at 28 days post-hatch.

		Experiment 1						Experiment 2			
Treatment		Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Treatment		Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Sorghum	SMBS (g/kg)					Sorghum	SMBS (g/kg)				
MP	0.00	9.59bc	10.05b	13.84	15.41c	HP	0.00	6.22	10.42	14.17	15.41
	1.75	7.63b	10.81bc	14.08	15.66c		1.75	4.67	9.58	13.28	15.15
	3.50	9.14bc	12.38c	15.06	15.65c		3.50	6.64	9.88	12.71	13.74
JM	0.00	5.09a	6.89a	10.95	12.54a	Tiger	0.00	3.89	9.87	12.89	13.62
	1.75	6.81a	9.74b	12.89	13.66b		1.75	4.00	8.50	12.25	12.83
	3.50	10.73c	12.41c	14.48	15.06c		3.50	4.61	9.26	12.13	13.20
						HFQ	0.00	6.73	11.14	13.67	14.24
							1.75	5.72	9.14	12.76	13.77
							3.50	6.33	9.94	12.82	13.49
					SEM		0.8967	0.6051	0.3240	0.4553	
SEM		0.7546	0.5950	0.5022	0.3547	Sorghum					
Sorghum						HP		5.84b	9.96	13.39b	14.77b
MP		8.79	11.08	14.33b	15.57	Tiger		4.17a	9.21	12.43a	13.22a
JM		7.54	9.68	12.77a	13.75	HFQ		6.26b	10.07	13.08b	13.84a
SMBS						SMBS					
0.00		7.34	8.47	12.40a	13.98	0.00		5.61	10.48b	13.58b	14.42b
1.75		7.22	10.28	13.49b	14.66	1.75		4.80	9.08a	12.76a	13.92ab
3.50		9.94	12.40	14.77c	15.35	3.50		5.86	9.69a	12.55a	13.48a
Significance (P=)						Significance (P =)					
Sorghum		0.053	0.007	0.001	<0.001	Sorghum		0.016	0.178	0.002	<0.001
SMBS		0.001	<0.001	<0.001	0.002	SMBS		0.326	0.025	<0.001	0.049
Interaction		0.001	0.036	0.075	0.011	Interaction		0.846	0.910	0.783	0.397

ab Means within columns not sharing a common suffix are significantly different ($P < 0.05$).

versus 10.48 g/bird/day) at 3.5 g/kg. Protein disappearance from Tiger-based diets in the proximal ileum was significantly slower than HP by 7.17% (12.43 versus 13.39 g/bird/day) and HFQ by 4.97% (12.43 versus 13.08 g/bird/day). Sodium metabisulphite significantly retarded proximal ileal protein disappearance by 6.04% (12.76 versus 13.58 g/bird/day) at 1.75 g/kg and by 7.58% (12.55 versus 13.58 g/bird/day) at 3.5 g/kg. Distal ileal protein disappearances from Tiger- and HFQ-based diets were significantly retarded in comparison to HP by 10.5% (13.22 versus 14.77 g/bird/day) and HFQ by 6.30% (13.84 versus 14.77 g/bird/day). At 3.50 g/kg, sodium metabisulphite significantly retarded protein disappearance by 6.52% (13.48 versus 14.42 g/bird/day) and 1.75 g/kg sodium metabisulphite was statistically similar to both 0 and 3.5 g/kg.

The effects of dietary treatments on starch digestibility coefficients in four small intestinal segments are shown in Table 10. In Experiment 1 there were significant treatment interactions in three sites but this was not the case in the distal jejunum ($P > 0.35$). In this segment sorghum MP supported superior starch digestibility by 10.0% (0.824 versus 0.749; $P < 0.001$). Sodium metabisulphite significantly ($P < 0.001$) enhanced starch digestibility by 9.53% (0.793 versus 0.724) at 1.75 g/kg and by 16.4% (0.843 versus 0.724) at the 3.50 g/kg inclusion level. This pattern of responses was observed in the remaining three intestinal segments. Essentially, JM sorghum was superior and sodium metabisulphite enhanced starch digestibility but these responses were subject to significant interactions. This was because starch digestibilities were more responsive to sodium metabisulphite in birds offered JM sorghum in comparison to MP sorghum-based diets. For example, 3.50 g/kg sodium metabisulphite enhanced proximal jejunal starch digestibility by 28.4% (0.863 versus 0.672), proximal ileal starch digestibility by 13.2% (0.927 versus 0.819), and distal ileal starch digestibility by 9.60% (0.948 versus 0.865).

In Experiment 2, there were not any significant interactions; however, sorghum variety significantly influenced starch digestibility coefficients in the distal jejunum ($P < 0.001$) and proximal ileum ($P < 0.02$). Tiger sorghum starch digestibility (0.774) was superior to both HP sorghum (0.732) and HFQ sorghum (0.687) in the distal jejunum. In the proximal ileum, starch digestibility of sorghum HP diets (0.790) was statistically inferior to Tiger (0.819) and HFQ (0.813) sorghum-based diets. Sodium metabisulphite did not statistically influence starch digestibility coefficients.

The effects of dietary treatments on starch disappearance rates in four small intestinal segments are shown in Table 11. There was a treatment interaction ($P < 0.015$) in the proximal jejunum because sodium metabisulphite accelerated starch disappearance rates in sorghum JM to a greater extent than in diets based on sorghum MP. In the three caudal small intestinal segments sodium metabisulphite significantly ($P < 0.001$) accelerated starch disappearance rates. In the distal ileum, sorghum MP supported faster starch disappearance rates by 3.84% (42.69 versus 41.11 g/bird/day; $P < 0.03$) than sorghum JM. Sodium metabisulphite significantly ($P < 0.001$) accelerated starch disappearance rates in the distal ileum by 17.8% (44.70 versus 37.96 g/bird/day) at 1.75 g/kg and by 21.3% (46.05 versus 37.96 g/bird/day) at the 3.50 g/kg inclusion level.

In Experiment 2, there were significant differences ($P < 0.025$) in proximal jejunal starch disappearance rates between sorghum varieties with Tiger sorghum supporting a 21.9% more rapid rate (24.97 versus 20.49 g/bird/day) than in birds offered HFQ sorghum-based diets. Similarly, in the distal jejunum, the rate for Tiger sorghum was 15.1% more rapid than

Table 10

Effects of sodium metabisulphite (SMBS) inclusions on apparent starch digestibility coefficients in four small intestinal segments of broilers offered sorghum-based diets at 28 days post-hatch.

Treatment		Experiment 1				Treatment		Experiment 2			
		Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum			Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Sorghum	SMBS (g/kg)					Sorghum	SMBS (g/kg)				
MP	0.00	0.668a	0.704	0.833ab	0.880ab	HP	0.00	0.598	0.767	0.811	0.799
	1.75	0.723ab	0.750	0.847abc	0.894ab		1.75	0.512	0.683	0.767	0.771
	3.50	0.708a	0.792	0.869bc	0.890ab		3.50	0.607	0.745	0.791	0.797
JM	0.00	0.672a	0.744	0.819a	0.865a	Tiger	0.00	0.653	0.780	0.807	0.802
	1.75	0.788b	0.835	0.879c	0.906b		1.75	0.611	0.764	0.821	0.821
	3.50	0.863c	0.894	0.927d	0.948c		3.50	0.586	0.778	0.829	0.847
						HFQ	0.00	0.476	0.710	0.820	0.793
							1.75	0.553	0.665	0.805	0.805
							3.50	0.516	0.686	0.813	0.808
						SEM		0.0500	0.0258	0.0129	0.0183
SEM		0.0258	0.0224	0.0133	0.0092	Sorghum					
Sorghum						HP		0.572	0.732b	0.790a	0.789
MP		0.700	0.749a	0.850	0.888	Tiger		0.617	0.774c	0.819b	0.823
JM		0.774	0.824b	0.875	0.906	HFQ		0.515	0.687a	0.813b	0.802
SMBS						SMBS					
0.00		0.670	0.724a	0.826	0.872	0.00		0.576	0.752	0.813	0.798
1.75		0.756	0.793b	0.863	0.900	1.75		0.559	0.704	0.798	0.799
3.50		0.785	0.843c	0.898	0.919	3.50		0.570	0.737	0.811	0.817
Significance (P=)						Significance (P =)					
Sorghum		0.001	<0.001	0.025	0.023	Sorghum		0.053	<0.001	0.016	0.088
SMBS		<0.001	<0.001	<0.001	<0.001	SMBS		0.917	0.055	0.300	0.385
Interaction		0.015	0.394	0.033	0.001	Interaction		0.396	0.698	0.207	0.641

ab Means within columns not sharing a common suffix are significantly different ($P < 0.05$).

Table 11

Effects of sodium metabisulphite (SMBS) inclusions on accumulative apparent starch disappearance rates (g/bird/day) in four small intestinal segments of broilers offered sorghum-based diets at 28 days post-hatch.

Treatment		Experiment 1				Treatment		Experiment 2			
		Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum			Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Sorghum	SMBS (g/kg)					Sorghum	SMBS (g/kg)				
MP	0.00	29.92a	31.49	37.30	39.39	HP	0.00	25.71	32.98	34.86d	34.38de
	1.75	34.85b	36.02	40.76	42.99		1.75	21.19	28.27	31.78b	31.98abcd
	3.50	36.33b	40.63	44.58	45.68		3.50	23.00	28.20	29.95a	30.18a
JM	0.00	28.31a	31.46	34.60	36.52	Tiger	0.00	26.39	31.61	32.70bc	32.47abcde
	1.75	35.10b	37.22	39.19	40.40		1.75	24.66	30.88	33.26bcd	33.29cde
	3.50	42.23c	43.76	45.39	46.10		3.50	23.86	31.74	33.83cd	34.54e
						HFQ	0.00	18.38	27.50	31.80b	30.73ab
							1.75	22.77	27.30	33.05bc	33.03cd
							3.50	20.32	27.06	32.09b	31.87abc
						SEM		1.9459	0.9496	0.5969	0.8773
SEM		1.2040	1.747	0.9672	0.2668	Sorghum					
Sorghum						HP		23.30ab	29.82b	32.20	32.18
MP		33.70	36.05	40.88	42.69b	Tiger		24.97b	31.41c	33.26	33.44
JM		35.21	37.48	39.73	41.11a	HFQ		20.49a	27.29a	32.31	31.88
SMBS						SMBS					
0.00		29.11	31.48a	35.95a	37.96a	0.00		23.49	30.70b	33.12	32.53
1.75		34.98	63.62b	39.98b	44.70b	1.75		22.87	28.82a	32.70	32.77
3.50		39.28	42.20c	44.99c	46.05c	3.50		22.40	29.00a	31.96	32.20
Significance (P=)						Significance (P=)					
Sorghum		0.134	0.146	0.154	0.029	Sorghum		0.024	<0.001	0.067	0.081
SMBS		<0.001	<0.001	<0.001	<0.001	SMBS		0.788	0.036	0.064	0.729
Interaction		0.011	0.409	0.197	0.076	Interaction		0.242	0.055	<0.001	0.005

ab Means within columns not sharing a common suffix are significantly different ($P < 0.05$).

Table 12
Effects of sodium metabisulphite (SMBS) inclusions on starch:protein disappearance rate ratios in four small intestinal segments of broilers offered sorghum-based diets at 28 days post-hatch.

Treatment		Experiment 1				Treatment		Experiment 2			
		Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum			Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Sorghum	SMBS (g/kg)					Sorghum	SMBS (g/kg)				
MP	0.00	3.20a	3.15a	2.70a	2.59a	HP	0.00	4.32	3.23	2.47abcd	2.23ab
	1.75	4.46ab	3.35a	2.89ab	2.75b		1.75	4.86	2.96	2.39abc	2.12a
	3.50	4.12ab	3.29a	2.97bc	2.92c		3.50	3.61	2.91	2.36ab	2.20a
JM	0.00	6.85c	5.08b	3.20d	2.92c	Tiger	0.00	1.49	3.24	2.54cd	2.39bc
	1.75	5.34bc	3.84a	3.04bcd	2.96c		1.75	2.62	3.73	2.72e	2.60d
	3.50	4.06ab	3.54a	3.14cd	3.08d		3.50	6.03	3.55	2.80e	2.62d
						HFQ	0.00	2.82	2.53	2.33a	2.16a
							1.75	4.20	3.06	2.61d	2.41c
							3.50	3.33	2.78	2.51bcd	2.37bc
SEM		0.6485	0.3017	0.0730	0.0342	SEM		1.9154	0.2419	0.0619	0.0577
Sorghum						Sorghum					
MP		3.99	3.26	2.86	2.74	HP		4.27	3.03a	2.41	2.18
JM		5.42	4.15	3.13	2.99	Tiger		3.38	3.51b	2.69	2.54
SMBS						HFQ		3.45	2.79a	2.48	2.32
0.00		5.03	4.12	2.95	2.74	SMBS					
1.75		5.00	3.59	2.97	2.85	0.00		2.88	3.00	2.44	2.26
3.50		4.09	3.41	3.06	3.00	1.75		3.89	3.25	2.58	2.38
Significance (P=)						3.50		4.32	3.08	2.55	2.40
Sorghum		0.011	0.001	0.037	<0.001	Significance (P=)					
SMBS		0.278	0.068	0.120	<0.001	Sorghum		0.821	0.003	<0.001	<0.001
Interaction		0.018	0.019	0.032	0.021	SMBS		0.639	0.456	0.027	0.013
						Interaction		0.627	0.430	0.016	0.021

ab Means within columns not sharing a common suffix are significantly different ($P < 0.05$).

HFQ sorghum but sodium metabisulphite significantly ($P < 0.05$) depressed starch disappearance rates by up to 6.12% (28.82 versus 30.70 g/bird/day) at 1.75 g/kg.

There was a significant treatment interaction ($P < 0.001$) in the proximal ileum because sodium metabisulphite depressed starch disappearance rates in birds offered sorghum HP-based diets but did not statistically influence diets based on Tiger and HFQ sorghums. There was also a significant treatment interaction ($P < 0.01$) in the distal ileum, again because sodium metabisulphite decreased starch disappearance rates in birds offered sorghum HP-based diets. Sodium metabisulphite did not influence starch disappearance with Tiger sorghum-based diets but, curiously, 1.75 g/kg sodium metabisulphite significantly increased starch disappearance rates by 7.48% (33.03 versus 30.73 g/bird/day) with HFQ sorghum-based diets.

The effects of dietary treatments on starch:protein disappearance rate ratios in four small intestinal segments are shown in Table 12 and, in Experiment 1, there were significant treatment interactions ($P < 0.035$) in all four sites. Sodium metabisulphite significantly increased starch:protein disappearance rate ratios in birds offered sorghum MP-based diets in the proximal and distal ileum by up to 10.0% and 12.7%, respectively. With sorghum JM-based diets, sodium metabisulphite narrowed starch:protein disappearance rate ratios by up to 40.7% in the proximal jejunum and by 30.3% in the distal jejunum. In contrast, however, sodium metabisulphite widened starch:protein disappearance rate ratios by up to 5.48%.

In experiment 2, there were no significant treatment effects in the proximal jejunum. In the distal jejunum, diets based on sorghum Tiger had the widest disappearance rate ratio by an average of 20.6% (3.51 versus 2.91). There were significant treatment interactions ($P < 0.025$) in both ileal segments. Sodium metabisulphite did not statistically influence starch:protein disappearance rate ratios of HP sorghum-based diets in the proximal ileum. However, 3.50 g/kg sodium metabisulphite increased the ratio by 10.2% (2.80 versus 2.54) from sorghum Tiger-based diets and 1.75 g/kg sodium metabisulphite increased the starch:protein disappearance rate ratio by 12.0% (2.61 versus 2.33) from sorghum HFQ-based diets. The pattern of results was similar in the distal ileum where 3.50 g/kg sodium metabisulphite increased the ratio by 9.62% (2.62 versus 2.39) with sorghum Tigers and 1.75 g/kg sodium metabisulphite increased the starch:protein disappearance rate ratio by 9.72% (2.37 versus 2.16) with HFQ sorghum.

4. Discussion

In the present study, sodium metabisulphite significantly enhanced AME by an average of 0.40 MJ at 1.75 g/kg in Experiment 1 and by 0.38 MJ at 3.50 g/kg in Experiment 2, which is in contrast to the numerical reduction 0.42 MJ in AME reported by Truong et al. (2015a). The different pattern of responses to sodium metabisulphite in the two experiments is curious. Nevertheless, responses in energy utilisation (AME, ME:GE ratios, AMEn) were quite consistent across both experiments with maximum AME responses of 0.40 MJ (MP) and 0.41 MP (JM) in Experiment 1 and 0.34 MJ (HP), 0.29 MJ (Tiger) and

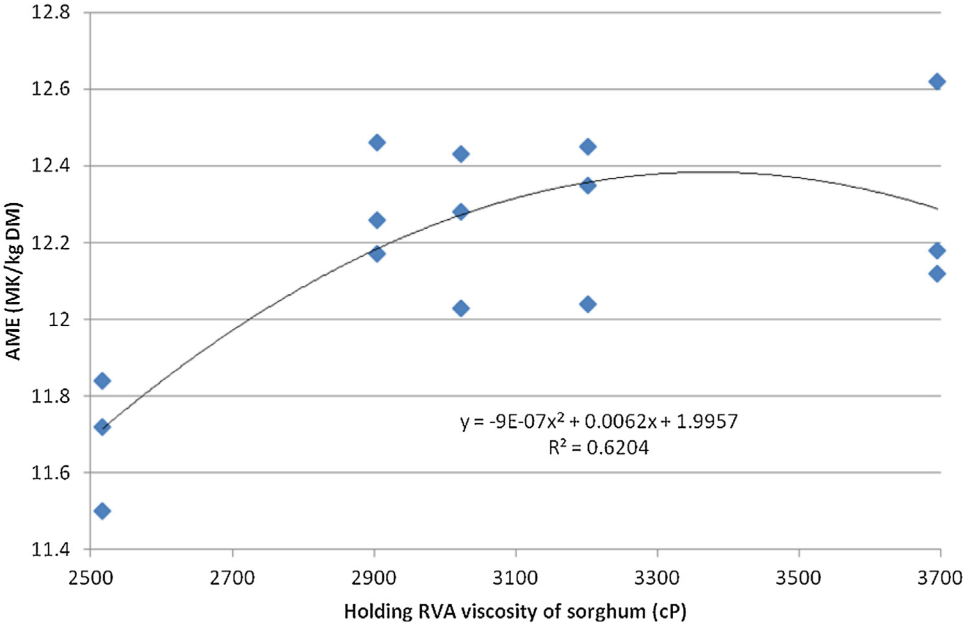


Fig. 1. Quadratic relationship between holding RVA viscosity of five sorghum varieties with AME (r = 0.788; P = 0.003) in broiler chickens offered sorghum-based diets without and with sodium metabisulphite in both Experiments. The quadratic equation predicts the maximal AME of 12.34 MJ/kg would be generated by a sorghum with an RVA holding viscosity of 3333 Cp.

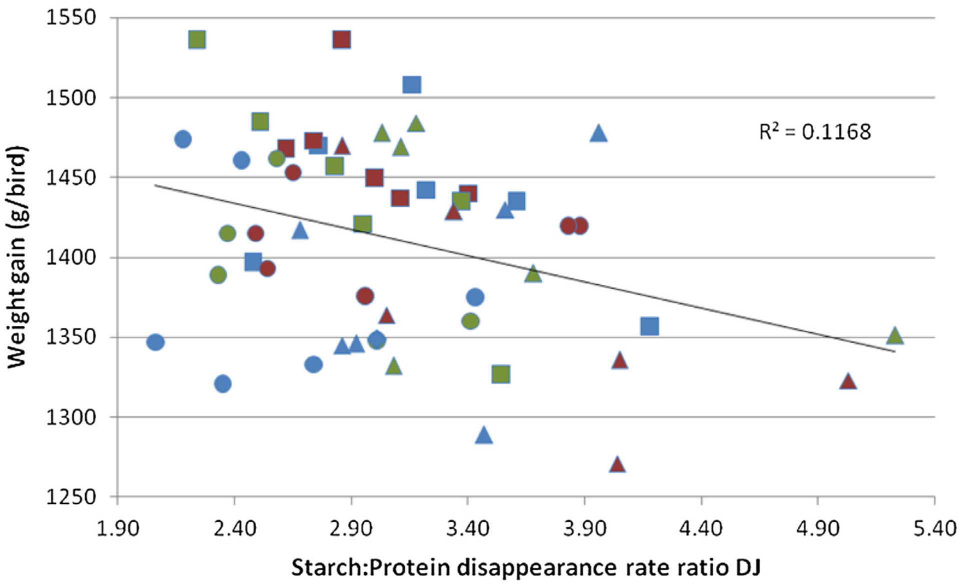
0.50 MJ (HFQ) in Experiment 2. Moreover, there were no suggestions of any treatment interactions for energy utilisation parameters in both experiments.

There is one possible reason that positive responses were observed in the present study as opposed to the Truong et al. (2015a) study. In both studies, the dietary treatments were formulated to an energy density of 12.95 MJ/kg. In the earlier study the AME of the control diet was 12.89 MJ/kg which effectively met the target. However, it is noticeably higher than the average AME of 11.97 MJ/kg in the five control diets in the present study. It follows that birds offered diets with low inherent energy densities would be more likely to respond to sodium metabisulphite. Also, this emphasises the shortfalls in energy utilisation that may occur in sorghum-based broiler diets and a major contributing factor is probably that the digestibility of sorghum starch is consistently inferior to that of maize in poultry (Truong et al., 2015b).

Unlike energy utilisation, several significant treatment interactions were observed for other parameters. These included feed intake, FCR (Experiment 2), protein (N) digestibility coefficients (Experiment 1), starch digestibility coefficients (Experiment 1) and some starch:protein disappearance rate ratios in both experiments. As discussed, sodium metabisulphite has the capacity to both reduce disulphide cross-linkages in proteins and to depolymerise starch, most of which was derived from sorghum. Moreover, this dual mode of action is complicated by the likelihood that kafirin is impeding starch digestion via the formation of protein structures involving disulphide cross-linkages in sorghum endosperm that impede starch gelatinisation and digestion (Hamaker and Bugusu, 2003). Therefore, it is perhaps not surprising that responses to sodium metabisulphite will be variable depending on the inherent properties of the grain sorghum in question and which mode of action of the reducing agent is the more dominant. For example, sodium metabisulphite significantly increased feed intakes in Experiment 1 but did not influence feed intakes in Experiment 2. Also, FCR of birds offered HP sorghum–based diets (Experiment 2) were markedly improved by sodium metabisulphite but this was not the case for the other sorghum varieties.

It is evident in Table 2 that the characteristics of the five sorghums are reasonably similar as phytate concentrations range from 6.31 to 8.94 g/kg and kafirin from 48.0 to 51.3 g/kg. In a previous study, a sorghum with a kafirin content of 61.5 g/kg was distinctly inferior as a feed grain for broilers in comparison to a sorghum variety with a lower kafirin content of 50.7 g/kg (Truong et al., 2015c). The amino acid profiles of kafirin from sorghums MP and HP (Table 3) are in close agreement with three data sets published by Xiao et al. (2015) where the paucity of lysine and the abundance of leucine are noteworthy. Predictably, the white sorghum HFQ contains less polyphenolic compounds than the four red sorghums but, conversely, substantially more phenolic acids (Table 4). HFQ contained 1008 μg/g total phenolic acids and 838 μg/g total ferulic acid as opposed to corresponding average concentrations of 437 and 332 μg/g in the four red sorghum varieties.

Probably the most notable variation across the five sorghum grains is their RVA starch pasting profiles where peak viscosity ranged from 2591 cP in sorghum HP to 5559 cP in sorghum JM (Table 3). Interestingly, HP sorghum–based diets supported the lowest AME of 11.69 MJ/kg which suggests there may be an association between low peak RVAs and low AMEs in sorghum-based broiler diets. There was no significant difference in AME or AMEn between the two experiments which permits consideration of the overall data. Holding viscosity was positively correlated with AME (r = 0.607; P = 0.016) and



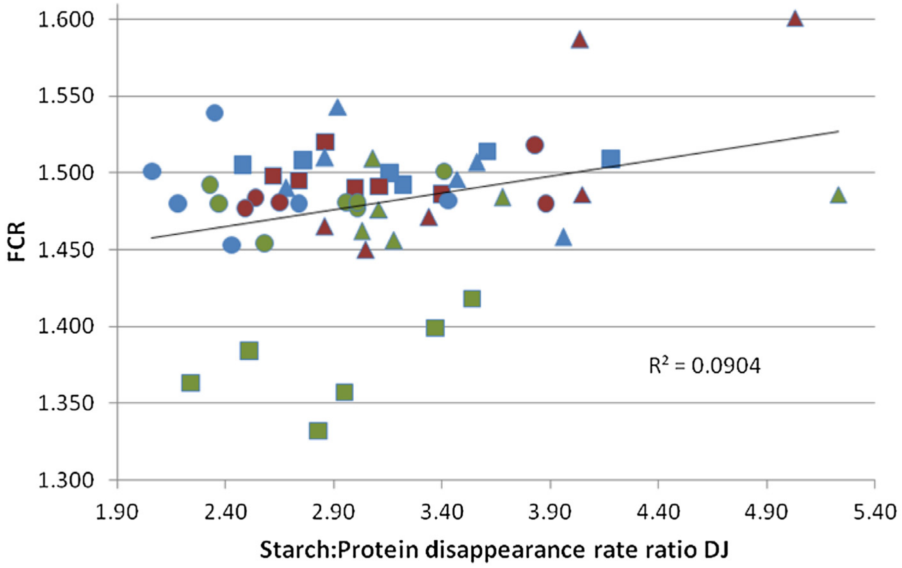


Fig. 2. Correlations between distal jejunal starch:protein disappearance rate ratios with weight gain (r = –0.341; P = 0.012) and FCR (r = 0.300; P = 0.028) from 7 to 28 days post-hatch (Experiment 2).

AMEn (r = 0.539; P = 0.038) on a linear basis. However, the quadratic relationship between holding viscosity and AME was more significant (r = 0.788; P = 0.003) and this relationship is shown in Fig. 1. The quadratic equation predicts that sorghum with an RVA holding viscosity of 3333 Cp would generate the maximal AME of 12.34 MJ/kg. Similarly, breakdown viscosity (the difference between peak and holding viscosities) was positively correlated with AME (r = 0.523; P = 0.046) and AMEn (r = 0.541; P = 0.037). These outcomes are consistent with data presented by Selle et al. (2016) and the proposition that sorghums with high RVA starch pasting profiles are associated with enhanced energy utilisation in broiler diets.

There is the contention that starch and protein digestive dynamics or the bilateral bioavailability of glucose and amino acids at sites of protein synthesis are pivotal to broiler performance (Liu and Selle, 2015). This is illustrated in Experiment 2, where distal jejunal starch:protein disappearance rate ratios were significantly related to both weight gain (r = –0.341; P = 0.012) and FCR (r = 0.300; P = 0.028) from 7 to 28 days post-hatch (Fig. 2). Also, as shown in Fig. 3, there was a similar relationship between distal ileal starch:protein disappearance rate ratios with weight gain (r = –0.368; P = 0.006) where the relationship with FCR closely approached significance (P = 0.052). However, static distal ileal starch and protein digestibility

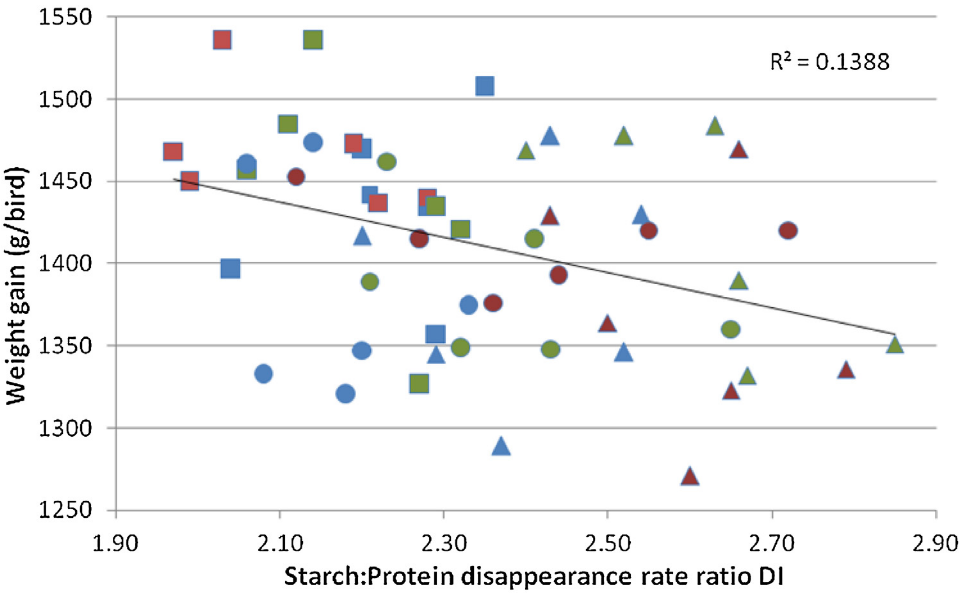


Fig. 3. Correlations between distal ileal starch:protein disappearance rate ratios with weight gain (r = –0.368; P = 0.006) from 7 to 28 days post-hatch (Experiment 2). In Figs. 2 and 3 the following legend applies: Treatment 1 = blue square; Treatment 2 = red square; Treatment 3 = green square; Treatment 4 = blue triangle; Treatment 5 = red triangle; Treatment 6 = green triangle; Treatment 7 = blue circle; Treatment 8 = red circle; Treatment 9 = green circle.

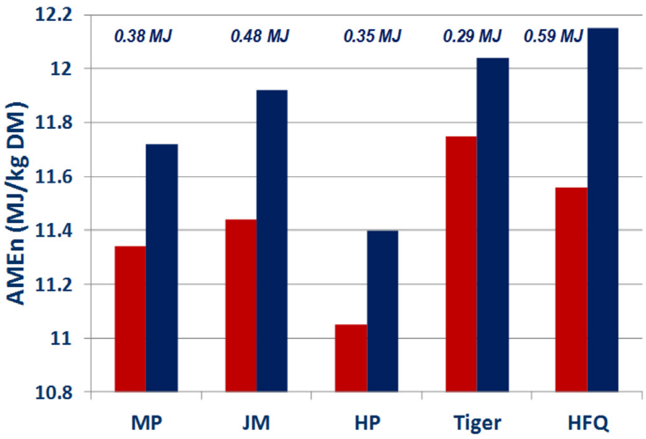


Fig. 4. The impact of 3.5 g/kg sodium metabisulphite (red bars = 0 g/kg; blue bars = 3.50 g/kg) on N-corrected AME in Experiments 1 and 2, where absolute responses (MJ) are shown in italics.

coefficients were not correlated with weight gain to significant extents. This contrast emphasises the merits of kinetic versus static digestibility parameters in assessing broiler performance and supports the concept that digestion/absorption of starch/glucose is quite often too rapid in relation to that of protein/amino acids. Thus tapering starch:protein disappearance rate ratios (or basically more protein absorption relative to starch) were associated with better broiler growth performance.

Remarkably, 3.50 g/kg sodium metabisulphite significantly increased starch digestibility coefficients of JM diets along the four small intestinal segments by 28.4, 20.2, 13.2 and 9.60% but responses of similar magnitudes were not observed in the other four sorghums. Therefore, it is noteworthy that peak RVA viscosity of JM sorghum was 44% greater than the average of the other four sorghums (5559 versus 3859 cP) Also, 3.50 g/kg sodium metabisulphite significantly increased protein (N) digestibility coefficients of JM sorghum-based diets by 110, 80, 31.6 and 19.3% along the four small intestinal segments but, again, the other four sorghums did not respond in a similar manner. Curiously, these marked improvements in protein (N) and starch digestibility coefficients of JM sorghum-based diets did not translate into growth performance responses improvements. However, sodium metabisulphite compromised N retention of JM diets by 8.32 percentage units at 3.50 g/kg, which implies that the pronounced increases in protein (N) digestibility did not translate into comparable increases in protein deposition.

It is noteworthy that Zhang and Hamaker (1998) reported that wet-cooking four sorghum varieties in the presence of sodium metabisulphite significantly increased *in vitro* starch digestibility which was not the case with maize. Further, Hamaker et al. (1987) reported that wet-cooking sorghum depressed *in vitro* pepsin digestibility (24.5%) to a greater extent than barley maize rice and wheat (average of 7.9%). Moreover, a sulphite reducing agent (2-mercaptoethanol) increased pepsin digestibility of sorghum by 25.1% as opposed to an average increase of only 1.9% in the other four cereals. Collectively, these outcomes suggest that the impact of sulphite reducing agents may be ‘sorghum-specific’ and may not extend to other cereals. All these cereals contain high starch contents and disulphide bonds exist in their protein fractions so sorghum is by no means exclusive in both respects. It appears that the unique feature of sorghum is its discrete kafirin protein bodies with high cysteine/cystine levels in the peripheral β- and γ-fractions. Thus there is the real possibility that the prime mode of action of sodium metabisulphite is the reduction of disulphide cross-linkages in β- and γ-kafirin with the inference that the reducing agent is indeed ‘sorghum-specific’.

In conclusion sodium metabisulphite enhanced energy utilisation as, across both experiments, 3.50 g/kg sodium metabisulphite increased AME by 0.34 MJ from 11.97 to 12.31 MJ/kg and increased N-corrected AME by 0.42 MJ from 11.43 to 11.85 MJ/kg. The AMEn responses are depicted in Fig. 4. Given that the inclusion cost of the reducing agent is largely offset by its replacement of sodium bicarbonate to maintain dietary sodium levels, this ‘energy-sparing’ effect would appear to be economically viable. Thus the inclusion of sodium metabisulphite in sorghum-based broiler diets merits consideration although it is evident that responses to the reducing agent will vary across sorghum cultivars.

Conflict of interest statement

The authors declare that there is no conflict of interests.

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