Effect of thermal ammoniation and heat treatment of high-tannin grain sorghum on the TME value for roosters and relative nutritive value for rats

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An experiment was conducted to (i) determine the effect of heat treatment and thermal ammoniation on dry-matter digestibility and true metabolizable energy content of high-tannin grain sorghum in a digestion trial with roosters and (ii) to determine the relative nutritive value of the different test components in a slope ratio assay with rats. Thermal ammoniation reduced the tannin content of high-tannin grain sorghum by 55,1% whereas heat treatment *per se* had no effect. Dry-matter digestibility and true metabolizable energy content were significantly ($P \leq 0,01$) improved by 4,7 and 8,9% by thermal ammoniation, respectively. In the slope ratio assay with rats, no significant differences were obtained in the relative nutritive value of the test components.

'n Eksperiment is uitgevoer om (i) die invloed van hittebehandeling en termiese ammonifisering op die droë-materiaalverteerbaarheid en ware metaboliseerbare energie-inhoud van hoë-tannien graansorghum in 'n vertering-studie met hoenders te bepaal en om (ii) die relatiewe voedingswaarde van die verskillende toetskomponente in 'n groeistudie met rotte te bepaal. Termiese ammonifisering het die tannieninhoud van hoë-tannien graansorghum met 55,6% gereduseer, terwyl hittebehandeling *per se* geen invloed gehad het nie. Termiese ammonifisering het die droëmateriaalverteerbaarheid en ware metaboliseerbare energie-inhoud betekenisvol ($P \leq 0,01$) verhoog met 4,7 en 8,9% onderskeidelik. In die groeistudie met rotte kon egter geen betekenisvolle verskille in die relatiewe voedings-waarde van die verskillende toetskomponente gevind word nie.

Keywords: Relative nutritive value, sorghum, tannin content, thermal ammoniation.

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Introduction

The production of grain sorghum in South Africa for the 1986/87 season amounted to 430000 ton, of which bird-proof sorghum (BPS) contributed 11–15% (Grain Sorghum Board, 1988). The tannin in BPS provides a natural protection system against bird depredation (McMillan, Wiseman, Burns, Harris & Greene, 1972) and the production of BPS is important in countries where these stresses are severe. However, the tannin in BPS depresses protein, energy, and amino acid digestibilities (Kemm, Ras & Daiber, 1984; Myer, Gorbet & Combs, 1986; Rostagno, Featherson & Rogler, 1973), metabolizable energy content (Gous, Kuyper & Dennison, 1982; Halley, Nelson Kirby & York, 1986), growth rate (Kemm *et al.*, 1984; Myer & Gorbet, 1985) and feed conversion (Grosjean & Castaing, 1984; Rostagno *et al.*, 1973) when incorporated into pig diets.

The negative effect of tannin on the performance of growing pigs was also observed by various other research workers (Almond, Smith, Savage & Lawrence, 1979; Cousins, Tanksley, Knabe & Zebrowska, 1981; Ford, 1977; Tanksley, 1975).

The deleterious effects of tannins in a diet seem to be related to their interaction with dietary proteins. Tannin–protein complexes are believed to be responsible for growth depression and low protein digestibility (Desphande, Cheryan & Salunkhe, 1986; McLeod, 1974). Various methods were applied to reduce the tannin content in forages (Kumar & Singh, 1984). Although digestibility was improved and tannin content reduced in the most cases, the improvements had no beneficial effect on the feedlot performance of pigs (Kemm, Daiber & Ras, 1981; Mitaru, Reichert & Blair, 1984). Treatment was not economical as a result of the cost of the chemicals used (Kemm & Ras, 1985; Myer *et al.*, 1986) or resulted in a loss of dry matter during the removal process (Sing & Arora, 1978; Panda, Sahu & Mohapatro, 1979).

According to Price, Butler, Featherson & Rogler (1978), ammoniation of high-tannin grain sorghum with concentrated NH_4OH (28–30% NH_3) has been shown to decrease assayable tannin to approximately 30% of the original level and to greatly improve the growth rate and efficiency when fed to rats and chicks. Swiegers, Davie, Kühn & Slabbert (1987) used a practical and rapid ammoniation method, where hot anhydrous ammonia (NH_3) is circulated through the grain in an oven. In their study they used both whole and grinded sorghum at two moisture levels (10 and 20%). They treated the grain with 0, 7,5, 15 and 30 g NH_3/kg DM. They found that the physical condition of the grain had no influence on the treatment. An increase in moisture content resulted in a decrease in tannin content, but had no influence on digestibility. They concluded from their study that ammoniation at a 1,5% NH_3 level was the most effective to deactivate the tannin. The tannin content of BPS was reduced from 1,33 to 0,33% and *in vitro* digestibility improved by 6%.

This experiment was therefore conducted to determine the effect of thermal ammoniation and heat treatment on

the true metabolizable energy (TME) content for roosters and relative nutritive value (RNV) for rats. The treatment conditions, recommended by Swiegers (1987), were used.

Experimental Procedures

Grain from a high-tannin sorghum cultivar (class GH sorghum) with a polyphenol content of 1,24% was used in the study. The polyphenol content was determined by the modified Jerumanis procedure as described by Daiber (1975). The sorghum was milled through a 3-mm screen, divided into three batches and treated as follows:

1. BPS (no treatment applied).
2. BPS thermally ammoniated (NH_3BPS) in a commercial An-Stra-Verter® oven. The sorghum was placed in the chamber and the door closed airtight. Anhydrous ammonia was let in at a level of 15 g NH_3/kg DM. The ammonia gas was circulated in the chamber for 15 h and temperature increased to 90°C. The heat element was then switched off and while the temperature declined the ammoniation process continued for 4 h. The chamber was ventilated with fresh air for another 4 h after which the process was completed. Treated sorghum was stored for a few days in order to let the excess ammonia evaporate, before use.
3. The same process as in 2, except that no ammonia gas was released into the chamber (HBPS).

Digestion trial

Digestion trials were carried out with 13 adult roosters (Amber-Link egg-laying strain), approximately 14 months of age. They were housed in single cages equipped with water nipples and special feed troughs to minimize spillage. Room temperature was not controlled and fluctuated between 12 and 28°C. A 16 h lighting regime was applied. The Dual-Semi-Quick (DSQ) feeding method, as described by Du Preez, Minnaar & Duckitt (1984), was used. The roosters were allowed free access to the three test diets during a four-day test period. Measurements of feed consumption and excreta collection were only carried out during the last three days of the experimental period. Test materials were mixed with a maize basal diet in the proportion of 3:7. The basal diet consisted of 98% maize and a 0,2% vitamin/mineral premix.

The true ME values of the feedstuffs were calculated by correcting apparent ME for endogenous energy losses. In the present experiment this was done by feeding dextrose during a three day collection period.

Gross energy determinations were carried out on a Gallenkamp adiabatic bomb calorimeter. Excreta samples were not pooled and the BPS, HBPS and NH_3BPS were fed to 4, 5 and 6 roosters, respectively. Differences between treatment means were tested for significance by analysis of variance (Snedecor & Cochran, 1980).

Growth trial

The growth assay, to determine the RNV of the experimental diets, was carried out with rats. Sixty 27-day-old SPF (Specific Pathogen Free) Wistar rats were used. The rats were individually housed in metabolism cages at 20°C with a 12 h lighting regime. Lactalbumin (LA), BPS, HBPS and NH_3BPS were used in combination with maize starch, sunflower oil and a vitamin and mineral mixture to formulate 17 experimental diets to contain different amounts of protein (Table 1).

The rats were divided into 17 groups of approximately equal mass with two females and one male in every group (the group that received the N-free diet contained an additional three rats) and were then allocated randomly to the experimental diets. Six rats (the initial slaughter group) were asphyxiated at the onset of the experiment and the chemical composition was determined according to the method described by Davie (1988).

The experimental diets were fed *ad libitum* for 20 days, after which the rats were killed and treated similarly to the initial slaughter group. Body protein was chosen as the measure of response to the dietary protein, because the most meaningful response criterion is based on carcass nitrogen deposition (Sauer & Ozimek, 1986). Protein quality was determined by means of a multipoint slope-ratio assay developed by Hegsted, Neff & Worcester (1968) as used by Siebrits, Esterhuyse & Kemm (1985).

Table 1 Composition of the experimental diets on an air-dry basis (%)

	Lactalbumin					Bird-proof sorghum[a]			
Components	0	2% CP	4% CP	6% CP	8% CP	2% CP	4% CP	6% CP	8% CP
Maize starch	85	81,9	78,7	75,6	72,5	67,6	50,2	32,8	15,4
Sunflower oil	10	10	10	10	10	10	10	10	10
Mineral & vitamin premix[b]	5	5	5	5	5	5	5	5	5
Lactalbumin	–	3,1	6,3	9,4	12,5	–	–	–	–
Sorghum	–	–	–	–	–	17,4	34,8	52,2	69,6

[a] Diets contained BPS, HBPS or NH_3BPS in the quantities given below. Dietary crude protein corrected for increased nitrogen due to ammoniation.

[b] Supplied per kg feed: Vitamin A, 2,0 IU; Vitamin D, 1 000 IU; Vitamin E, 35 mg; Vitamin K, 50 g; Thiamin hydrochloride, 1,25 mg; Vitamin B6, 7 mg; Vitamin B12, 5 g; Calcium pantothenate, 8 mg; Niacin, 15 mg; Choline chloride, 750 mg; Cu, 5 mg; Mn, 50 mg; Zn, 12 mg; I, 0,15 mg; Fe, 35 mg; Se, 0,04 mg; Mg, 0,4 g; P, 4,0 g; K, 1,8 g; Na, 0,5 g; Ca, 5,0 g.

The slope-ratio technique was used, because it has the advantage that it assigns an availability value of nutrients based directly on the performance of the growing animal. The initial body protein contents of the final slaughter groups were derived from the linear regression equation that was fitted to the data of the initial slaughter group. The growth response used was the accumulation of body protein determined by subtracting initial body protein from final body protein (Siebrits *et al.*, 1985). The efficiency of protein utilization for protein deposition was estimated as the coefficient (b) of the linear regression (y = a + bx) of body protein gain (y) and protein intake (x). Data from rats fed the protein-free diet were included in the regression calculation. A common intercept was fitted for all the protein sources. The RNV is the ratio of the regression coefficient (b) for the test diet to that of the reference diet (Hegsted *et al.*, 1968; Siebrits *et al.*, 1985). The regression lines were statistically analysed by analysis of co-variance (Snedecor & Cochran, 1980).

Results and Discussion

The effect of the different treatments on the chemical composition of BPS is presented in Table 2. Treatment with heat and NH_3 reduced the polyphenol content of the grain from 1,24 to 1,14 and 0,55%, respectively. These reductions were appreciably lower than the 75% reduction in assayable tannin found by Swiegers (1987) and the 93% reduction found by Ford & Hewitt (1979a). They used 36 g ammonia solution, *ca.* 350 g NH_3/kg per 320 g sorghum grain and allowed it to stand for seven days at room temperture. Reductions of 94 and 87% respectively, were found by Price, Butler, Rogler & Featherson (1979), who used concentrated NH_4OH and 2,5M-NH_4OH and treated the grain for 22 and 18 days respectively, at room temperature. The difference in the extent of tannin reduction may possibly be due to factors such as grain variety (Mitaru, Reichert & Blair, 1985) and moisture content (Teeter, Sorani, Smith & Hibberd, 1986). Owing to the presence of a large number of polar groups, the tannin molecule is soluble in polar solvents such as water and it appears that moist conditions enhance the detoxification reaction as shown by Price *et al.*, (1978). They found that treatment of BPS with gaseous NH_3 improved the nutritional quality of high-tannin sorghum to a lesser degree than grain moistened with NH_4OH.

Table 2 Chemical composition (on dry-matter basis) of the test components

Component	Dry matter (%)	Crude fibre (%)	Crude protein (%)	Neutral detergent fibre (%)	Starch (%)	Tannin (%)	Gross energy (MJ/kg)
BPS	90,16	2,45	11,50	6,28	76,26	1,24	16,95
HBPS	91,32	2,55	11,75	5,91	72,02	1,14	17,49
NH_3BPS	90,22	2,05	13,63	5,92	72,65	0,55	17,54

Ammoniation increased the crude protein (CP) content of BPS from 11,5 to 13,63% which is in accordance with the 17,2% increase found by Ford & Hewitt (1979b). Heat treatment *per se* led to a small increase in CP content, which was possibly due to residual ammonia in the oven. It was assumed that ammonia was of no value to the chickens and rats and was excreted as urea. Therefore the CP content of the experimental diets was corrected for increased nitrogen content due to ammoniation.

Digestion trial

Results on digestibility data for the BPS, HBPS and NH_3BPS-containing diets fed during the digestion trial are summarized in Table 3. Ammoniation and heat treatment had no significant effect on total dry-matter (DM) intake, although DM intake was slightly higher in the NH_3- and heat treatment group. Gandhi, Cherian, Mulky & Menon (1975) processed sal seed meal with NH_3 to depolymerize tannins and observed that the processed meal was more palatable. Heat treatment had no significant influence on DM digestibility whereas NH_3 treatment improved the DM digestibility significantly ($P \leq 0,01$). The present results appear to be slightly higher than the improvement of 2,5% found by Reichert, Flemming & Schwabb (1980) who treated BPS with 0,25N-NH_4OH for 48 h at 25°C. Besides the improvement in DM digestibility due to the reduction in tannin content, thermal ammoniation may also have caused ammonolysis of ester groups (Buettner, Lechtenberg, Hendrix & Hertel, 1982), which may have further improved digestibility.

Heat treatment *per se* had a small and non-significant effect on the TME content of BPS. Thermal ammoniation, however, improved the TME content of BPS significantly ($P \leq 0,01$) by 8,9%.

Table 3 Means (± *SD*) for the parameters measured with the DSQ method using adult roosters during the digestion trial

Measurement	Diets		
	BPS (control)	HBPS	NH_3BPS
Dietary crude protein content [a] (%)	11,5	11,5	11,5
DM intake (g)	245,3 ± 39,5	277,8 ± 54,4	277,5 ± 35,8
DM digestibility (%)	78,33 ± 0,86	77,87 ± 0,86	82,05[b] ± 1,16
True metabolizable energy (MJ/kg)	12,61 ± 0,17	12,58 ± 0,09	13,72[b] ± 0,21

[a] Dietary crude protein corrected for increased nitrogen content due to ammoniation.

[b] Means significantly ($P \leq 0,01$) higher than control.

Growth trial

The regression equations describing the relationships between protein ingested and deposition by the rats on the different dietary treatments as well as RNV values are given in Table 4. A common intercept (o) was fitted for all the test components. The initial body protein contents of the final slaughter groups were calculated from the linear regression equation

$y = 15{,}241 - 0{,}016x$ (Syx = 0,49; R^2 = 0,11)

obtained from the data of the initial slaughter group.

Table 4 Regression equations of the retationships between protein consumed and protein deposited by rats on different dietary treatments as well as the relative nutritive values (RNV)

Diets containing	Regression[a] Equation ± *SD*	R^2	Syx	RNV
Lactalbumin	Y[b] = 0,305[c]x ± 0,02	0,937	1,263	1
BPS	Y = 0,188 x ± 0,03	0,800	0,540	0,616*
HBPS	Y = 0,172 x ± 0,03	0,758	0,602	0,564*
NH_3BPS	Y = 0,182 x ± 0,03	0,729	0,622	0,597*

[a] Intercept = 0.

[b] Protein deposited.

[c] Protein intake.

* Differ significantly ($P \leq 0{,}01$) from lactalbumin.

Results obtained from the slope-ratio assay indicate that heat treatment *per se* reduced the RNV of BPS from 0,616 to 0,597. The difference was not significant. Price, Butler & Hagerman (1980) also found that rats fed boiled high-tannin grain sorghum gained even less weight than rats fed untreated high-tannin grain. Thermal ammoniation also had a small and non-significant effect on the RNV of BPS. The RNV of BPS was reduced by thermal ammoniation, but not to the extent of heated BPS. These results are in contrast with results from Reichert *et al.*, (1980) who reported an improvement of 19,7 to 71,4 g in weight gain, 12,52 to 5,02 g in feed efficiency rate and 0,79 to 1,86 in protein efficiency ratio in a four-week rat growth performance trial with ammoniated high-tannin sorghum. Price *et al.* (1979) also found similar feed efficiencies and weight gains with chickens fed an untreated low-tannin sorghum diet and a high-tannin ammoniated sorghum diet. Ford & Hewitt (1979a) found an improvement in the RNV of high-tannin sorghum from 43 to 96 after ammoniation with ammonia solution, as measured microbiologically with *Streptococcus zymogenes*.

Conclusions

The mechanism by which ammoniation detoxifies high-tannin sorghum, as well as the action of tannin in lowering the nutritional quality of high-tannin diets, have not yet been solved. From our studies it could be concluded that heat *per se* had a detrimental effect on the nutritional value of grain sorghum. Thermal ammoniation, however, improved DM digestibility and TME content significantly. No improvements were, however, expressed in terms of a higher RNV. This phenomenon could be the consequence of damage of lysine as a result of the Maillard reaction between the free carboxyl groups of reducing sugars and the free amino groups of proteins. The Maillard reaction occurs when conditions are suitable and temperatures high (± 90°C) with moderate moisture (Mauron, 1972; Hurrell & Carpenter, 1974). The Maillard reaction also occurs during thermal ammoniation (Krawelitzki & Nehring, 1973).

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