POULTRY CRC LTD

FINAL REPORT

Project No: 2.9.3

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Sub-Project Title: Combined inclusion of a reducing agent and an exogenous protease in poultry diets

DATE OF COMPLETION: April 2016

ISBN 1 921010 89 4

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Published in 2017

Executive Summary

The effects of tandem inclusions of a sulphite reducing agent, sodium metabisulphite, and an exogenous protease, Ronozyme® ProAct, in wheat- and sorghum-based diets for broiler chickens were investigated. Male Ross 308 chicks were offered nutritionally-equivalent experimental diets from 7 to 28 days post-hatch in a 2x2x2 factorial array of dietary treatments, which comprised: (i) diets based on wheat or sorghum, (ii) without and with 2.75 g/kg sodium metabisulphite, (iii) without and with 1000/kg units of protease activity. The parameters assessed included growth performance, nutrient utilisation, protein (N) digestibility coefficients and disappearance rates (g/bird/day) in four small intestinal segments, starch digestibility coefficients and disappearance rates in two small intestinal segments and starch:protein disappearance rate ratios in the proximal jejunum and distal ileum.

The outstanding outcomes were significant grain type by sodium metabisulphite interactions (P = 0.015–0.005) observed for apparent metabolisable energy (AME), metabolisable energy:gross energy ratios (ME:GE), nitrogen (N) retention and N-corrected AME (AMEn). The inclusion of sodium metabisulphite in sorghum-based diets enhanced AME by 0.18 MJ (12.47 versus 12.29 MJ/kg), ME:GE ratios by 1.20% (0.761 versus 0.752), N retention by 1.89 percentage units (65.80 versus 63.91%) and AMEn by 0.09 MJ (11.17 versus 11.08 MJ/kg). In contrast, sodium metabisulphite inclusion in wheat-based diets depressed AME by 0.43 MJ (11.88 versus 12.31 MJ/kg), ME:GE ratios by 3.65% (0.713 versus 0.740), N retention by 2.65 percentage units (61.75 versus 64.40%) and AMEn by 0.40 MJ (10.53 versus 10.93 MJ/kg). A similar interaction was observed for feed conversion ratios (FCR) that approached statistical significance (P = 0.061) where sodium metabisulphite improved FCR of sorghum-based diets by 1.28% (1.541 versus 1.561) but depressed FCR of wheat-based diets by 3.68% (1.577 versus 1.521).

Clearly, therefore, the benefits of including sodium metabisulphite in sorghum-based diets do not extend to wheat-based broiler diets. This suggests, as discussed, that sodium metabisulphite-generated reductions of disulphide cross-linkages in β- and γ-fractions of kafirin located in the periphery of protein bodies are pivotal to sodium metabisulphite responses in sorghum-based diets. It does appear that the positive effects of sodium metabisulphite may be

"sorghum specific" and stem from biophysical and/or biochemical starch-protein interactions involving starch granules and kafirin protein bodies which are both embedded in the glutelin protein matrix of sorghum endosperm.

As a main effect, protease significantly increased feed intake by 2.91% (2408 versus 2340 g/bird; $P < 0.05$) and tended to increase protein (N) digestibility coefficients by 9.37% (0.537 versus 0.491; $P = 0.052$) in the proximal jejunum. Thus overall responses to an exogenous protease were subtle.

In a preliminary investigation, concentrations of free amino acids and glucose in the portal (anterior mesenteric vein) and systemic (brachial vein) blood-flows were determined in birds offered control wheat-based diets and the same diet supplemented with sodium metabisulphite and protease. The two feed additives in tandem significantly depressed plasma amino acid concentrations of the following amino acids: alanine, cystine, glutamine, glycine, histidine, isoleucine, methionine, proline, serine and valine. The two feed additives numerically disadvantaged FCR by 4.56% (1.582 versus 1.513) and there are some instructive correlations between free amino acid concentrations in portal blood and FCR of broiler chicks.

Finally, it is our intention to submit a paper based on this study to *Animal Nutrition*. The tentative title is "Inclusions of sodium metabisulphite and exogenous protease, individually and in combination, generate interactions between sodium metabisulphite and grain type in wheat- and sorghum-based broiler diets for parameters of nutrient utilisation" by Peter H Selle, Ha H Truong, Amy F Moss and Sonia Yun Liu.

Objectives

The primary objective of this project was to investigate the tandem inclusions of a sulphite reducing agent, sodium metabisulphite, and an exogenous protease, Ronozyme® ProAct, in diets for broiler chickens. The rationale was that because exogenous proteases do not have the capacity to reduce disulphide cross-linkages they could be advantaged by the simultaneous inclusion of a reducing agent. The secondary objective was to ascertain whether or not the benefits of including sodium metabisulphite in sorghum-based diets extend to wheat-based broiler diets.

Methodolgy

Wheat, sorghum and soybean meal were characterised and wheat- and sorghum-based diets were formulated to standard nutrient specifications as shown in Table 1. Sodium metabisulphite (2.75 g/kg) and/or an exogenous protease (500 g/kg) were included in the two basal diets that were steam-pelleted at a conditioning temperature of 80°C and crumbled. The trial design consisted of a 2x2x2 factorial array of dietary treatments; wheat or sorghum-based diets, without and with Na metabisulphite, without and with exogenous protease. The enzyme used was Ronozyme® ProAct (CT), a serine protease produced by a genetically modified strain of *Bacillus licheniformis*, and marketed by DSM. The addition rate provided 1000 units of protease activity per kg of feed.

Each of the eight dietary treatments was offered to six replicates (6 male Ross 308 chicks per caged replicate) from 7 to 28 days post-hatch in an environmentally-controlled housing facility. Growth performance and nutrient utilisation [AME (MJ/kg and MJ/day), ME:GE ratios, N retention, AMEn] were determined by standard procedures. Similarly, apparent digestibility coefficients of protein (N) in four small intestinal sites (proximal jejunum, distal jejunum, proximal ileum, distal ileum) and disappearance rates (g/bird/day) were determined. Apparent digestibility coefficients and disappearance rates of starch were determined in the proximal jejunum and distal ileum and starch:protein disappearance rate ratios in these two sites were calculated. Blood samples from two treatment groups were taken from the anterior mesenteric and brachial veins to determine concentrations of free amino acids and glucose, which is considered separately.

Experimental data was analysed using the IBM SPSS Statistics 20 program (IBM Corporation, Somers, NY, USA). The feeding study complied with specific guidelines approved by the Animal Ethics Committee of the University of Sydney.

Results

The effects of grain type and dietary inclusions of sodium metabisulphite and protease on growth performance are shown in Table 2. Overall, bird performance was highly satisfactory with a weight gain of 1532 g/bird, feed intake of 2370 g/bird, FCR of 1.549 from 7 to 28 days post-hatch with a low mortality/cull rate of 0.78%. The only significant treatment effect observed was that protease increased feed intake by 2.91% (2408 versus 2340 g/bird; $P < 0.05$). However, the interaction between grain type and sodium metabisulphite closely approached significance ($P = 0.061$) for FCR. This was because sodium metabisulphite improved FCR of sorghum-based diets by 1.28% (1.541 versus 1.561) but depressed FCR of wheat-based diets by 3.68% (1.577 versus 1.521).

The effects of dietary treatments on parameters of nutrient utilisation are shown in Table 3. As a main effect, sorghum-based diets supported better AME by 0.29 MJ (12.38 versus 12.09 MJ/kg; $P < 0.01$), ME:GE ratios by 3.99% (0.756 versus 0.727; $P < 0.001$), N retention by 1.77 percentage units (64.85 versus 63.08%; $P < 0.04$) and AMEn by 0.30 MJ (11.03 versus 10.73 MJ/kg; $P < 0.001$). However, there were significant grain type by sodium metabisulphite interactions ($P = 0.015 – 0.005$) that impacted on the above outcomes of all four parameters. The inclusion of sodium metabisulphite in sorghum-based diets enhanced AME by 0.18 MJ (12.47 versus 12.29 MJ/kg), ME:GE ratios by 1.20% (0.761 versus 0.752), N retention by 1.89 percentage units (65.80 versus 63.91%) and AMEn by 0.09 MJ (11.17 versus 11.08 MJ/kg). In total contrast, sodium metabisulphite inclusion in wheat-based diets depressed AME by 0.43 MJ (11.88 versus 12.31 MJ/kg), ME:GE ratios by 3.65% (0.713 versus 0.740), N retention by 2.65 percentage units (61.75 versus 64.40%) and AMEn by 0.40 MJ (10.53 versus 10.93 MJ/kg). Dietary treatments did not influence energy intake expressed as MJ/day.

The effects of dietary treatments on apparent protein (N) digestibility coefficients in four small intestinal segments at 28 days post-hatch are shown in Table 4. There were no significant effects recorded. Although, protease increased proximal jejunal protein (N) digestibility coefficients by 11.0% (0.537 versus 0.491; $P = 0.052$), which closely approached significance.

The effects of dietary treatments on protein (N) disappearance rates in four small intestinal segments are shown in Table 5. Wheat-based diets supported a higher distal ileal protein disappearance rate by 13.9% (27.0 versus 23.7 g/bird/day; $P < 0.001$). There was a significant interaction ($P < 0.025$) between grain type and Na metabisulphite in the distal jejunum. Sodium metabisulphite increased protein disappearance rates by 8.64% (23.9 versus 22.0 g/bird/day) in sorghum-based diets; but decreased protein disappearance rates by 7.08% (22.3 versus 24.0 g/bird/day) in wheat-based diets.

The effects of dietary treatments on starch digestibility coefficients and starch disappearance rates in the proximal jejunum and distal ileum are shown in Table 6. Sorghum-based diets supported higher distal ileal starch digestibility coefficients by 4.64% (0.880 versus 0.841; $P < 0.05$) and starch disappearance rates by 7.74% (69.6 versus 64.6 g/bird/day; $P < 0.02$) than wheat-based diets. There was a significant sodium metabisulphite by protease interaction ($P = 0.007$) for starch disappearance rates in the distal ileum. Protease alone retarded starch disappearance rates from 68.8 to 65.7 g/bird/day; whereas, in combination with sodium metabisulphite, protease accelerated rates from 62.8 to 71.0 g/bird/day.

The effects of dietary treatments on starch:protein disappearance rate ratios in proximal jejunum and distal ileum are shown in Table 7. There were no significant treatment effects in the proximal jejunum. In the distal ileum wheat based diets supported "narrower" disappearance rate ratios (2.41 versus 2.96; $P < 0.001$). There was a significant sodium metabisulphite by protease interaction ($P < 0.001$). Protease alone decreased disappearance rate ratios (2.53 versus 2.81) but increased disappearance rate ratios (2.89 versus 2.52) in the presence of sodium metabisulphite. Starch:protein disappearance rate ratios in the proximal jejunum were negatively correlated with weight gain and positively correlated with FCR to significant extents as illustrated in Figure 1.

Transfer of amino acids into the portal circulation

As a preliminary investigation blood samples were taken from the anterior mesenteric and brachial veins to determine concentrations of free amino acids and glucose in the portal and systemic circulations, respectively. Thus three birds at random from each caged replicate were sampled that had been offered the control wheat-based diets or the same diet containing both sodium metabisulphite and protease. The choice of these two treatments proved serendipitous as the control wheat-based diet supported the best FCR numerically but the poorest following the inclusion of both feed additives. Feed conversion ratios (FCR) were increased by 4.56% from 1.513 in negative control diet to 1.582 in the supplemented diet and this difference approached significance (P = 0.084).

As shown in Tables 8 and 9, sodium metabisulphite plus protease significantly depressed (P < 0.05) the "gross portal transfer" (concentrations of amino acids in plasma taken from the anterior mesenteric vein) of 10 ex 20 amino acids which included histidine, isoleucine, methionine, valine, alanine, cystine, glutamine, glycine, proline and serine. sodium metabisulphite plus protease did not significantly influence systemic concentrations of free amino acids in plasma taken from the brachial vein. Instructively, concentrations of certain amino acids in the anterior mesenteric vein were significantly correlated with FCR (Table 3). Of the essential amino acids 6 ex 10 were significantly negatively correlated (P < 0.05) with FCR (isoleucine, leucine, methionine, phenylalanine, threonine, valine) and a further 3 (arginine, histidine, lysine) tended to be correlated (P < 0.10). Threonine had the strongest negative relationship with FCR (r = -0.773; P = 0.005) and this is illustrated in Figure 2.

Therefore, the gross portal transfer or post-enteral availability of certain amino acids were related to FCR; probably the single most important parameter of chicken-meat production. It follows that the tandem inclusion of sodium metabisulphite plus protease may have been influencing either (i) protein digestion, (ii) amino acid absorption and/or (iii) catabolism of amino acids in the gut mucosa; thereby compromising feed conversion efficiency. Interestingly, sodium metabisulphite plus protease depressed proximal jejunal protein (N) disappearance rates by 14.8% (16.1 versus 18.9 g/bird/day; P = 0.132) but promoted proximal jejunal starch disappearance rates by 21.6% (37.1 versus 30.5 g/bird/day; P = 0.256); thereby, altering starch:protein digestive dynamics. The outcomes of this preliminary investigation must be treated with caution; nevertheless, taking blood samples from the anterior mesenteric vein of broiler chickens to determine plasma concentrations of amino acids in the portal circulation appears to be a valid approach to investigate the impacts of dietary treatments on the catabolism of amino acids in the gut mucosa. Either glucose or amino acids, especially glutamic acid/glutamine, undergo catabolism in the gut mucosa, in part to meet the copious energy requirements for gut function. It seems likely that if this ratio could be manipulated towards glucose as a fuel for the gut then the post-enteral availability of amino acids for protein accretion would be enhanced. While speculative, it may be that a combination of slower starch but more rapid protein digestive dynamics could be a dietary means of manipulating the catabolism of glucose versus amino acids in the gut mucosa.

The inclusion of sodium metabisulphite wheat-based broiler diets (unpublished data)

Truong et al. (2015) reported on the individual and combined additions of sodium metabisulphite and exogenous phytase to sorghum-based broiler diets. However, in this study the effects of sodium metabisulphite and phytase in wheat-based diets were also determined although this data was not reported in the paper and has not been published. A summary of the pertinent outcomes is presented as Tables 11 and 12. There was a significant treatment interaction for ME:GE ratios where phytase alone significantly enhanced energy utilisation by 9.16% (0.846 versus 0.775) but sodium metabisulphite addition to the phytase-supplemented diet significantly depressed energy utilisation by 7.09% (0.786 versus 0.846). There were no other treatment interactions. The only significant main effects of sodium metabisulphite were to depress AME (12.83 versus 13.14; $P < 0.04$) and AMEn (11.87 versus 12.27; $P < 0.01$). Individually, Na metabisulphite numerically compromised FCR by 1.87% (1.583 versus 1.554). In short, there were not any indications that the inclusion of sodium metabisulphite in wheat-based diets, either alone or in tandem with phytase, was advantageous. In contrast, phytase generated robust responses in protein (N) digestibility coefficients along the small intestine.

Truong HH, Cadogan DJ, Liu SY, Selle PH (2015) Addition of sodium metabisulfite and microbial phytase, individually and in combination, to a sorghum-based diet for broiler chickens from 7 to 28 days post-hatch *Animal Production Science* dx.doi.org/10.1071/AN14841

Discussion

The tandem inclusions of sodium metabisulphite and protease in sorghum-based diets numerically advantaged weight gain by 3.09% (1533 versus 1487 g/bird), FCR by 1.09% (1.538 versus 1.555), AME by 0.18 MJ (12.46 versus 12.28 MJ/kg) and ME:GE ratios by 1.33% (0.760 versus 0.750). In contrast, tandem inclusions in wheat-based diets disadvantaged weight gain by 0.77% (1541 versus 1553 g/bird), FCR by 4.56% (1.582 versus 1.513), AME by 0.55 MJ (11.93 versus 12.48 MJ/kg) and ME:GE ratios by 6.00% (0.705 versus 0.750). Therefore, while there is a cautious case to be made for sodium metabisulphite and protease inclusions in sorghum-based diets, this does not appear applicable for wheat-based diets.

The individual inclusion of 2.75 g/kg sodium metabisulphite in sorghum-based diets fractionally advantaged weight gain by 0.40% (1493 versus 1487 g/bird), FCR by 0.64% (1.545 versus 1.555), AME by 0.20 MJ (12.48 versus 12.28 MJ/kg) and ME:GE ratios by 1.73% (0.763 versus 0.750). Again in contrast, sodium metabisulphite alone in wheat-based diets disadvantaged weight gain by 1.55% (1529 versus 1553 g/bird), FCR by 3.97% (1.573 versus 1.513), AME by 0.44 MJ (12.04 versus 12.48 MJ/kg) and ME:GE ratios by 3.73% (0.722 versus 0.750).

The responses to inclusions of sodium metabisulphite and protease in sorghum-based diets ***only*** in respect of starch are instructive (Table 6). Individually, sodium metabisulphite (0.868 versus 0.875) and protease (0.873 versus 0.875) did not influence distal ileal starch digestibility coefficients to any extent. However, in combination, they improved starch digestibility by 3.31% (0.904 versus 0.875) as opposed to an additive response of -1.03%. Similarly, sodium metabisulphite (70.7 versus 68.5 g/bird/day) and protease (64.6 versus 68.5 g/bird/day) did not greatly influence distal ileal starch disappearance rates. The combination increased starch disappearance by 8.91% (74.6 versus 68.5 g/bird/day; P = 0.148) as opposed to an additive response of -2.48%. Both outcomes suggest that synergistic responses to tandem inclusions sodium metabisulphite and protease in sorghum-based diets may be observed.

Sulphite reducing agents, including sodium metabisulphite, have the capacity to depolymerise starch via oxidative-reductive reactions. However, starch digestibility coefficients and disappearance rates were not influenced by sodium metabisulphite to significant extents (Table 6) in the present study. It seems possible that any starch depolymerisation induced by reducing agents is of little consequence in poultry performance. Sodium metabisulphite has the capacity to reduce disulphide cross-linkages which are ubiquitous in the protein components of all relevant feedstuffs. However, as illustrated in Figures 3 and 4, this capacity to reduce disulphide cross-linkages appears to be reflected in the *in vitro* protein and starch digestibility of sorghum, but not maize and wheat. The genesis of this pivotal difference may be the presence of disulphide cross-linkages in the β- and γ-fractions of kafirin protein bodies. These spherical protein bodies are located in sorghum endosperm where the β- and γ-fractions encapsulate the central core of α-kafirin. Starch granules and kafirin protein bodies are both embedded in the glutelin protein matrix of sorghum endosperm. It is generally accepted that kafirin impedes starch utilisation in sorghum via biophysical and biochemical starch-protein interactions involving disulphide cross-linkages in β- and γ-kafirin. Thus the benefits of sulphite reducing agents in poultry diets may be "sorghum-specific" because of these unique structural factors in grain sorghum endosperm.

Therefore, the tentative conclusion from this study, coupled with the unpublished data, is that the established benefits of sodium metabisulphite in sorghum-based diets do not extend to wheat-based broiler diets. The advantages from sodium metabisulphite inclusions in sorghum-based diets may fundamentally, and quite specifically, stem from the reduction of disulphide cross-linkages of β- and γ-kafirins located in the periphery of protein bodies.

In the present study, the significant impacts of protease as a main effect were limited to a 2.91% increase in feed intake and a widening of the starch:protein disappearance rate ratio from 1.74 to 2.16 in the proximal jejunum. However, our findings have generally indicated that a narrowing of starch:protein disappearance rate ratios is more likely to advantage broiler performance. Also, in a previous study we found that an alternative exogenous protease generated more promising results in sorghum-based diets than the one used in the present study. However, protease would have been disadvantaged by quite high inclusions of synthetic amino acids. Synthetic amino acids represented approximately 27% of total lysine, 50% of methionine, 21% of threonine and 32% of arginine across the two diets in the present study and, notionally, these amino acid proportions are completely digestible. In another previous study we found a protease significantly increased the digestibility of 14 ex 16 amino acids. The two exceptions were lysine and methionine, the total dietary levels of which received contributions from synthetic forms of these two amino acids.

Acknowledgements

We would like to thank Ms Moreen Ali for formulating the diets, Ms Judy O'Keefe for providing the protease feed enzyme, Ms Joy Gill and her team from the Poultry Research Foundation for their technical assistance and the Poultry CRC for funding the project.

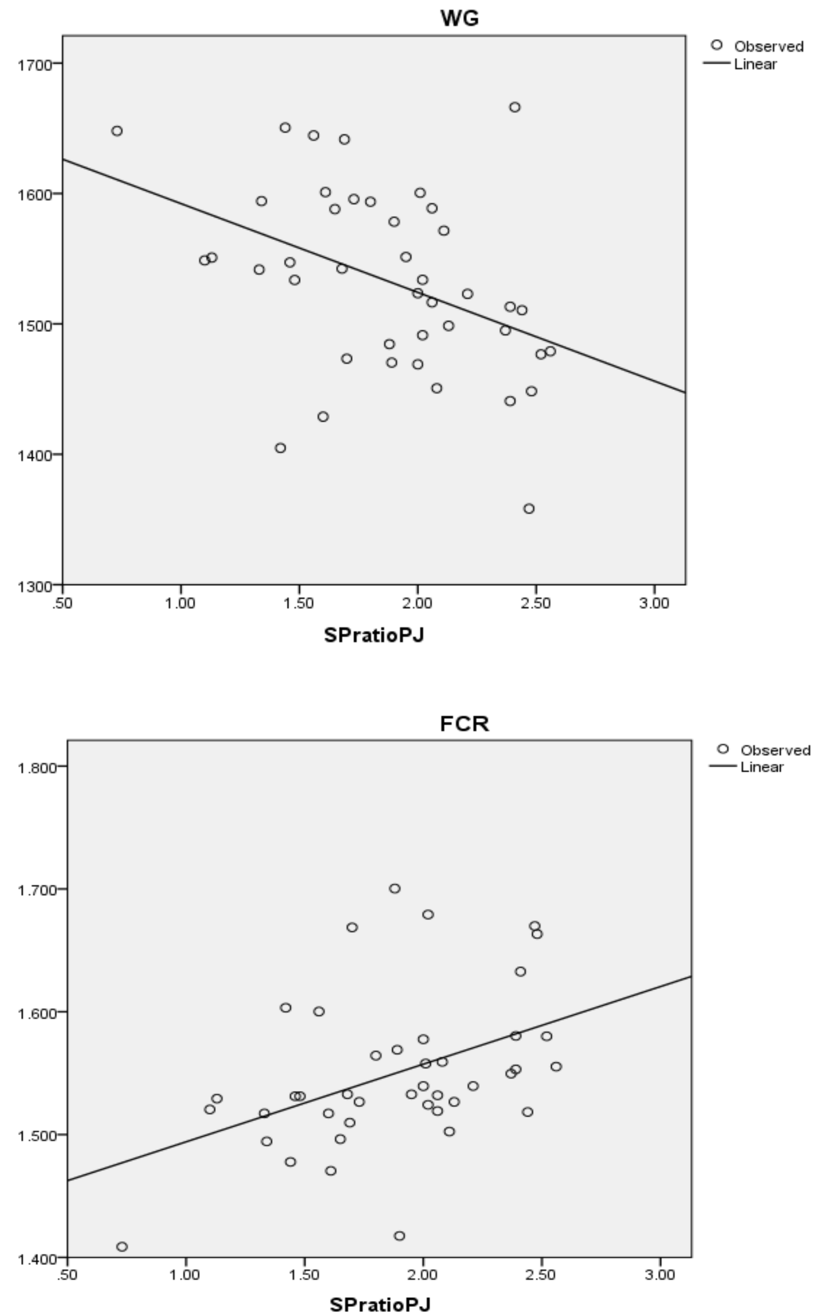

Figure 1 Linear relationships between proximal jejunal starch:protein disappearance rate ratios and weight gain (r = -0.414; P = 0.006) and FCR (r = 0.431; P = 0.004)

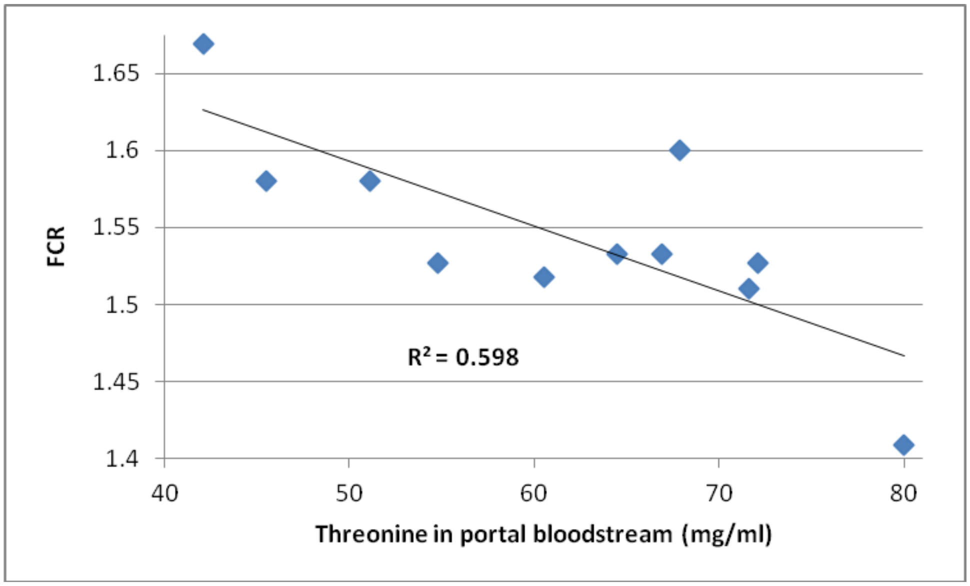

Figure 2 Linear relationship (r = -0.773; P = 0.005) between free threonine concentrations in blood samples taken from the anterior mesenteric vein and FCR in broiler chickens from 7 to 28 days post-hatch

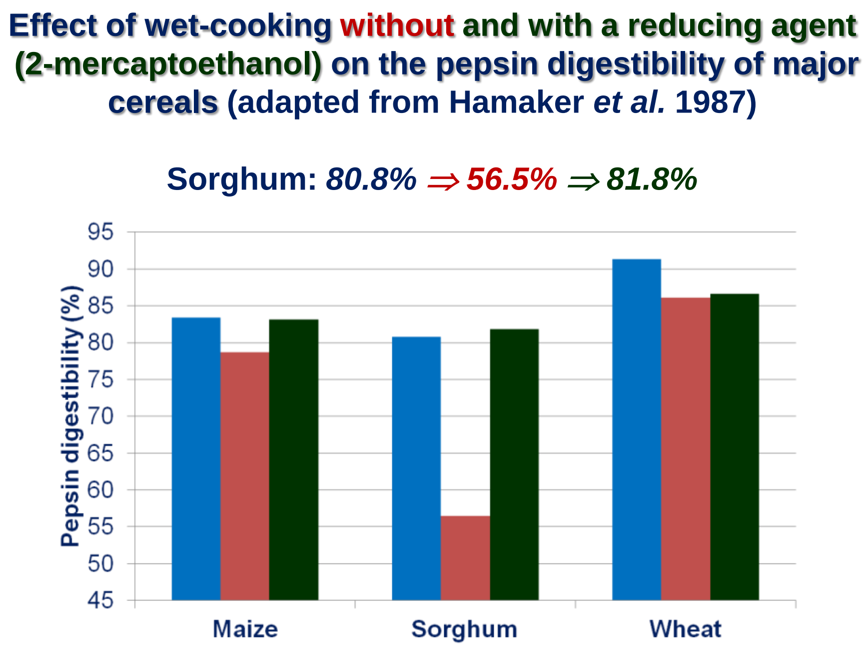

Figure 3 The effect of wet-cooking and a sulphite reducing agent on the *in vitro* pepsin digestibility of maize, sorghum and wheat (adapted from Hamaker et al. 1987) Legend: Blue = control grain, Red = wet-cooked grain, Green = wet cooked grain with sulphite reducing agent

Reference

Hamaker BR, Kirleis AW, Butler LG, Axtell JD, Mertz ET (1987) Improving the *in vitro* protein digestibility of sorghum with reducing agents. *Proceedings of the National Academy of Sciences of the United States of America* **84**, 626-628.

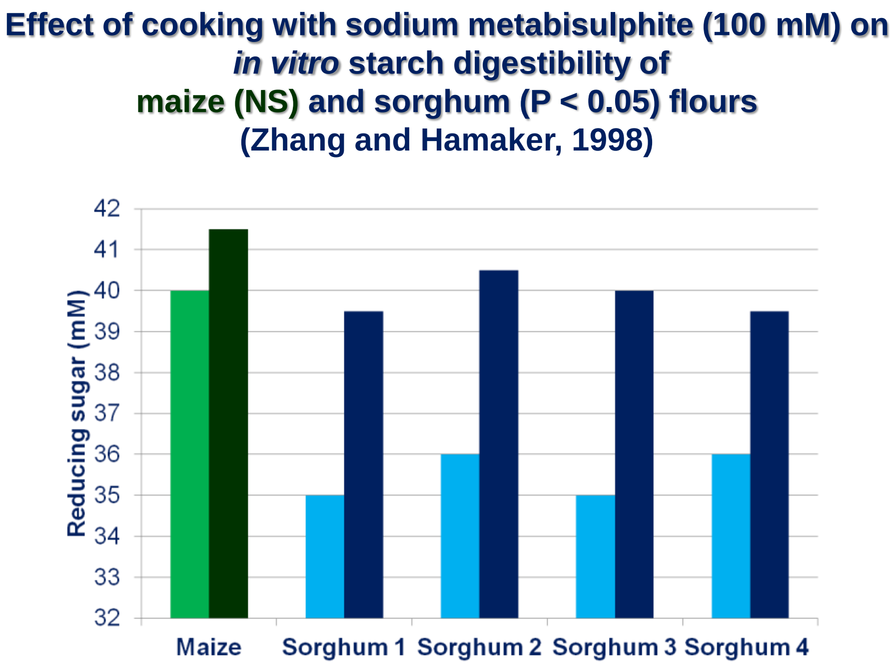

Figure 4 The effect of wet-cooking and a sulphite reducing agent on the *in vitro* starch digestibility of maize and sorghums (adapted from Zhang and Hamaker, et al. 1998)

Legend: Mid-green and mid-blue: wet-cooked grain. Dark-green and dark-blue: wet-cooked grain with sulphite reducing agent

Reference

Zhang G, Hamaker BR (1998) Low α-amylase starch digestibility of cooked sorghum flours and the effect of protein. *Cereal Chemistry* **75**, 710-713.

Table 1 Composition and nutrient specifications of the basal wheat-and sorghum-based diets

Feed ingredient (g/kg)	Wheat-based diet	Sorghum-based diet
Wheat	638.2	-
Sorghum	-	682.3
Soybean meal	255.0	235.0
Canola oil	45.0	20.0
Limestone	13.0	13.0
Dicalcium phosphate	12.0	12.0
Sodium bicarbonate[1]	6.0	5.7
Lysine HCl	3.4	4.5
Methionine	2.6	3.0
Threonine	1.6	1.6
Arginine	5.6	5.0
Choline chloride	0.6	0.9
Vitamin-trace mineral premix[2]	2.0	2.0
Celite[3]	15.0	15.0
Metabolisable energy (MJ/kg)	13.06	12.92
Protein	215.1	215.7
Calcium	8.05	7.81
Total phosphorus	6.54	6.22
Available phosphorus	4.05	3.85
Sodium	1.79	1.80
Digestible amino acids		
Lysine	11.40	11.50
Methionine	5.40	5.78
Methionine + cystine	8.61	8.66
Tryptophan	2.13	2.13
Arginine	17.2	15.6
Threonine	7.68	7.68
Leucine	12.6	17.5
Isoleucine	8.18	8.31

[1]Sodium metabisulphite (2.75 g/kg) replaced 2.34 g/kg sodium bicarbonate to maintain Na levels, difference corrected with Celite [2]Vitamin-trace mineral premix supplied per tonne of feed; [million international units, 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 [3]Protease added at the expense of Celite

Table 2 The effects of grain type and dietary inclusions of sodium metabisulphite (SMBS) and protease on parameters of growth performance from 7 to 28 days post-hatch

Treatment			Growth performance			
Grain	SMBS (g/kg)	Protease (units/kg)	Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Mortality/culls (%)
Wheat	0	0	1553	2347	1.513	0.00
	0	1000	1589	2430	1.529	3.33
	2.75	0	1529	2400	1.573	0.00
	2.75	1000	1541	2436	1.582	0.00
Sorghum	0	0	1487	2311	1.555	0.00
	0	1000	1539	2411	1.566	3.33
	2.75	0	1493	2304	1.545	0.00
	2.75	1000	1533	2355	1.538	0.00
SEM			24.459	44.725	0.0273	1.5373
Main effects Grain						
Wheat			1553	2403	1.549	0.83
Sorghum			1513	2345	1.551	0.83
Na metabisulphite						
0			1542	2374	1.541	1.67
2.75			1524	2374	1.559	0.00
Protease						
0			1516	2340a	1.546	0.00
1000			1551	2408b	1.554	1.67
Significance (P =)						
Grain			0.067	0.075	0.916	1.000
Na metabisulphite			0.410	0.985	0.354	0.136
Protease			0.106	0.040	0.713	0.136
Grain x Na metabisulphite			0.401	0.338	0.061	1.000
Grain x Protease			0.600	0.798	0.788	1.000
Na metabisulphite x Protease			0.666	0.455	0.749	0.136
Grain x Na metabisulphite x Protease			0.895	0.992	0.882	1.000

ab Means within columns not sharing a common suffix are significantly different at the 5% level of probability

Table 3 The effects of grain type and dietary inclusions of sodium metabisulphite (SMBS) and protease on parameters of nutrient utilisation within 7 to 28 days post-hatch

Treatment			Nutrient utilisation				
Grain	SMBS (g/kg)	Protease (units/kg)	AME (MJ/kg)	ME:GE ratio	AME (MJ/day)	N retention (%)	AMEn (MJ/kg)
Wheat	0	0	12.48	0.750	2.14	65.48	11.06
	0	1000	12.15	0.729	2.06	63.32	10.81
	2.75	0	12.04	0.722	1.99	62.04	10.71
	2.75	1000	11.93	0.705	1.94	61.47	10.35
Sorghum	0	0	12.28	0.750	1.92	62.76	11.10
	0	1000	12.31	0.754	1.99	65.06	11.06
	2.75	0	12.48	0.763	1.94	65.95	11.19
	2.75	1000	12.46	0.760	1.98	65.64	11.15
SEM			0.1431	0.0086	2.2913	1.1036	0.1357
Main effects Grain							
Wheat			12.09	0.727	2.03	63.08	10.73
Sorghum			12.38	0.756	1.96	64.85	11.13
Na metabisulphite							
0			12.30	0.746	2.03	64.16	11.01
2.75			12.17	0.737	1.96	63.77	10.85
Protease							
0			12.32	0.746	2.00	64.06	11.01
1000			12.16	0.737	1.99	63.87	10.84
Significance (P =)							
Grain			0.008	< 0.001	0.115	0.030	< 0.001
Na metabisulphite			0.205	0.189	0.201	0.630	0.112
Protease			0.130	0.138	0.870	0.814	0.083
Grain x Na metabisulphite			0.005	0.006	0.179	0.006	0.015
Grain x Protease			0.104	0.120	0.223	0.141	0.179
Na metabisulphite x Protease			0.901	0.856	0.944	0.745	0.761
Grain x Na metabisulphite x Protease			0.889	0.681	0.757	0.188	0.790

Table 4 The effects of grain type and dietary inclusions of sodium metabisulphite (SMBS) and protease on protein (N) digestibility coefficients in four small intestinal segments at 28 days post-hatch

Treatment			Protein (N) digestibility coefficients			
Grain	SMBS (g/kg)	Protease (units/kg)	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Wheat	0	0	0.464	0.741	0.773	0.780
	0	1000	0.515	0.706	0.759	0.790
	2.75	0	0.490	0.692	0.753	0.786
	2.75	1000	0.601	0.695	0.743	0.761
Sorghum	0	0	0.499	0.690	0.749	0.790
	0	1000	0.513	0.712	0.780	0.760
	2.75	0	0.511	0.700	0.767	0.777
	2.75	1000	0.520	0.721	0.743	0.783
SEM			0.0334	0.0193	0.0136	0.0146
Main effects Grain						
Wheat			0.517	0.708	0.757	0.779
Sorghum			0.511	0.705	0.760	0.777
Na metabisulphite						
0			0.498	0.712	0.765	0.780
2.75			0.530	0.701	0.752	0.777
Protease						
0			0.491	0.705	0.761	0.783
1000			0.537	0.708	0.756	0.773
Significance (P =)						
Grain			0.769	0.794	0.801	0.880
Na metabisulphite			0.166	0.401	0.211	0.765
Protease			0.052	0.785	0.674	0.373
Grain x Na metabisulphite			0.329	0.157	0.706	0.428
Grain x Protease			0.144	0.147	0.457	0.854
Na metabisulphite x Protease			0.551	0.470	0.246	0.983
Grain x Na metabisulphite x Protease			0.491	0.498	0.177	0.092

ab Means within columns not sharing a common suffix are significantly different at the 5% level of probability

Table 5 The effects of grain type and dietary inclusions of sodium metabisulphite (SMBS) and protease on protein (N) disappearance rates (g/bird/day) in four small intestinal segments at 28 days post-hatch

Treatment			Protein (N) disappearance rates (g/bird/day)			
Grain	SMBS (g/kg)	Protease (units/kg)	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Wheat	0	0	18.9	24.7	25.9	26.8
	0	1000	17.4	23.4	25.1	26.9
	2.75	0	17.5	23.2	25.2	26.8
	2.75	1000	16.1	21.5	23.0	27.3
Sorghum	0	0	15.8	22.6	24.5	22.1
	0	1000	15.7	21.4	23.5	25.4
	2.75	0	18.2	23.2	25.7	23.5
	2.75	1000	14.0	24.5	25.4	24.0
SEM			1.2541	1.0362	1.2330	1.0415
Main effects Grain						
Wheat			17.5	23.2	24.8	27.0b
Sorghum			15.9	22.9	24.8	23.7a
Na metabisulphite						
0			16.9	23.0	24.7	25.3
2.75			16.5	23.1	24.8	25.4
Protease						
0			17.6	23.4	25.3	24.8
1000			15.8	22.7	24.2	25.9
Significance (P =)						
Grain			0.083	0.728	0.995	< 0.001
Na metabisulphite			0.613	0.905	0.993	0.902
Protease			0.051	0.335	0.229	0.151
Grain x Na metabisulphite			0.361	0.021	0.110	0.918
Grain x Protease			0.686	0.296	0.612	0.289
Na metabisulphite x Protease			0.261	0.518	0.825	0.421
Grain x Na metabisulphite x Protease			0.239	0.355	0.530	0.268

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Table 6 The effects of grain type and dietary inclusions of sodium metabisulphite (SMBS) and protease on starch digestibility coefficients and starch disappearance rates (g/bird/day) in proximal jejunum and distal ileum at 28 days post-hatch

Treatment			Starch digestibility coefficients		Starch disappearance rates	
Grain	SMBS (g/kg)	Protease (units/kg)	Proximal jejunum	Distal ileum	Proximal jejunum	Distal ileum
Wheat	0	0	0.404	0.887	30.5	69.0
	0	1000	0.437	0.805	33.1	60.7
	2.75	0	0.416	0.821	31.2	61.2
	2.75	1000	0.467	0.853	37.1	67.5
Sorghum	0	0	0.358	0.875	28.1	68.5
	0	1000	0.358	0.868	29.2	70.7
	2.75	0	0.407	0.873	30.9	64.6
	2.75	1000	0.367	0.904	30.4	74.6
SEM			0.0492	0.0273	4.051	2.736
Main effects Grain						
Wheat			0.431	0.841a	33.0	64.6a
Sorghum			0.373	0.880b	29.6	69.6b
Na metabisulphite						
0			0.389	0.859	30.2	67.2
2.75			0.414	0.863	32.4	67.0
Protease						
0			0.396	0.864	30.2	65.8
1000			0.407	0.858	32.4	68.4
Significance (P =)						
Grain			0.100	0.046	0.258	0.015
Na metabisulphite			0.475	0.825	0.464	0.888
Protease			0.746	0.740	0.445	0.199
Grain x Na metabisulphite			0.904	0.494	0.949	0.901
Grain x Protease			0.375	0.323	0.509	0.082
Na metabisulphite x Protease			0.875	0.052	0.886	0.007
Grain x Na metabisulphite x Protease			0.674	0.318	0.673	0.387

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Table 7 The effects of grain type and dietary inclusions of sodium metabisulphite (SMBS) and protease on starch:protein disappearance rate ratios in proximal jejunum and distal ileum at 28 days post-hatch

Treatment			Starch:protein disappearance rate ratios	
Grain	SMBS (g/kg)	Protease (units/kg)	Proximal jejunum	Distal ileum
Wheat	0	0	1.87	2.57
	0	1000	1.88	2.26
	2.75	0	1.88	2.30
	2.75	1000	1.87	2.64
Sorghum	0	0	1.96	3.06
	0	1000	1.90	2.79
	2.75	0	1.81	2.74
	2.75	1000	1.85	3.15
SEM			0.2056	0.1095
Main effects Grain				
Wheat			1.87	2.44a
Sorghum			1.88	2.93b
Na metabisulphite				
0			1.90	2.67
2.75			1.85	2.71
Protease				
0			1.88	2.66
1000			1.87	2.71
Significance (P =)				
Grain			0.956	< 0.001
Na metabisulphite			0.731	0.651
Protease			0.973	0.550
Grain x Na metabisulphite			0.724	0.812
Grain x Protease			0.976	0.735
Na metabisulphite x Protease			0.900	< 0.001
Grain x Na metabisulphite x Protease			0.839	0.914

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Table 8 The effects of feed additives (sodium metabisulphite plus protease) in wheat-based diets on concentrations of essential amino acids and glucose in the portal and systemic circulation of broiler chickens at 28 days post-hatch

Amino acid (mg/ml)	Portal circulation (anterior mesenteric vein)				Systemic circulation (brachial vein)			
	Control	Additives	SEM	P =	Control	Additives	SEM	P =
Arginine	108.9	85.4	12.117	0.200	97.5	99.5	8.385	0.867
Histidine	12.2	2.3	0.923	0.023	5.9	5.6	0.670	0.732
Isoleucine	18.6	15.5	0.976	0.048	9.6	9.5	0.729	0.912
Leucine	26.1	23.7	1.153	0.180	14.9	15.1	0.903	0.889
Lysine	47.0	39.3	3.321	0.133	33.4	34.8	2.830	0.728
Methionine	15.8	12.5	0.829	0.017	14.2	12.5	1.506	0.452
Phenylalanine	21.0	17.9	1.116	0.075	13.9	12.9	0.953	0.475
Threonine	63.8	50.8	7.446	0.247	56.8	56.7	5.704	0.994
Tryptophan	6.2	6.0	0.477	0.791	4.7	4.5	0.365	0.707
Valine	27.8	23.3	1.305	0.037	17.6	17.2	0.953	0.782
Glucose (mmol/L)	17.4	19.3	1.427	0.365	15.4	14.6	0.600	0.311

Table 9 The effects of feed additives (sodium metabisulphite plus protease) in wheat-based diets on concentrations of non-essential amino acids in the portal and systemic circulation of broiler chickens at 28 days post-hatch

Amino acid (mg/ml)	Portal circulation (anterior mesenteric vein)				Systemic circulation (brachial vein)			
	Control	Additives	SEM	P =	Control	Additives	SEM	P =
Alanine	88.7	66.7	5.911	0.025	53.0	53.2	4.189	0.972
Aspartic acid	13.1	23.5	3.830	0.084	9.0	10.0	1.033	0.502
Asparagine	30.0	21.4	4.474	0.202	22.5	17.7	3.216	0.318
Cystine	13.6	9.9	0.852	0.001	11.8	10.10.	0.678	0.116
Glutamic acid	47.9	52.2	2.680	0.280	31.0	27.7	1.136	0.068
Glutamine	196.6	143.8	10.734	0.006	171.2	149.3	12.819	0.253
Glycine	51.9	37.7	2.463	0.002	37.2	31.8	1.887	0.075
Proline	69.2	48.3	5.099	0.016	53.9	42.7	5.252	0.161
Serine	59.2	48.1	3.299	0.039	49.2	47.9	3.905	0.830
Tyrosine	27.6	27.2	2.400	0.909	22.0	23.8	2.274	0.588

Table 10 Correlations between gross portal transfers of amino acids and feed conversion ratios of broilers offered wheat-based diets without and with sodium metabisulphite plus protease from 7 to 28 days post-hatch

Essential amino acids	Correlation coefficient (r =)	Significance (P =)	Non-essential amino acids	Correlation coefficient (r =)	Significance (P =)
Arginine	-0.563	0.072	Alanine	-0.157	0.644
Histidine	-0.568	0.068	Aspartic acid	-0.019	0.956
Isoleucine	-0.643	0.033	Asparagine	-0.606	0.048
Leucine	-0.621	0.042	Cystine	-0.329	0.322
Lysine	-0.575	0.064	Glutamic acid	+0.295	0.379
Methionine	-0.660	0.027	Glutamine	-0.501	0.116
Phenylalanine	-0.683	0.021	Glycine	-0.692	0.018
Threonine	-0.773	0.005	Proline	-0.802	0.003
Tryptophan	-0.247	0.464	Serine	-0.499	0.118
Valine	-0.657	0.028	Tyrosine	+0.085	0.804

Table 11 Effects of sodium metabisulphite and phytase inclusions in wheat-based diets on growth performance, toe ash and nutrient utilisation from 7 to 28 days post-hatch (unpublished data from other work of Truong et al. 2015).

Treatment		Weight gain	Feed intake	FCR	Toe ash (%)	AME (MJ/kg)	ME:GE ratio	N retention	AMEn (MJ/kg)
SMBS	Phytase								
0	0	1527	2364	1.554	12.05	12.99	0.775a	68.36	12.11
	1000	1506	2342	1.559	11.60	13.29	0.846b	67.42	12.44
1.75	0	1517	2401	1.583	12.49	12.83	0.790a	69.17	11.82
	1000	1512	2350	1.558	11.49	12.83	0.786a	67.92	11.92
SEM		41.73	46.43	0.0358	0.3044	0.0187	0.0112	1.132	0.1449
Main effects SMBS									
0		1517	2353	1.557	11.82	13.14b	0.810	67.89	12.27b
1.75 g/kg		1515	2376	1.570	11.99	12.83a	0.788	68.55	11.87a
Phytase									
0		1522	2382	1.569	12.27b	12.91	0.782	68.76	11.96
1000 FTU/kg		1509	2346	1.558	11.54a	13.06	0.816	67.67	12.18
Significance (P =)									
Na metabisulphite		0.967	0.633	0.698	0.590	0.033	0.101	0.567	0.010
Phytase		0.759	0.477	0.767	0.027	0.288	0.016	0.343	0.145
SMBS x Phytase		0.851	0.759	0.661	0.383	0.276	0.008	0.893	0.439

Table 12 Effects of sodium metabisulphite and phytase inclusions in wheat-based diets on apparent digestibility coefficients of starch and protein (N) in four small intestinal segments [proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI), distal ileum (DI)] at 28 days post-hatch (unpublished data from other work of Truong et al. 2015).

Treatment		Starch	Starch	Starch	Starch	Protein (N)	Protein (N)	Protein (N)	Protein (N)
SMBS	Phytase	PJ	DJ	PI	DI	PJ	DJ	PI	DI
0	0	0.631	0.742	0.770	0.829	0.531	0.575	0.693	0.720
	1000	0.614	0.745	0.761	0.817	0.571	0.655	0.736	0.756
1.75	0	0.593	0.741	0.757	0.796	0.480	0.568	0.687	0.706
	1000	0.633	0.745	0.761	0.815	0.596	0.650	0.741	0.727
SEM		0.0378	0.0267	0.0239	0.0169	0.0339	0.0239	0.0207	0.0169
Main effects SMBS									
0		0.623	0.743	0.765	0.823	0.551	0.615	0.714	0.738
1.75 g/kg		0.613	0.743	0.759	0.806	0.538	0.609	0.714	0.717
Phytase									
0		0.612	0.742	0.764	0.813	0.506a	0.572a	0.690a	0.713
1000 FTU/kg		0.624	0.745	0.761	0.816	0.583b	0.652b	0.738b	0.742
Significance (P =)									
Na metabisulphite		0.794	0.998	0.803	0.327	0.708	0.796	0.987	0.160
Phytase		0.755	0.892	0.920	0.855	0.033	0.003	0.036	0.064
SMBS x Phytase		0.439	0.984	0.796	0.392	0.278	0.961	0.828	0.618