Growth-Suppressing and Related Effects on Rats of Unextracted and Ethanol-Extracted Grains of Certain Sorghum Cultivars

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SUMMARY

Samples of the grains of 6 different sorghum cultivars, whole as well as ethanol-extracted to reduce tannin content, were fed in balanced diets to young male rats (12 per sample) in an experiment aimed at assessment of the effect of sorghum grain feeding on growth rate and liver lipid content, as well as determination of the digestibility of the sorghum protein. The possible involvement of tannins in the biological utilisation of the sorghum grains was also considered.

The results revealed statistically significant differences among cultivars, mainly in respect of effect on growth rate and protein digestibility. With regard to the possible cause(s) of such differences it was found that there were significant correlations between (*i*) growth rate and protein digestibility; (*ii*) protein digestibility and dietary tannin content; and (*iii*) growth rate and dietary tannin content. The degree of correlation observed varied in descending order from (*i*) to (*iii*).

It was concluded that differences among cultivars in respect of effect on growth were essentially due to differences in protein digestibility, and that the digestibility figure provides the most convenient basis for selection of cultivars for breeding purposes.

Observed sample-to-sample variations in protein digestibility and effects on growth were only partially explicable in terms of variations in dietary tannin content.

The data obtained on liver lipid content could not be explained on the basis of the reaction involving detoxification of gallic acid through O-methylation and the consequent reduction of available supplies of the methyl donors, methionine and choline.

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Local production of grain sorghum (*Sorghum vulgare*) during the 1971/72 season was estimated at 653 000 metric tons. Of the 322 000 tons exported, 162 428 tons (i.e. about 51%) were purchased by Japan.[1] These figures reflect the importance to South Africa of exports to the Orient, where the grain is used essentially as an ingredient of chick feeds. In South Africa grain sorghum is of direct as well as indirect importance to human nutrition: it is traditionally used in the brewing of Bantu beer and is also incorporated into livestock feeds.

Japanese buyers have from time to time found reason to complain to the Maize Board, which also controls grain sorghum production in South Africa, that some of our sorghum cultivars have growth-suppressing effects when incorporated into chick feeds. It has been suggested to the Board that the high tannin content of some of the newly-bred bird-resistant cultivars may be the cause of this effect.

Although it seems reasonable to suspect that attempts by the geneticists to breed bird-resistant cultivars might result in the eventual introduction of grains which are also chick-resistant, little experimental work appears to have been done up to the present towards elucidation of questions such as (*a*) are some of our grain sorghum cultivars in fact growth-suppressing?, (*b*) is the polyphenol (tannin) content implicated in such an effect, and if so, to what extent?, (*c*) what are the physiological mechanisms involved in the causation of the growth-suppressing effect? and (*d*) what would be the best criteria on the basis of which cultivars could be selected for use in breeding programmes directed at improvement of the nutritive value of grain sorghum?

In this paper the results are reported of a study aimed at finding answers to these questions.

MATERIAL AND METHODS

Briefly, the study entailed (*a*) a comparison of the growth rates of separate but equal groups of rats fed diets containing grains from various cultivars, unextracted as well as ethanol-extracted (to reduce tannin content); and (*b*) investigation of the relationship between growth rate and certain factors suspected of being implicated in growth suppression, viz. digestibility of the protein in the grain, polyphenol content of the diet, and the degree of fatty infiltration of the liver.

Grain Sorghum Cultivars and their Preparation for Use in the Trial

From samples of 40 cultivars obtained from a nearby experimental farm of the Department of Agricultural Technical Services and stored in bins in an outbuilding at

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the CSIR under insect-free conditions, 6 were selected for investigation. The choice of cultivars was based on polyphenol content, the aim being to obtain samples which, when mixed into the relevant experimental diets at the appropriate level, would yield a series of diets having polyphenol contents ranging from comparatively low to 'high' (Table I). The selected samples were designated I_1, CI_{43}, CI_{31}, CI_{23}, C_{26} and C_{45}.

Of each of the above samples an 'extracted' as well as an 'unextracted' meal was prepared for inclusion in the relevant experimental diets. The unextracted product consisted simply of whole grain which had been ground into a relatively fine meal in a hammer mill and then stored in the freezer in tightly-closed containers. Preparation of the extracted meal was a more complicated operation. First the dark-coloured outer layer of the grain was removed as completely as was possible in a rice-pearling machine. The dark-coloured fraction thus removed was then collected quantitatively and kept overnight in a glass beaker in 60% (v/v) aqueous ethanol. The following morning the liquid fraction containing the extractable polyphenol in solution was separated from the dark-coloured, fibrous material by filtration through a Buchner filter. After filtration the filter cake was washed thoroughly 3 or 4 times with ethanol solution. The above procedure was repeated. The extracted outer layer fraction of the grain was then dried in an air circulation oven at room temperature and thereafter recombined with the decorticated inner portion. The recombined fractions were ground into a meal in a hammer mill. The meal was then first homogenised thoroughly in a mechanical mixer and finally placed in the freezer in dry, tightly-closed containers. Details regarding the nitrogen and extractable tannin contents of both the extracted and the unextracted meals from each of the relevant cultivars are given in Table I.

Analytical and Histological Methods

Nitrogen determinations were done on grain samples, experimental diets and rat faeces according to the Kjeldahl method, mercuric sulphate being used as catalyst. Generally the procedures followed were as previously described by Dreyer.[2,3]

Moisture contents of all experimental rations were determined by means of a Brabender apparatus. Drying time and temperature were, respectively, 2 hours and 90°C.

Tannin. Sorghum grain samples, ethanol-extracted as well as unextracted, were analysed for their content of 1% (v/v) HCl methanol-extractable tannin. These determinations were done on 3 - 5-g samples obtained from bigger samples which had previously been ground to meals. Each sample was agitated mechanically in 100 ml of the solvent for 24 hours at 25°C. The liquid phase was then filtered off through Whatman 40 filter paper; 1 ml of the filtrate was used to determine the tannin content. The determination was done according to the AOAC method[4] for alcoholic beverages. In Table I are listed the extractable tannin contents (expressed as tannic acid equivalents) of the unextracted and ethanol-extracted meals used in the study as well as the extractable tannin contents of the experimental diets incorporating these meals.

Liver lipid contents were determined on samples of approximately 1 g of freeze-dried material through extraction for about 24 hours in a Soxhlet apparatus, petroleum ether (boiling point 30 - 60°C) being used as the solvent.

For histological examination of livers with regard to lipid deposits, 10-μm sections were cut from formalin-fixed specimens by means of a freeze microtome. The sections were first rinsed in distilled water, then stained successively with Oil Red O and haematoxylin, and were finally mounted in glycerine for inspection.

Experimental Diets

All experimental diets were of the semisynthetic type used in this laboratory on a routine basis in protein quality studies;[2] such diets contain adequate amounts of all known nutritionally essential minerals, vitamins and energy-yielding nutrients. The dextrin component was replaced weight-for-weight with (*a*) defatted whole egg to bring the protein content to exactly 4%; and (*b*) sorghum grain meal to raise the protein level by the addition of 5,3 parts of sorghum protein per 100 parts of diet. The experimental diets were therefore equal in protein as well as total nutrient content, but different in tannin and dextrin content (Table I).

One reason for the inclusion of egg protein in all the diets was to ensure that diets would be capable of supporting a certain measure of growth, the *suppression* of growth not lending itself readily to investigation if there is no growth in the first place. Another reason was to ensure complete consumption of the food offered to the rats, since it is essential to the type of study performed that the mass of food consumed per unit body mass per day should be approximately the same for all the experimental animals.

In addition to the experimental diets there was also a control diet, the use of which was essential to the assessment of the digestibility of the sorghum protein. The composition of this diet was the same as that of the experimental diets except that none of the dextrin component had been replaced with sorghum grain meal.

Rats and Rat Experiments

The rats used were young males from litters representing the F_2 generation of a cross between two inbred strains, BD IX and BD V. Previous work done in this laboratory[5] had shown that on a given diet rats of this breed respond in a remarkably uniform fashion with regard to urinary nitrogen output, i.e. to protein retention. The animals were bred and raised to weaning age (21 days) by the SABS Small-Animal Unit, Groenkloof, Pretoria, under excellent conditions of housing, feeding and general hygiene.

The rat experiment was in reality three experiments in one, since the animals were used for the determination of effects on growth rate and liver lipids as well as for the assessment of the digestibility of the sorghum protein components of the experimental diets.

The experimental procedure was briefly as follows: from 237 rats, 180 rats falling in a body mass range of 39 - 52 g were selected. These animals were then separated into 15 groups of 12 rats each and of close to equal average body mass (45,8 - 46,1 g). Three of these groups were allocated at random to the control diet (4% egg protein level) and each of the remaining 12 to a given experimental diet (4% egg protein + 5,3% sorghum protein level).

The mass of the food offered per rat per day throughout the experiment was 10% of the body mass, the latter figure being obtained regularly every 4 days during the course of an experimental period of 32 days. Drinking water was offered *ad libitum* to all animals for the full duration of the trial.

Each rat was housed in a screen-bottomed metabolism cage[6] in an air-conditioned room at a temperature of 26 ± 1°C and a relative humidity of 50 ± 5%.

On the morning of day 5 all rats were transferred to newly-washed cages for (*a*) measurement of food consumption; and (*b*) quantitative collection of faeces for the following 8 days, as described previously.[8] The data thus obtained were needed for assessment of the digestibility of the sorghum protein.

At the end of the collection period the 36 rats fed the control (4% egg protein) diet were eliminated from the experiment since, as has already been indicated, their use was limited to their being the controls in the protein digestibility trial. The rats fed the experimental (sorghum-containing) diets were, however, transferred to newly-cleaned cages and kept in these cages till the end of the 32-day experimental period.

As certain investigations pertaining to the histopathology and composition of certain organs of these rats were also envisaged, the animals were then killed with ether for collection of the relevant specimens. As far as these investigations are concerned, only the results bearing on liver lipid content and the histological examination of livers will be recorded here.

From each experimental group 4 rats were selected for investigation of liver histology. Portions of the livers were excised and kept in 10% formalin until the eventual cutting and staining of sections could be performed. The liver of each of the remaining 8 rats in each group was first dissected out in its entirety; it was then weighed and finally dried under vacuum at sub-zero temperature in a freeze-drier. This material was used for determination of liver lipid content.

The relative growth rate of each individual rat was calculated from the regularly-recorded body mass data. This value, as applied in this report, can be defined as the average of the gains made in mass, expressed in g per 100 g body mass per unit time during the eight 4-day periods of the experiment. The unit of body mass was the average of the results (in g) of the two successive weighings (one at the beginning and the other at the end) pertaining to a particular 4-day period; the unit of time was 1 week.

The digestibility of the sorghum protein was estimated from the nitrogen consumption and the faecal nitrogen data obtained during the 8-day collection period. These estimates were based on the principle previously demonstrated in this laboratory,[7,8] that there is a linear relationship between protein nitrogen intake (x axis) and the difference between nitrogen intake and faecal nitrogen excretion (y axis), all data being expressed per 100 g body mass and all the dry food consumption levels of the experimental animals (rats) per unit body mass being approximately equal. In the linear regression equation, $y = a + bx$, b represents the digestibility coefficient, i.e. the increase in the mass of nitrogen apparently absorbed per unit increase in the amount of nitrogen consumed.

In the present investigation the zero nitrogen intake points on the x axis were in reality the points of zero *sorghum nitrogen* intake yielded by a number of the rats fed the control diet (4% egg protein diet). These rats were a group of 12 selected on the basis of their dry food consumption from the 36 which had received the control diet; they were selected in such a way that their dry food consumption levels per unit body mass corresponded as closely as possible to those of the experimental rats.

Statistical Scrutiny of Results

The data obtained on the relative growth rate and liver lipid content of the rats, as well as those on the digestibility of the grain sorghum protein, were analysed statistically to ascertain whether there were significant differences with regard to the effects of the various samples investigated. In these tests the data were subjected to conventional analyses of variance. In addition it was found necessary to ascertain whether or not there were significant correlations between the various sets of observations, e.g. dietary tannin content versus growth rate of rats; or growth rate of rats versus digestibility of protein, etc. These investigations entailed, in each case, calculation of Kendall's rank correlation coefficient and estimation of the probability that the relevant correlation coefficient is significantly different from zero.

RESULTS

Table I contains the data on, *inter alia*, the nitrogen and tannin contents of the grain sorghum cultivars (unextracted and ethanol-extracted), as well as those of the relevant experimental diets. It should be noted that there were appreciable differences in respect of both nitrogen (protein) and extractable tannin content among the various cultivars. Furthermore, tannin contents as measured by the method applied were reduced substantially in all samples by extraction with the 60% ethanol solution.

Experimental diets were close to equal in nitrogen content. It is therefore improbable that the differences observed in the response of the animals could have been a function of the protein contents of the diets; such differences would more probably be due to the variations in tannic acid content or to other causes.

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TABLE I. DATA PERTAINING TO THE STUDY OF GROWTH-SUPPRESSING EFFECTS OF CERTAIN UNEXTRACTED AND ALCOHOL-EXTRACTED GRAIN SORGHUM CULTIVARS

	Unextracted						
	N content (%)		1% HCl (v/v) methanol-extractable tannin content (expressed as tannic acid equivalents)				
Cultivar	Cultivar	Exptl diet (grain sorghum N only)	Cultivar†	Ration	Relative growth rate (g/100 g body mass/week) ± SEM	Digestibility of protein (%) ±SEM	Liver lipid content* (% of dried liver) ± SEM
I_1	1,541	0,835	1,32	0,72	14,08 ± 0,351	75,69 ± 1,57	10,3 ± 0,595
CI_{43}	1,536	0,852	1,39	0,77	14,83 ± 0,262	66,96 ± 1,95	11,1 ± 0,666
CI_{34}	1,581	0,870	0,31	0,17	16,09 ± 0,275	87,73 ± 1,44	10,8 ± 0,391
CI_{23}	1,701	0,868	0,87	0,45	15,75 ± 0,274	85,92 ± 1,38	9,9 ± 0,362
C_{26}	1,177	0,847	1,94	1,37	12,90 ± 0,321	51,89 ± 1,83	13,7 ± 0,624
C_{45}	1,309	0,859	1,60	0,85	12,17 ± 0,269	45,21 ± 2,28	13,5 ± 0,538
	Extracted with 60% (v/v) ethanol						
I_1	1,579	0,842	0,80	0,42	14,91 ± 0,351	75,98 ± 1,41	12,7 ± 1,396
CI_{43}	1,543	0,848	0,76	0,43	13,91 ± 0,332	70,94 ± 1,29	11,4 ± 0,638
CI_{34}	1,560	0,873	0,22	0,15	15,89 ± 0,383	91,92 ± 1,32	11,7 ± 0,712
CI_{23}	1,716	0,842	0,63	0,34	15,54 ± 0,368	80,56 ± 1,29	11,5 ± 0,950
C_{26}	1,194	0,870	0,98	0,72	13,70 ± 0,176	67,66 ± 1,28	13,3 ± 0,986
C_{45}	1,318	0,849	0,74	0,48	13,18 ± 0,243	67,90 ± 1,16	10,7 ± 0,913

* Averages of 8 individual observations with the exception of I_1 (ethanol-extracted) and C_{26} (extracted) which, due to accidental loss of one sample each, are based on 7 observations each.
† Average of 2 determinations.

5%) differences among unextracted as well as extracted grains in respect of effect on growth rate. A similar result was obtained in the analysis of the protein digestibility data. It appears, therefore, that among the grains investigated there were some cultivars which were significantly poorer in nutritive value than others, and that the reduction of tannin content did not eliminate differences in nutritive value.

Both growth performance and protein digestibility were, however, improved by the ethanol extraction in the case of cultivars C_{26} and C_{45}, the unextracted grains of which were, respectively, first and second in the order of tannin contents. However, even in these two cases the degree of improvement secured through ethanol extraction was by no means sufficient to raise the nutritive values to that of CI_{34}, the best of the samples investigated. In one instance the effect of ethanol extraction on the digestibility of the protein was anomalous: it will be seen that extraction resulted in an appreciable decrease in digestibility of the protein in CI_{23}, while in all the others there was a tendency toward higher values.

Histological examination of the livers of the 4 rats selected for this purpose from each experimental group yielded clear evidence of typical fatty infiltration in all instances. Superficially, liver lipid contents appeared to differ from one experimental group to another. The figures pertaining to the individual observations were, however, highly variable, as can be judged from the standard errors of the means recorded in Table I. Presumably because of this great variability, significant differences ($P \leqslant 5\%$) in respect of liver lipid content were observed only among experimental groups fed the unextracted grains. A noteworthy reduction of liver lipid content following ethanol extraction of the grain was observed only in one cultivar, viz. C_{45}. In all the others, lipid levels either remained essentially unchanged or tended to rise as a result of ethanol extraction of the grain fed.

The degree of correlation between the members of specific pairs of the various sets of data collected in this study is shown in Table II. Three of the correlation coefficients calculated were significantly different from zero, viz. growth rate versus dietary tannin content or digestibility of dietary protein; and digestibility of dietary protein versus dietary tannin content. The most closely correlated variables were growth rate and protein digestibility, these being followed in decreasing order by the values obtained for digestibility versus tannin content, and growth rate versus tannin content.

Liver lipid content generally appeared not to be associated with any of the other variables, least of all with dietary tannin content.

DISCUSSION

From the above results it is clear that the grain sorghum samples investigated differed from one another in respect of ability to support growth in the young rat, as well as in the digestibility of the protein component. The significant correlation between growth rate and

TABLE II. DEGREE OF CORRELATION BETWEEN CERTAIN VARIABLES PERTAINING TO BIOLOGICAL EFFECTS OF CERTAIN SORGHUM GRAIN CULTIVARS

Variables	Kendall's rank correlation coefficient	P%
Growth rate *v.* digestibility of protein	0,8182	<0,1*
Growth rate *v.* tannin content of diet	−0,6260	0,2*
Growth rate *v.* liver lipid content	−0,3333	7,6
Digestibility of protein *v.* tannin content of diet	−0,8092	<0,1*
Digestibility of protein *v.* liver lipid content	−0,3333	7,6
Tannin content of diet *v.* liver lipid content	0,1985	18,5

* Significant correlation at $P \leqslant 5\%$.

digestibility and dietary tannin content and digestibility suggests that under the dietary conditions imposed upon the animals in this experiment, growth was essentially dependent on the digestibility of the dietary protein, while growth rate and digestibility were inversely dependent on dietary tannin content. In view of this it appears that the primary cause of the poor growth associated with some of the grains investigated is a high tannin content.

According to Fuller *et al.*,[9] high tannin contents are characteristic of especially the bird-resistant brown-seeded grain sorghum varieties. Several tannin-like compounds, mainly flavones, flavans and anthocyanins, as well as their derivatives, have already been isolated from the grain pericarp.[10-17] The predominance of the above polyphenols in such isolates suggests that the grain sorghum tannins are essentially of the type classified as 'condensed tannins',[18] although the presence of members of the 'hydrolysable' types cannot as yet be excluded as a possibility.

In view of the main characteristic of tannins alone (their protein-binding property) it is reasonable to expect that ingestion of these compounds will have significant biological effects. Apart from a possible effect on palatability, the tannins or their derivatives could conceivably combine with dietary proteins to form indigestible complexes, thus rendering the protein unavailable for biological utilisation; they could block active sites in digestive and other enzymes; or they could be absorbed through the gut wall to become toxicants on a metabolic level. Reports indicating that ingestion by monogastric animals of certain tannin-containing grain sorghum cultivars,[9,19,20] isolated tannins[21] or certain tannic acid metabolites[22] causes suppression of growth, therefore concord with what might at first glance have been expected. As regards the mode of action of tannins, both impairment of protein digestibility and specific metabolic activities are apparently implicated.

Cummins[23] showed in *in vitro* dry matter digestibility (IDVM) tests with a bird-resistant (high-tannin) and a low-tannin cultivar that there is an inverse relationship between digestibility and tannin content. He also found that ensiling caused a decrease in tannin content and a concomitant increase in the digestibility. Using growing chicks as experimental animals, Chang and Fuller[19] found in *in vivo* trials a slight depression of protein digestibility, while comparing high-tannin sorghum-containing diets with sorghum-containing diets of lower tannin content. The magnitude of the depression was considered insufficient to cause the concomitant growth retardation noted. It should be pointed out, however, that the validity of the technique employed by these authors in their digestibility trials for separation of the urinary and faecal nitrogen in the excreta — through precipitation with uranyl acetate after oxidation with potassium permanganate — has been seriously challenged by Nesheim and Carpenter.[24]

The results of the present study, obtained with animals (rats) not presenting similar methodological problems, support the view that the HCl-methanol-extractable tannin content is inversely related to protein digestibility and that reduction of the tannin content of high-tannin samples results in an increase in digestibility.

The degree of correlation observed between dietary tannin content and protein digestibility was not found to be such that variations in digestibility could be explained entirely on the basis of variations in tannin content. The lack of a perfect correlation observed in respect of these two criteria could be due either to an effect on digestibility of factors other than tannins or to a possibility that the analytical method employed in the determination of the tannin contents of samples is not sensitive to all the various tannins present in the grains. From a study of the various methods available for determination of the tannin contents of sorghum grains, Maxson and Rooney[25] recently concluded that the biochemical compounds being measured are 'unknown', and that each method measures a different compound.

Of equal importance is the finding that the correlation between dietary tannin content and growth rate was not of a high degree; tannin content does not appear to be as good an index of the growth-suppressing properties of sorghum grains as is protein digestibility. The digestible protein content of the grain therefore appears to be the most suitable criterion for use in the selection of cultivars for breeding, this figure being more readily procurable than the growth rate data and being apparently more reliable than determinations of tannin contents. Furthermore, rapid *in vitro* methods for digestibility determination could be developed to expedite the selection procedure.

From studies on the effects of feeding certain sorghum grains to young rats, Breuer and Dohm[20] recently concluded that there is an inverse correlation between growth rate and protein digestibility. Commenting on their results these authors concluded that ' . . . there may be no advantage so far as the nutrition of non-ruminants are [*sic*] concerned in producing sorghum grain with high levels of digestible protein until improvements are made in the balance of amino acids present in sorghum grain or methods are developed for the specific supplementation of the sorghum grain protein'. This statement could well apply to the grains studied by Breuer and Dohm.[20] The reported digestibility figures varied from sample to sample over only a small range (74 - 86%) and were all of a comparatively high order of magnitude. In such cases

amino acid composition is likely to be the most important factor with respect to ability to support growth in animals. However, as is undoubtedly the case with regard to the results obtained in our study, protein content and digestibility are of primary importance to nutritive value when protein content and digestibility are as low as the values found by us in respect of some of the cultivars tested. From a nutritional viewpoint the South African grain sorghum crop will therefore certainly be improved greatly if future production could be based on those cultivars containing protein of comparatively high digestibility at high levels.

Available information indicates that some tannins could have specific metabolic effects. Booth *et al.*[26] found that in rats gallic acid, propyl gallate or tannic acid undergo metabolic conversion (*O* - methylation) to 4-*O*-gallic acid. The authors speculated that ingestion of such compounds could induce an increased dietary requirement of the methyl donors choline and methionine, marginal supplies being reduced to the low levels usually associated with fatty infiltration of the liver. In further studies Booth and co-workers[27] did in fact find that in rats fed low-methionine, low-choline diets containing gallic acid at a 1% level, liver lipid content was substantially increased. This increase was prevented by addition of choline to the diet. Pyrogallol, however, had no effect on liver lipid content under comparable dietary conditions, but nevertheless repressed growth rate.

Corroborative observations were subsequently made by Chang and Fuller,[19] and by Rayudu *et al.*[22] On feeding sorghum-containing diets varying in tannin content to growing chicks, the former authors induced fatty livers in the test animals, the lipid contents of which were in direct proportion to dietary tannin content. Similar results obtained with tannic acid were considered to be due to the fact that tannic acid yields, *inter alia,* gallic acid as a degradation product. Supplementation with choline and methionine hydroxy analogue prevented the fatty infiltration. The results of Rayudu *et al.*[22] were in exact agreement with those of Booth *et al.*,[27] in that chick liver fat content was found to be raised by gallic acid or tannic acid fed at a 1% dietary level, but not by pyrogallol.

Another important point emanating from the work of Rayudu *et al.*[22] is the observation that fatty infiltration of the liver is not the only — and also not the most harmful — effect of dietary tannins. These authors noted that those tannins causing fatty infiltration are in fact least harmful because of their detoxification through methylation. The other two metabolites of tannic acid, pyrogallol and pyrocatechol, were found to be much more harmful in respect of their effect on growth and mortality in chicks, as these compounds are not detoxified in the body.

As, according to our results, there was no statistically significant relationship between dietary tannin content and liver lipid content, the mechanism involving partial or complete elimination of lipotropic factors (methyl donors) from the diet through methylation of tannins does not appear to be implicated in the production of the fatty livers under our experimental conditions. Rather it would appear that there was an over-all inadequacy of lipotropic factors in all the diets, the presence in the diets of egg protein as well as choline chloride notwithstanding. Another observation which concords with the idea that the tannins in the sorghum grains studied by us were not primarily responsible for the fatty infiltration, is that partial removal of tannin from the grains did not result in a general reduction of the liver lipid contents of the rats fed the diets containing these grains.

In general, therefore, it would appear that protein content and digestibility, and not effect on liver lipid content, would be the most reliable criteria for the ranking of South African grain sorghum cultivars in order of value.

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