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THE RELATIONSHIP BETWEEN TANNIC ACID CONTENT AND METABOLIZABLE ENERGY CONCENTRATION OF SOME SORGHUM CULTIVARS

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OPSOMMING: DIE VERWANTSKAP TUSSEN TANNIENSUUR EN METABOLISEERBARE ENERGIE KONSENTRASIES IN VERSKILLENDE GRAANSORGHUMCULTIVARS

Die skynbare en ware metaboliseerbare energie konsentrasies van 22 graansorghumcultivars, wat van tanniensuurinhoud verskil het, is bepaal deur monsters van die verskillende variëteite aan volwasse hane te voer. A hoogsbetekenisvolle negatiewe korrelasie tussen die M.E. van die graan en sy tanniensuurkonsentrasie is gevind. Hierdie verwantskap is te wyte aan 'n verlaagde verteerbaarheid met 'n vermeerdering van die tanniensuur konsentrasie.

Die bevinding word bespreek met betrekking tot die moontlike oorsake van sulke verlaagde verteerbaarheid, metodes waardeur die nadelige gevolge van tanniensuur verminder kan word, en die belangrikheid daarvan om die tanniensuur konsentrasie van sorghum grane wat vir die voeding van nie-herkouers bestem is, te bepaal.

SUMMARY:

The apparent and true metabolizable energy concentrations of 22 cultivars of sorghum grains, differing in their tannic acid content, were determined by feeding samples of the different varieties to adult roosters. A highly significant negative correlation was found to exist between the M.E. of the grain and its tannic acid content, this relationship being due to a decreased digestibility with increasing tannic acid concentration.

This finding is discussed in relation to the possible causes of such reduced digestibility, means by which the adverse effects of tannic acid can be reduced, and the importance of determining the tannic acid content of sorghum grains destined to be fed to non-ruminants.

In an effort to reduce the amount of sorghum grains consumed by wild birds prior to harvesting, plant geneticists have selected and propagated sorghum cultivars containing high levels of polyphenols (tannins). These birdproof varieties contain from 5 to 25 times more tannin than cultivars of non-birdproof grain (Daiber & Van Bellinghen, 1976). Since they are often the only cereal crops grown in areas ravaged by gregarious birds and, moreover, produce superior yields, birdproof varieties make up about a third of the total South African sorghum harvest (Daiber & Van Bellinghen, 1976).

Unfortunately, the high concentrations of polyphenols in the bird-proof sorghum cultivars reduce the value of these sorghums for feeding purposes. A number of reports have indicated the adverse effects on growth rate and feed conversion efficiency in both rats and poultry (Chang & Fuller, 1964; Armstrong, Featherston & Rogler, 1974; Dreyer & Van Niekerk, 1974; Rogler, Featherston, Elkin & Giles, 1978). In an effort to overcome these effects a number of workers have reported on the effect of supplementing diets containing high-tannin sorghums with methyl donors, including methionine, methylation being a recognised pathway for polyphenol detoxification. Some of these reports have indicated that methionine ameliorates the growth depression occasioned by tannic acid (Chang & Fuller, 1964; Rogler *et al.*, 1978). However, others (Armstrong *et al.*, 1974; Queiroz, Rostagno, Fialho & Fonseca, 1978) were not able to overcome the growth depressing effects of high tannins by methionine supplementation. The latter workers obtained an enigmatic response to lysine supplementation, but the mechanism of this effect remains unclear.

With regard to the effect of tannins on the metabolizable energy (M.E.) of sorghums, Sibbald (1977) published values of 3870 (16,19) and 3380 kcal/kg (14,14 MJ/kg) for the True metabolizable energy (T.M.E.) of low tannin – and high tannin – sorghums respectively. Queiroz *et al.*, (1978) found the values to be 3091 (12,93) and 2886 kcal/kg (12,07 MJ/kg) for the Apparent metabolizable energy (A.M.E.) of the equivalent sorghum grains. (T.M.E. differs from A.M.E. in that, in calculating the former value, the metabolic faecal energy (FE_m) and endogenous urinary energy (UE_e) voided during the test period is estimated and subtracted from the total energy voided in the urine and faeces).

In this report, 22 cultivars of sorghum grains were fed to cockerels in order to determine the A.M.E. and T.M.E. values of each variety, and these values were then compared with the polyphenol content of the respective varieties in order to determine if the previously-published relationship holds for a large number of sorghum cultivars of different tannin content. In addition, the M.E. of a high-tannin sorghum cultivar was measured prior to, and following treatment by means of a process patented by the C.S.I.R. (1976), which has the effect of improving the digestibility of protein (Kemm, Daiber & Ras, 1981). By comparing the M.E. concentrations of the same cultivar, but containing either high or low tannin concentrations, an improved estimate of the effect of tannic acid on the M.E. of sorghums could be determined.

Experimental procedure

Samples of 22 sorghum cultivars were obtained from the Department of Agriculture and Fisheries for this study. Ten of these samples were known to contain high concentrations of tannins, the remaining samples being relatively free of this compound.

Determination of M.E.

The method of Sibbald (1976) was used to determine both the A.M.E. and the T.M.E. of the 22 varieties of sorghum. The method was essentially as follows: after a 24-hour fasting period, adult Amber-link cocks whose mean body mass was 3125 g were each fed a 40 g pelleted sample of each sorghum variety. The excreta voided during the following 24 h were collected, freeze-dried and the dry mass of each sample was then measured. The gross energy of each excreta sample was determined in duplicate, as was the gross energy of each sorghum variety, by means of a Parr adiabatic bomb calorimeter. An estimate of the ($F.E._m + U.E._e$) was obtained by measuring the total gross energy of the faeces voided by birds housed together with the test birds, during the latter 24 h of a 48 h fasting period. Six birds were chosen at random and used for this purpose during each test period. With 50 cockerels available and one test being conducted each week, the experiment was completed during a 3-week period, with 2 replicates of each sorghum variety being fed each week.

The T.M.E. was calculated according to the following equation:

$$T.M.E.\ (MJ/kg) = \frac{(G.E._f \times X) - (Ye - (F.E._m + U.E._e))}{X}$$

Where $G.E._f$ is the gross energy of the feed
X is the amount of feed supplied (g)
Y_e is the gross energy voided as excreta.

The A.M.E. is calculated from the above equation, but ignoring ($F.E._m + U.E._e$).

Determination of polyphenol content

The total polyphenol content of each sorghum cultivar was determined according to the modified method of Jerumanis (1972) as outlined by Daiber (1975). Duplicate samples (250 mg per sample) of each cultivar were mixed with 5 ml 75 percent dimethylformamide by rotation for one hour, then centrifuged for 10 min. The determination of tannic acid concentrations was carried out in triplicate for each sample, 3 ml of the supernatant being divided into 1 ml aliquots in 3 test tubes. Distilled water, 0,1% polyethylene glycol 1500 BDH, 1% ferric ammonium citrate BDH and 8% ammonia were added in the ratio of 1 (sample): 4,4 : 1,2 : 0,8 : 0,8. Mixing between the addition of constituents was carried out on a "Whirlimix". Absorbance was read at 520 μm. The sample blank contained distilled water in place of the ferric ammonium citrate and sorghum aliquot. The concentration of polyphenols was calculated from the standard curve obtained with similarly-treated solutions (0–5 mg/ml) of tannic acid.

Formaldehyde treatment of birdproof sorghum

Samples of birdproof sorghum, class KF, cultivar SSK2, with a total polyphenol content of 1,32% were treated with formaldehyde in order to reduce the polyphenol content. One sample was steeped for 4 h in 0,09% W/W formaldehyde at 17°C (the optimal treatment), whilst a second sample was steeped for 6 h in the same concentration of formaldehyde at 18°C (double the optimal treatment). After the predetermined time the dilute formaldehyde was drained and the grain was rinsed with tap water for about 15 min., after which the grain was dried for 10 h at 50° to 60°C in a force draught kiln.

This process was not undertaken by the authors, but samples of the untreated and 2 treated grains were obtained from K.H. Daiber (Sorghum Beer Unit of the C.S.I.R.). The polyphenol contents of these 3 samples presented in this paper were determined by the C.S.I.R., using the technique outlined above.

Table 1

Tannic acid concentrations of the sorghum cultivars, mass of excreta voided, and apparent and true metabolizable energy concentrations of grains

Sorghum cultivar	Tannic acid concentration (percent)	Excreta mass (g)	A.M.E. (MJ/kg)	T.M.E. (MJ/kg)
SSK 30	1,53	9,88 ± 1,35[1]	11,73 ± 1,63[1]	13,13
SSK 52	1,53	7,91 ± 1,88	11,49 ± 2,73	12,88
SSK 2	1,51	9,45 ± 1,91	10,71 ± 2,16	12,11
SSK 20	1,41	10,67 ± 1,76	10,35 ± 2,68	11,75
SSK 28	1,31	10,67 ± 1,78	10,90 ± 1,82	12,29
H 731	1,23	10,96 ± 1,67	11,06 ± 2,69	12,46
SSK 22	1,10	9,88 ± 1,26	11,48 ± 1,46	12,87
NK 300	0,96	7,80 ± 1,42	11,82 ± 2,06	13,22
DC 99	0,80	7,64 ± 1,02	11,99 ± 1,55	13,39
DC 72	0,75	8,89 ± 1,02	12,19 ± 2,24	13,59
C 42[A]	0,28	6,85 ± 1,93	11,91 ± 3,06	13,31
BARNARD RED	0,26	6,20 ± 1,02	13,35 ± 2,23	14,75
NK 202	0,16	7,74 ± 0,94	12,76 ± 1,57	14,16
NK 222	0,10	6,46 ± 2,09	13,32 ± 2,31	14,72
PNR 8324	0,10	6,21 ± 1,66	13,51 ± 3,96	14,91
NK 283	0,08	6,23 ± 1,31	13,19 ± 2,62	14,59
PNR 8311	0,06	6,48 ± 1,14	12,84 ± 2,23	14,24
DC 34	0,06	7,87 ± 0,97	12,99 ± 1,61	14,39
DC 59	0,04	7,79 ± 1,94	13,26 ± 3,10	14,66
DC 36	0,02	6,84 ± 0,96	13,80 ± 2,00	15,20
G 766W	0,00	5,98 ± 0,81	12,87 ± 1,74	14,27
C 40	0,00	6,23 ± 6,09	12,88 ± 1,40	14,28
SSK 2[2]				
Untreated	1,32	8,93 ± 2,55	11,67 ± 3,33	13,07
Optimal treated	0,24	7,38 ± 1,17	12,14 ± 1,92	13,54
Over treated	0,13	6,31 ± 1,81	12,79 ± 3,67	14,19

[1] Mean ± standard error of the mean

[2] Effect of formaldehyde treatment of cultivar SSK2. Tannic acid concentrations of these three samples were determined at the C.S.I.R. (K.H. Daiber, personal communication)

Results and Discussion

In calculating the T.M.E. of feedingstuffs an estimate of the daily ($F.E._m$ + $U.E._e$) excretion by the birds is required. In this study, the value of the above excretion voided by the birds was estimated to be 14,64 ± 0,4 kJ/kg body mass d. This value was used in calculating the T.M.E. of the sorghum grains.

The gross energy contents of the different sorghum cultivars proved to be very similar to one another, the mean and standard error of the mean of all 22 varieties being 16,26 ± 0,304 MJ/kg. The mass of excreta voided during the 24 hours following the feeding of each sample varied considerably between samples, reflecting large differences in the digestibility of the various sorghum cultivars. Values for excreta voided and the resultant A.M.E. and T.M.E. concentrations are given in Table 1, together with the tannic acid concentrations of the respective sorghum cultivars.

This data is also presented in graphic form to illustrate the relationship between A.M.E. and tannic acid concentration (Fig. 1). The equation expressing the regres-

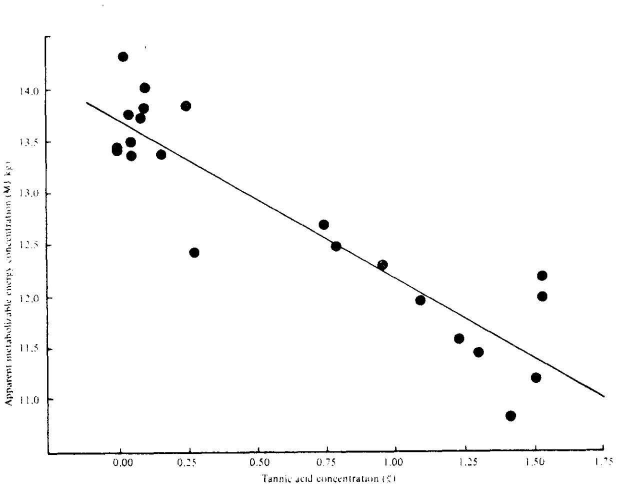

Fig. 1 *Regression of apparent metabolizable energy concentration (MJ/kg) on the tannic acid concentration (%) of 22 sorghum cultivars*

sion of A.M.E. (Y) on tannic acid concentration (X) is as follows :

$$Y = 13{,}19 - 1{,}497\ X$$

This regression proved to be highly significant, as can be seen from the analysis of variance table presented in Table 2. Also shown in Table 2 is an analysis of variance of the regression relating mass of excreta voided (Y) to tannic acid concentration (X), the regression equation in this case being :

$$Y = 6{,}61 + 2{,}049\ X$$

This equation also proved to be highly significant in describing excreta mass voided in terms of tannic acid concentration.

It might be argued that the tannic acid concentrations of the various sorghum cultivars were such that 2 divergent populations existed in respect of such concentrations, leading to an unjustifiably good relationship between A.M.E. and tannic acid concentration. Two important considerations would militate against such an argument. First, although the majority of samples had either high tannic acid concentrations or virtually none at all, there were nevertheless sorghum cultivars whose tannic acid contents satisfactorily covered the range between these 2 "populations", and the relationship between A.M.E. and tannic acid in these intermediate cultivars followed almost exactly the regression line shown in Fig. 1. Second, the fact that, by reducing the tannic acid concentration by means of formaldehyde treatment, the A.M.E. of the sorghum was improved, albeit only slightly, (Table 1), indicates that a negative relationship appears to exist between these 2 varieties. The large variation in the A.M.E. concentrations of the treated SSK samples does not allow definite conclusions to be drawn as to the relationship between A.M.E. and tannic acid concentration, but further research on this subject is definitely warranted. The tannic acid concentrations of birdproof varieties of sorghums determined in our laboratory correlate well with values published previously (Daiber, 1975), although the actual concentrations are in most cases between 85 and 90 percent of those published previously. Such a discrepancy would have the effect of altering the slope of the regression line expressing

Table 2

Analyses of variance for the linear regressions of A.M.E. and mass of excreta on tannic acid concentrations of sorghum cultivars

Source	D.F.	S.S.	M.S.	F
A.M.E. vs tannic acid concentration:				
Due to regression	1	17,25	17,252	88,02**
Deviations (residual)	20	3,93	0,196	
Total	21	21,182	1,008	
Excreta mass vs tannic acid concentration:				
Due to regression	1	33,59	33,586	28,27**
Deviations	20	23,76	1,188	
Total	21	57,34	2,731	

A.M.E. in terms of tannic acid concentration, causing it to fall less steeply than it does in Fig. 1, but would not alter the significant negative correlation between A.M.E. and tannic acid concentration.

Dreyer & Van Niekerk (1974) suggested that some of the possible causes of the reduced protein digestibility of birdproof sorghum cultivars, and the consequent poor growth performance of chickens and rats fed such grains, (Chang & Fuller, 1964; Armstrong *et al.*, 1974; Rogler *et al.*, 1978) could be the effect on palatability; the tannins or their derivatives combining with dietary proteins to form indigestible complexes, thus rendering the protein unavailable for biological utilization, the blocking of active sites in digestive and other enzymes; or, following their absorption through the gut wall, they could become toxicants on a metabolic level.

In studying the problem using the technique employed here, the first and last of the above causes of impaired performance could both be discarded : the first, because an exact amount of feed was placed in the crop of each bird thereby overcoming any palatability difference between samples; the second because the mass of excreta voided was significantly greater among birds fed birdproof sorghum grains, indicating that the problem originates prior to, or during, absorption of the grain. A further reduction in nutritive value is, of course, possible due to toxicants being produced within the gut wall, but it would not be possible to prove their presence using the above technique. It would be possible to study the effect of the presence of tannic acid on the uptake of individual amino acids using *in vitro* techniques, but to date no such studies have been undertaken. The present study would indicate that in order to further elucidate the reason for the impaired performance of chickens fed sorghums containing high concentrations of tannic acid, *in vitro* studies of the effect of tannic acid on absorption of nutrients might well be the next appropriate step, thereby separating the processes of digestion and absorption. It is our opinion that tannic acid causes more damage to the feedstuff either during digestion or absorption, than after nutrients (and tannic acid?) have been absorbed into the body.

The reduction in the apparent digestibility of birdproof sorghum grains, making use of the equation relating excreta voided vs tannic acid concentration given above, amounted to 64,8 percent (6,61 g voided at zero tannic acid concentration vs 10,195 g voided at 1,75 percent tannic acid). Part of this reduction apparently can be overcome by supplementing the diet with methionine or choline (Chang & Fuller, 1964) and the use of this method of detoxifying the tannins present in the sorghum would obviously be of benefit when birdproof sorghum varieties are being fed. Detoxification by means of treatment with formaldehyde appears to improve the A.M.E. of the birdproof sorghum grains, but the "overtreated" sample appeared to be more satisfactorily treated than the sample treated "optimally". Further evaluation of the formaldehyde process on reducing the deleterious effects of feeding birdproof sorghum varieties apparently is required. The method of evaluation presented in this paper, together with *in vitro* absorption studies would appear to be amoung the most effective and inexpensive methods available for such an evaluation.

The important consequence of the results of this study is that, whereas in the feed industry in South Africa all sorghum cultivars are assigned the same M.E. value, (protein concentrations are regularly adjusted according to the results of chemical determinations conducted at most of the larger feed mills) no account is taken of the deleterious effect of tannin content on either M.E. or protein digestibility of the grain. If large amounts of sorghum grains are included in the diets of pigs or poultry the M.E. of such diets could be significantly different from the expected value, depending on the tannic acid content of the sorghum cultivar being used. For this reason it would be an advantage if those feed compounders using large quantities of sorghums in diets of non-ruminants would include in their quality control programme the chemical determination of total polyphenols in the sorghum grain being used in the mill, and accordingly make appropriate adjustments to the M.E. and protein contents assigned to the sorghum.

The routine determination of tannic acid content of sorghum grains would serve a dual purpose : use could be made of a regression equation of the type presented in this publication, and this would provide an improved estimate of the M.E. content of the sorghum grain being used without the necessity of conducting biological tests at regular intervals; and it would indicate to the feed compounder whether the protein digestibility would be likely to be affected (in the case of birdproof sorghum varieties), in which case remedial action could take place. In the long term it might be preferable for all birdproof sorghums destined to be fed to non-ruminants to be treated with formaldehyde, or some such detoxifying process, if it can be conclusively shown that this would lead to an improvement in the utilization of sorghum grains by non-ruminants.

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