Influence of increasing protease supplementation on two different types of sorghum

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Executive Summary

The performance young pigs offered sorghum based diets is up to 20% lower than for pigs offered wheat based diets (Premium Grains for Livestock Program, Final Report). A major reason for the difference is thought to be due to the encapsulation of starch granules within sorghum by a protein envelope consisting of relatively indigestible kafirin proteins. An experiment was conducted to test the hypothesis that preliminary digestion of the kafirin envelope with a protease, Subtilisin, would improve the performance of young pigs offered sorghum based diets. The experiment was a 2 x 4 factorial array, with the treatments being sorghum type (White or Liberty cultivar and high kafirin red sorghum, the cultivar Buster) and Subtilisin protease dose (0, 50, 100 and 500ppm).

Over the 0 to 21 day experimental period, pigs offered the wheat control out performed animals on the Liberty and Buster controls in daily weight gain (P<0.05) by 16.5% and 20.8%, respectively. The Liberty sorghum supported poorer FCR (P<0.05) than the wheat control, but feed intakes were similar. With Buster the opposite effects were noted. Pigs on the Buster control diet ate less (P<0.05) than pigs on the wheat control but there was no significant difference in FCR. There were no significant differences in growth performance between the two sorghum grains after 21 day. Increasing the protease dose linearly improved FCR (P=0.017). The highest level of enzyme (500 ppm) significantly improved the FCR of pigs offered both sorghum types, producing a similar FCR to that of the wheat control. There was no overall influence of protease on daily gain or feed intake, although the 50 ppm dose significantly increased intake on the Buster sorghum, however intake significantly dropped when the enzyme dose doubled, indicating feed wastage may have been a contributing factor at the low dosage.

The Buster sorghum was measured to be significantly lower in DE and diet digestibility compared to the white Liberty sorghum and the wheat (P<0.001). In fact, DE of the wheat and Liberty based were exactly the same at 14.87 MJ/kg. There was an interaction (P=0.048) between protease and sorghum type, with the enzyme increasing (linear effect; P=0.058) both diet digestibility and DE. The majority of the improvement was observed in the first 100 ppm of the added protease.

Overall, the unsupplemented sorghum diets depressed the performance of young male pigs and the highest dose rate of protease added to both the sorghum varieties improved feed efficiency to the levels of the wheat based diet. The two sorghums did respond differently to the increasing level of protease, with the most notable being the significant improvement in DE for the red sorghum, whereas the enzyme had no effect on the digestibility of the white sorghum. The results indicate the protease can reduce the nutritional barriers caused by the structural and storage proteins in sorghum, and improve feed efficiency to the level supported by wheat based diets. The most cost effective dose rate of the protease is most likely to be between 50 and 500 ppm but would appear to vary with variety. Further research is warranted to establish the optimum level of enzyme inclusion and to relate this to the chemical composition of the sorghum grain.

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1. Introduction

Sorghum is grown in a wide variety of soils and climates in Australia, which can affect the filling and therefore starch content of the grain. A recent review by Selle et al (2010) indicated there are three factors affecting the digestibility of sorghum, tannin (polyphenols), phytate and kafirin (structural protein), which may have attributed to the significant difference in weaner and grower growth when offered different sorghum types (Finn et al, 2007). The white sorghum (cultivar Liberty) has low tannin and has proven to produce superior growth performance in pigs and poultry, compared to the red sorghum counterparts. The main limiting factor in sorghum, however, could be the poorly digested structural protein (kafirin) surrounding the starch granule. This protein can vary considerably in sorghum, depending on the variety and growing conditions from approximately 35% to 65% of the total protein content. Kafirin is a proamine protein, which is hydrophobic (poorly solubilised) and contains disulphide bonds which are poorly degraded by pig endogenous proteases. Kafirin content is higher in the vitreous endosperm, compared to the starchy or floury endosperm. The floury endosperm is higher in content in grains of higher weight and size, and these “softer” grains are generally more digestible. The main problem is modern sorghums are being bred to contain less starchy endosperm to limit mould growth (to increase yields), and therefore producing smaller grains and more kafirin. In theory, sorghum could be become less digestible in the future. An in-vitro experiment (Pork CRC project 1B-105) conducted by Avril Finn showed when hydrated sorghum was mixed with a protease, called Subtilisin, for a hour, almost all the kafirin was digested.

The hypothesis was that the red sorghum variety will produce a significantly lower growth performance and will respond substantially better to the increasing level of protease. If the hypothesis is correct, significant improvements in FCR and nutrient intake in progeny pigs will lower cost of production of pork.

2. Methodology

The experiment was a 2 x 4 factorial array, with the treatments being sorghum type (White or Liberty cultivar and high kafirin red sorghum, the cultivar Buster) and Subtilisin protease dose (0, 50, 100 and 500ppm). The Red or Buster sorghum was selected because it was analysed to contain high protein (11.5%), was hard grained and exhibited small size grains, all characteristic of a high kafirin sorghum. The Liberty sorghum protein was in the normal range (9.0%), and was soft and the grain of large diameter. A wheat plus xylanase positive control was also be incorporated to the experiment.

The experiment was conducted in the Rivalea’s individual weaner pens, using 16 pigs per treatment, a total of 144 entire males. The very nutritional sensitive model of using male pigs between 8 and 20 kg was considered the best method to test the hypothesis. The grains were incorporated in the diets at 65%. The remaining diet contained very high quality protein, energy and micro-nutrients to obtain optimum growth performance. An indigestible marker was added to the diets. The diets were manufactured at QLD DPI&F.

Table 1 - Composition of the experimental diets (on as-fed basis)

Diet	Diet base		
	Wheat	Liberty Sorghum	Buster Sorghum
Ingredients, %			
Wheat	65.00	65.00	65.00
Whey powder	139.50	139.50	139.50
Tallow	0.80	0.80	0.80
SDPP	3.50	3.50	3.50
Meat meal	3.60	3.60	3.60
Fishmeal	9.7	9.7	9.7
Blood meal	0.5	0.5	0.5
Limestone	0.38	0.38	0.38
Salt	0.10	0.10	0.10
L-Lysine	0.62	0.62	0.62
DL-Methionine	0.22	0.22	0.22
L-Threonine	0.24	0.24	0.24
L- Isoleucine	0.07	0.07	0.07
L-Tryptophan	0.08	0.08	0.08
Zinc Oxide	0.30	0.30	0.30
Titanium Oxide	0.10	0.10	0.10
Acidifier	0.03	0.03	0.03
Mineral/Vitamin	0.10	0.10	0.10
Calculated composition:			
DE (MJ/kg)	14.5	14.6	14.6
Crude protein (%)	22.5	19.8	21.3
Available lysine (%)	1.55	1.55	1.55
Analyzed composition:			
DE (MJ/kg)	14.8	14.7	14.3
Crude protein (%)	20.3	18.8	20.6
Crude fat (%)	3.1	3.8	3.8

[1]Supplied per kg of diet: 60.0 mg Fe ($FeSO_4$); 10.0 mg Cu ($CuSO_4$); 40.0 mg Mn (MnO); 100.0 mg Zn (ZnO); 0.30 mg Se (Na_2SeO_3); 0.50 mg I (KI); 0.20 Co ($CoSO_4$); vitamin A, 7,000 IU; vitamin D_3, 1,400 IU; vitamin E, 20.0 mg; vitamin K_3, 1.0 mg; thiamin, 1.0 mg; riboflavin, 3.0 mg; pyridoxine, 1.5 mg; vitamin B_{12}, 0.015 mg; pantothenic acid, 10.0 mg; Folic acid, 0.2 mg; niacin, 12.0 mg and biotin 0.03 mg.

Pigs were selected at 22 days of age, housed in the individual pens, and placed on a commercial phase one nursery diet for 5 days, so the animals could adjust to the facility. Pigs were re-weighed and allocated to the 9 treatments, and offered the experimental diets adlibitum. The pigs were weighed weekly and feed disappearance recorded. The experiment was conducted over a 21 day period. Faeces were collected between days 14 to 16 so that dry matter, energy and protein digestibility could be measured.

Due to problems with 25% less protein/micro-nutrient base mix (product lost during packing and shipment), the amount of diet manufactured was reduced from 250 kg to 190kg. The reduction in feed available and excessive feed wastage by one or two individual animals meant that 6 pigs per treatment (from block 3) were only offered the diet for 14 days, instead of 21 days. These pigs were weighed at 14 days. The remaining 9 to 10 pigs per treatment remained on the study for the full 21 days.

Statistical analyses

All statistical analyses were performed using the GLM procedures of SPSS (SAS Inst. Inc., Cary, NC), with allotment block (random factor), grain type and protease dose as sources of variation. For all variables the individual pig was the experimental unit. Significance was accepted at $P < 0.05$.

3. Outcomes

During the first 14 days, one to two pigs pig per treatment were taken off the study due to poor health or performance and/or feed wasting. No feed wastage was evident on the wheat based diet, however most of the sorghum based diets had at least one pig exhibiting excessive feed wasting behaviour, particularly on the red or Buster sorghum.

After 14 days (Table 1), pigs offered the red sorghum control exhibited significantly inferior daily gain and FCR to the wheat control. The Liberty sorghum control diet produced a poorer FCR (P<0.05) to the Wheat Control, however there was no significant difference in daily gain or feed intake. The Liberty produced a superior daily gain (P=0.073) than the Buster sorghum, but there is no difference FCR or feed intake. The protease produced a quadratic effect on FCR (P=0.007; R^2=0.09), having a more pronounced effect at 100 and 500ppm. The enzyme also linearly decreased intake (P=0.019) as the dose rate increased. The protease did not influence daily gain during the 14 day growth period.

Growth rate and voluntary feed intake increased substantially across all treatments between 14 and 21 days (Table 2). Again, pigs offered the wheat based control grew faster than pigs on the control sorghum treatments (P<0.05). Pigs offered the Liberty sorghum control had a higher FCR (P<0.05) than those on the wheat control, however feed intakes were similar. In contrast pigs on the Buster sorghum control ate less (P=0.05), but there was no significant difference in FCR.

Animals offered the Liberty sorghum control diet exhibited higher feed intakes (P<0.05) than those on the Buster control diet but no significant differences in daily gain and FCR were not significant. There was a trend for an interaction on intake, between protease supplementation and sorghum type (P=0.082), by where the increase level of protease tended to decrease intake on the Liberty sorghum and generally increase feed intake on the Buster sorghum. When observing the general enzyme influence on both sorghums, the protease tended to linearly improve daily gain (P=0.073) and FCR (0.060), but had no significant effect on feed intake.

Over the total 0 to 21 day experimental period (Table 3), pigs offered the wheat control out performed animals on the Liberty and Buster controls in daily weight gain (P<0.05) by 16.5% and 20.8%, respectively. The Liberty sorghum supported poorer FCR (P<0.05) than the wheat control, but feed intakes were similar. With Buster the opposite effects were noted. Pigs offered the Buster control diet ate less (P<0.05) than pigs offered the wheat control but there was no significant difference in FCR. There were no significant differences in growth performance between the two sorghum grains after 21 day. Increasing the protease dose linearly improved FCR (P=0.017). The response to the protease tended to be more pronounced at lower levels when added to the Liberty sorghum, with the 50 ppm dose significantly improving FCR. The highest level of enzyme (500 ppm) significantly improved the FCR of pigs offered both sorghum types, producing a very similar FCR to that of the wheat control. There was no overall influence of protease on daily gain or feed intake, although the 50 ppm dose significantly increased intake on the Buster sorghum. However intake significantly dropped when the enzyme dose doubled, indicating feed wastage may have been a contributing factor at the low dosage.

The Buster sorghum was measured to be significantly lower in DE and diet digestibility (Table 4) compared to the white Liberty sorghum and the wheat ($P<0.001$). In fact, DE of the wheat and Liberty based were exactly the same at 14.87 MJ/kg. There was an interaction ($P=0.048$) between protease and sorghum type, with the enzyme increasing (linear effect; $P=0.058$) both diet digestibility and DE. The majority of the improvement was observed in the first 100 ppm of the added protease.

Table 2 - Growth performance of young male pigs during the 0 to 14 day period

Grain type	Protease (ppm)	14d weight (kg)	Daily gain (g/d)	FCR	Daily Intake (g/d)
Wheat Control	0	12.02^{a}	417^{a}	1.23^{b}	512^{abc}
Liberty Sorghum	0	11.42^{ab}	363^{ab}	1.51^{a}	523^{abc}
	50	11.51^{ab}	376^{ab}	1.42^{ab}	526^{ab}
	100	11.01^{b}	343^{b}	1.43^{ab}	486^{abcd}
	500	11.61^{ab}	385^{ab}	1.21^{b}	461^{bcd}
Buster Sorghum	0	10.80^{b}	333^{b}	1.52^{a}	473^{abcd}
	50	11.19^{b}	352^{b}	1.56^{a}	544^{a}
	100	10.82^{b}	321^{b}	1.39^{ab}	438^{dc}
	500	10.79^{b}	329^{b}	1.43^{ab}	430^{d}
SEM		0.151	7.9	0.032	22.9

Table 3 - Growth performance of young male pigs during the 14 to 21 day period

Grain type	Protease (ppm)	Daily gain (g/d)	FCR	Daily Intake (g/d)
Wheat Control	0	625^{a}	1.38^{bc}	839^{a}
Liberty Sorghum	0	493^{bc}	1.64^{a}	795^{ab}
	50	522^{bc}	1.55^{ab}	795^{ab}
	100	476^{bc}	1.48^{abc}	693^{ab}
	500	520^{bc}	1.50^{abc}	751^{abc}
Buster Sorghum	0	451^{c}	1.48^{abc}	659^{c}
	50	523^{bc}	1.51^{ab}	814^{ab}
	100	551^{ab}	1.48^{abc}	786^{ab}
	500	575^{ab}	1.26^{c}	715^{abc}

SEM		13.3	0.032	16.4

Table 4 - Growth performance of young male pigs during the 0 to 21 day period

Grain type	Protease (ppm)	21d weight (kg)	Daily gain (g/d)	FCR	Daily Intake (g/d)
Wheat Control	0	16.59^{a}	480^{a}	1.30^{b}	619^{a}
Liberty Sorghum	0	14.92^{b}	401^{b}	1.56^{a}	605^{ab}
	50	15.60^{ab}	432^{ab}	1.37^{b}	589^{ab}
	100	15.04^{b}	394^{b}	1.43^{ab}	559^{ab}
	500	15.77^{ab}	435^{ab}	1.31^{b}	566^{ab}
Buster Sorghum	0	14.46^{b}	380^{b}	1.43^{ab}	535^{b}
	50	15.37^{ab}	413^{ab}	1.49^{ab}	627^{a}
	100	14.56^{b}	384^{b}	1.39^{ab}	526^{b}
	500	15.31^{ab}	410^{ab}	1.33^{b}	536^{b}
SEM		0.231	9.2	0.023	11.6

Table 5 - The diet digestibility coefficients and measured digestible energy

Grain type	Protease (ppm)	Digestibility coefficient (%)	Digestible Energy (MJ/kg as fed)
Wheat Control	0	83.6^{a}	14.87^{a}
Liberty Sorghum	0	84.3^{a}	14.87^{a}
	50	83.9^{a}	14.82^{a}
	100	83.7^{a}	14.77^{a}
	500	83.4^{a}	14.73^{a}
Buster Sorghum	0	80.9^{b}	14.28^{b}
	50	81.0^{b}	14.29^{b}
	100	82.8^{a}	14.62^{a}
	500	83.3^{a}	14.70^{a}
SEM		0.24	0.043

The limitation of using sorghum in young pig diets, compared to wheat, has been confirmed again in the present study. The Liberty sorghum was expected to support superior growth performance to the Buster sorghum due to the later theoretically containing higher anti-nutritional kafirin levels, and the white sorghum was measured to be significantly higher in DE. Although pigs offered the diets based on the white sorghum numerically grew faster and ate more across all measured periods, the only actual significant difference in growth performance between the grains was observed in daily gain in the first 14 days and for feed intake during the final 7 days of the study.

Protease supplementation significantly improved feed efficiency for pigs on the sorghum based diets, and the influence was similar for both sorghum types. The highest enzyme dose of 500 ppm generally produced the best response, and achieved feed conversion rates similar to that of the wheat based diet, supplemented with xylanase. Moreover, the protease linearly increased the inferior DE of the Buster sorghum, from 14.28 to 14.70 MJ/kg (2.9%), similar to that of the wheat and Liberty sorghum based diets. Again the highest level of the enzyme produced the best response. The higher dose rates of protease limited feed intake, significant for the Buster sorghum.

The young male pig is sensitive to nutritional restrictions and anti-nutritional factors, and is ideal for assessing the effects of ingredients and supplements on growth and efficiency of feed use. However, the response in young pigs does not necessarily indicate how older pigs will respond on an absolute level, due to the gut of the young male still being immature. The influence of grain type and increasing enzyme dose rate numerically increased as the pigs gained in age, especially in the final 7 days, with daily gain and FCR improving by 21.5% and 14.8%, respectively for the Buster sorghum compared to the first 7 days. The Protease does not hydrolyse protein effectively below a pH of 5, therefore the longer the dwell time or small intestine length, the greater the effect of the enzyme. The length of the small intestine and dwell time increases as the pig grows, suggesting that the response to the protease may increase in older grower and finisher pigs.

In a study measuring the influence of 10 different sorghum varieties and/or location grown Finn et al (2007) reported that daily weight gain was significantly affected by sorghum type. The white sorghum produced a FCR of 1.30 for pigs of similar age and weight, similar to a wheat based diet (Finn et al, 2007). It had been suggested that white sorghum, cultivar Liberty, has lower amounts of anti-nutritional polyphenols and also lower in γ kafirin, the most indigestible storage protein in sorghum. In the present the white or Liberty sorghum supported a poorer FCR compared to wheat and was similar to that of the red Buster sorghum, even through the DE and diet digestibility of the white sorghum and wheat diets were similar.

Although most sorghum grains are measured to be higher in digestibility and DE than wheat, pig growth performance is equal to higher when the diets are iso-caloric with a similar essential amino balance (van Barneveld et al, 1997). There is a theory from several nutritionists and researchers that there is a lack of synchrony between the digestion and availability of starch and essential amino acids in sorghum based diets (Black et al 2005). Generally, starch solubility, and hence digestion, is lower in sorghum compared to wheat, and starch break occurs more in the distal region of the small intestine. Additionally, as sorghum is low in protein, and proportionally low in essential amino acids, higher quality protein is required in sorghum based diets to balance up the ration. Therefore, due to the diurnal feeding pattern of pigs, there is a potential essential amino acids are absorbed and arrive at tissue significantly earlier than glucose digested from sorghum starch, thus limiting protein deposition. The addition of protease, to hydrolyse the less digestible protein around sorghum starch granules, is likely to increase the rate of starch digestion and improve the synchrony of energy and protein at the tissue level.

Even though the protease improved FCR of both sorghum types, feed intake and daily gain were inferior to that of the wheat based diet, especially for feed intake of Buster based diet ($P<0.05$). One of the suggested reasons (Dr John Black personal comm.) for low feed intake of sorghum diet is due to the extremely high leucine levels relative to the other amino acids, especially isoleucine and valine. The leucine levels were 17% higher in the liberty and 19.5% higher in the Buster sorghum, compared to the wheat. High leucine levels have been reported to significantly reduce weaner pig feed intake and daily gain, but not FCR (Edmond's and Baker, 1987; Oestomer et al, 1973). Farran et al (2003) reported a significant reduction in all growth parameter in broiler chicks when leucine was increased to 6% above the control amino acid level. The main reason postulated by the authors is the significantly higher leucine content reduces blood plasma isoleucine and valine, thus creating a deficiency in both these branched chain amino acids. To correct this potential deficiency cause by high leucine levels in sorghum diets, isoleucine and valine levels need to be increased significantly. Further research is warranted to establish whether higher valine and isoleucine levels will improve feed intake and daily gain of young pigs. Currently the isoleucine to leucine and valine to leucine ratio in wheat based diet is approximately 0.54 and 0.66 respectively, where the ratios were 0.43 and 0.54 respectively for isoleucine:leucine and valine:leucine in the sorghum based diets.

4. Application of Research

Commercial applications

The commercial application is that the protease, which is registered in Australia, can be used to improve the consistency of growth of progeny pigs offer diets high in sorghum. Although pigs responded up to 500 ppm of protease, broken stick analysis suggests the cost effective rate approximately 100 ppm or 3600 units/kg of protease. The cost of application at this rate would be between $2.50 and $3.00/tonne.

Avril Finn (Pork CRC project 1B-105) showed that sorghum type significantly influence pig growth performance between 30 and 55 kg live weight. Although the percentage differences were not as high as the similar experimental design conducted in weaner pigs (Finn et al 2007), it does strongly suggest sorghum influence younger and older growing pigs in the same way. These observations also strongly indicate that older pigs will significantly respond to Subtilisin protease when offered diet with inferior quality sorghum.

The suggested benefits are an improvement in FCR of 0.05, which would reduce grower feed consumption, conservatively, by 10kg or $3.80/pig of feed (assuming average feed price of $380/tonne).

Research applications

Further research is warranted to establish the optimum protease dose rate for older progeny pigs, between 40 and 80 kg, where most of the feed is consumed. Additionally, additives like Sodium Sulphite, which reduce the cysteine bonds in kafirin potentially increasing digestion of the protein, may increase the response to the protease, or allow a lower dosage.

Lastly, the significant low isoleucine and valine to leucine ratios in sorghum based diets, mean that increasing either or both amino acids may promote feed intake and weight gain of progeny pigs. This needs to be tested in future control studies.

5. Conclusion

Overall, the unsupplemented sorghum diets depressed the performance of young male pigs and the highest dose rate of protease added to both the sorghum varieties improved feed efficiency to the levels of the wheat based diet. The two sorghums did respond differently to the increasing level of protease, with the most notable being the significant improvement in DE for the red sorghum, whereas the enzyme had no effect on the digestibility of the white sorghum. Interestingly, pigs offered the diet based on Liberty sorghum exhibited the most marked improvements in FCR and growth at the lowest Protease inclusion rate (50 ppm) whilst for Buster feed efficiency was improved most at the highest dose (500 ppm) and growth rate at the lowest dose. The results indicate the protease can reduce the nutritional barriers caused by the structural and storage proteins in sorghum, and improve feed efficiency to the level supported by wheat based diets. The most cost effective dose rate of the protease is most likely to be between 50 and 500 ppm but would appear to vary with variety, and further research is warranted to establish the optimum level of enzyme inclusion and to relate this to the chemical composition of the sorghum grain.

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