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Estimation of Tannins in Different Sorghum Varieties and Their Effects on Nutrient Digestibility and Absorption of Some Minerals in Caged White Leghorn Layers

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Abstract

Condensed tannin content of seven varieties of sorghum (White, Sandalbar, YS-98, JS-88, JS-263, JS-2002 and Hegari) found in Pakistan were determined and their effect on nutrient digestibility and absorption of minerals was investigated in White Leghorn Layers. For digestibility trial, 20 individually caged White Leghorn layers (30 weeks of age) were divided randomly into four experimental groups (5 birds/group) where each bird served as a replicate. Four isocaloric and isonitrogenous experimental diets i.e. control (C), low tannin (LT), medium tannin (MT) and high tannin (HT) were prepared. Diet C was without tannin; whereas, diet LT, MT and HT contained 1, 2 and 3 percent sorghum tannin, respectively. Among the seven varieties of sorghum grains the highest amount of tannin (5.34%) was observed in the variety Sandalbar; whereas, the lowest concentration of tannin was observed in white sorghum (0.02%). All tannin containing diets reduced the digestibility of nitrogen and starch but the effect was more severe with 3% tannin. However, ether extract digestibility remained unaffected. The mineral contents (calcium, phosphorus, sodium, potassium, magnesium and iron) in the feces of layers using sorghum tannins were significantly ($P<0.05$) more than those using diets without tannin. The results revealed that the effect of tannins on mineral absorption was more severe in birds fed high tannin diet (3%) than that of low tannin diets.

Keywords: Sorghum; Tannins; Layers; Minerals; Digestibility

Introduction

Tannins, naturally occurring polyphenolic compounds, are mostly found in dicotyledonous plants, most commonly in legumes. Important grains which are used for human and animal consumption are known to contain a significant amount of tannin contents like sorghum (*Sorghum bicolor*), millet (*Panicum milisceum*), barley (*Ordeum vulgare*) and a number of other legume seeds (Kamalak *et al.,* 2005; Arija *et al.,* 2006; Rana *et al.,* 2006; Kumar *et al.,* 2007).

Sorghum is unique among major cereal crops, which can be produced economically in hot and dry climates. Scarcity of cereal grains demands that sorghum is fully utilized both in human and animal diets. However, presence of anti-nutritional factors like phytate and tannins (Selle *et al.*, 2010) restricts its use as a food source. Sorghum tannins are of condensed type while hydrolysable tannins apparently do not occur in sorghum (Nyachoti *et al.,* 1997). In Pakistan seven different varieties of sorghum (White, Sandalbar, YS-98, JS-88, JS-263, JS-2002 and Hegari) are cultivated (Saeed and Zaki, 2003) but none of these has ever been analyzed for its tannin contents.

Multiple phenolic hydroxyl groups of tannins may form stable complexes with protein, metal ions and other macromolecules like polysaccharides (Choct and Hughes, 1999; Ozkan and Sahin, 2006; Kondo *et al.,* 2007). Stable complexes of tannins with proteins may lead to coagulation or precipitation of protein molecules. However, nature of tannins and that of proteins can determine the strength and degree of interaction between tannins and proteins (Hagerman, 1989). Tannins-carbohydrate interaction has also been observed particularly with starch molecules but their affinity is less than for proteins. The level of sorghum used has also shown a linear effect on digestibility of starch (Barekatain *et al.,* 2011).

Tannins also interact with a number of minerals, form precipitates and thus reduce their availability (Hassan *et al.,* 2003). The minerals which are ionised in the stomach (iron, calcium, magnesium, sodium and potassium) are prone to variety of absorption interferences (Arigator and Samman, 1994). Tannins-nutrient interaction may be one of the means by which tannins affect the digestive processes resulting into reduced availability of the nutrients in the gut.

Keeping in view the information above, an *in vitro* analysis was conducted to estimate concentration of tannins in local varieties of sorghum, particularly condensed

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tannins, and the *in vivo* effects, which sorghum tannins exhibit on digestibility of proteins, ether extract and starch, as well as on mineral absorption (calcium, phosphorus, sodium, potassium, magnesium and iron), in caged White Leghorn layers.

Materials and Methods

Collection, Drying and Grinding of Sorghum Grains for Tannin Analysis

Sorghum grains of seven different varieties (White, Sandalbar, YS-98, JS-88, JS-263, JS-2002 and Hegari) grown in Pakistan were procured from a grain market of Faisalabad (Pakistan). Grain samples from each variety were dried at 38°C in a forced draft oven. After drying, about 500 g of each sample material was ground to a level that it could pass through 2mm screen. The ground material was thoroughly mixed and about 100 g of this sample was reground to pass through a screen of 0.5 mm. This material was then analyzed for estimation of condensed tannins by using butanol-HCL method (Porter *et al.,* 1985).

Experimental Diets

Four experimental diets i.e. control (C), low tannin (LT), medium tannin (MT) and high tannin (HT) were prepared. Diet C was without tannin whereas diet LT, MT and HT contained 1, 2 and 3 percent sorghum tannin, respectively (Table 1). These diets were formulated according to the nutrient requirements recommended by NRC (1994). All the diets used for *in vivo* trials were isonitrogenous (CP 16.5%) and isocaloric (ME 2700 kcal/kg diet).

Digestibility Trial

The *in vivo* digestibility trial was conducted by using 30 weeks old White Leghorn layers. Twenty layers having similar body weight were obtained from a commercial layer farm and were reared in individual metabolic cages at Dr. Raja M. Akram, Animal Nutrition Research Centre, University of Agriculture, Faisalabad. These layers were allotted to four experimental diets randomly such that each diet was offered to 5 individually caged layers and each bird was taken as a replicate. Fecal samples were collected at the end of 30^{th} week of age for two consecutive days at the interval of 3 h, during which birds were fed same amount of feed.

The excreta samples collected during the collection period were analyzed for the estimation of crude protein, ether extract and starch contents using Kjeldhal proximate analysis method. The samples were also analyzed for calcium, phosphorus, potassium, magnesium, sodium and iron as described by AOAC (1990).

Statistical Analysis

The data thus collected were subjected to analysis of variance technique using Completely Randomized Design (CRD) whereas; the Least Significant Difference (Steel *et al.,* 1997) test was used for comparison of the treatment means.

Results

Seven varieties of sorghum grown in Pakistan were analyzed for their condensed tannin contents. The concentration of tannins in these varieties ranged from 0.02 to 5.34% (Table 2). The highest amount of tannin (5.34%) was found to be in the variety Sandalbar, whereas the lowest concentration of tannin was observed in white sorghum (0.02%). The amount of tannin in these varieties varied with the change in color of testa of the grains. The grains with white testa (white sorghum) showed the lowest amount of tannin while the tannin contents tended to increase with increase in color of the seed coat.

The apparent digestibility of crude protein, ether extract and starch in layers excreta is shown in (Table 3). All the diets containing sorghum tannins significantly ($P<0.05$) reduced the apparent digestibility of protein (nitrogen × 6.25) when compared to those fed tannin free diet. However, the reduction in digestibility was not so severe when the sorghum tannin had been incorporated at low level (1%) in the diet. The layers consuming diet containing 3% tannin showed the lowest nitrogen digestibility value followed by those of group MT (2% tannin) and group LT (1% tannin), respectively. Statistical analysis of the data revealed that levels of dietary sorghum tannin also affected the digestibility of protein. The digestibility of protein decreased as the level of tannins in the diets increased.

Dietary sorghum tannins exhibited a marked ($P<0.05$) effect on starch digestion in layers. The birds fed control diet digested starch more efficiently than those fed diet containing sorghum tannins. The birds consuming diets containing 2 and 3% sorghum tannins exhibited poorer starch digestibility than those fed control or diet containing 1% tannin, where the difference was non significant. Gradual decrease in starch digestibility value with increase in the tannin level in the diet also depicted the effect of tannin levels used in the diets. However, digestibility of the ether extract remained unaffected due to the dietary tannins.

Dietary sorghum tannins significantly ($P<0.05$) affected the absorption of minerals (calcium, phosphorus, sodium, potassium, magnesium and iron) in the layers (Table 4). The lowest absorption of these minerals was exhibited by the birds fed diet containing 3% tannin followed by the diets containing 2 and 1% tannin. The effect of tannins on mineral absorption was more severe in birds fed high tannin diet than that of low tannin diet. The only exception being that no difference regarding phosphorus absorption was observed in the birds using diet containing 1% tannins when compared to those maintained on 2% tannins. The result of the study revealed that the absorption of minerals was affected in the birds fed different levels of

Table 1: Chemical composition of feed offered during the experimental period

Ingredients (%)	Diet			
	C (Control)	LT (Tannin 1%)	MT (Tannin 2%)	HT (Tannin 3%)
Crude Protein %	16.84	16.81	16.78	16.76
Metabolizable Energy (Kcal/kg)	2895	2889	2878	2874
Calcium %	3.50	3.50	3.50	3.50
Phosphorus %	0.70	0.70	0.70	0.70
Crude Fiber %	3.53	3.53	3.53	3.53
Lysine %	0.79	0.79	0.79	0.79
Methionine %	0.42	0.42	0.42	0.42

C = control, without tannin (traces); LT = low tannin (1%); MT = medium tannin (2%); HT = high tannin (3%)

Table 2: Concentration of tannins in the various varieties of sorghum grown in Pakistan

Varieties	Dry Matter (%)	Tannin conc. (% dry matter)
White	94.2	0.02
Sandalbar	94.4	5.34
YS-98	94.2	1.24
JS-88	94.5	1.65
JS-263	92.6	3.31
JS-2002	93.6	1.95
Hegari	94.6	2.17

Table 3: Effects of different levels of sorghum tannins on the digestibility (%) of protein (N x 6.25), ether extract and starch in caged White Leghorn layers

Variables	Diets			
	C (Control)	LT (Tannin 1%)	MT (Tannin 2%)	HT (Tannin 3%)
Protein	$83.12^{a} \pm 4.15$	$74.16^{b} \pm 3.70$	$61.24^{c} \pm 3.05$	$47.05^{d} \pm 2.35$
Ether extract	90.61 ± 4.55	90.59 ± 4.52	89.92 ± 4.32	88.90 ± 4.16
Starch	$96.95^{a} \pm 4.85$	$93.05^{a} \pm 4.62$	$90.11^{b} \pm 4.53$	$88.34^{b} \pm 4.40$

Means with different superscripts in a row are significantly different ($P < 0.05$); C = control, without tannin (traces); LT = low tannin (1%); MT = medium tannin (2%); HT = high tannin (3%)

Table 4: Mean of absorption of various minerals in caged White Leghorn layers maintained under different concentrations of sorghum tannin

Variables	Diets			
	C (Control)	LT (Tannin 1%)	MT (Tannin 2%)	HT (Tannin 3%)
Calcium	$58.48^{a} \pm 3.42$	$47.89^{b} \pm 2.91$	$34.98^{c} \pm 1.74$	$26.30^{d} \pm 1.31$
Phosphorus	$53.83^{a} \pm 3.26$	$42.86^{b} \pm 2.90$	$37.63^{b} \pm 1.82$	$31.65^{c} \pm 1.32$
Iron	$61.39^{a} \pm 3.75$	$43.46^{b} \pm 2.91$	$32.90^{c} \pm 1.85$	$24.60^{d} \pm 1.39$
Sodium	$57.57^{a} \pm 3.24$	$49.99^{b} \pm 2.95$	$42.53^{b} \pm 1.96$	$34.03^{c} \pm 1.75$
Potassium	$54.44^{a} \pm 3.59$	$40.94^{b} \pm 2.74$	$33.48^{c} \pm 1.86$	$28.02^{d} \pm 1.40$
Magnesium	$55.41^{a} \pm 3.56$	$44.20^{b} \pm 2.85$	$36.66^{c} \pm 1.78$	$29.35^{d} \pm 1.42$

Means with different superscripts in a row are significantly different ($P < 0.05$); C = control, without tannin (traces); LT = low tannin (1%); MT = medium tannin (2%); HT = high tannin (3%)

sorghum tannins. There was a corresponding decrease in the mean absorption values with increase in tannin levels in the diets.

Discussion

The concentration of tannin contents in different cultivars of sorghum showed a great variation among the varieties analyzed. Dykes and Rooney (2006) observed that different cultivars of sorghum i.e. type I (without pigmented testa), type II (with pigmented testa) and type III (bird proof) were found to have 0.028, 0.448 and 1.195% tannins, respectively. They also found that amount of tannins and their location in plants vary considerably. This indicates that greater part of tannins in cereal grains and in legume seeds are located in outer covering of the grains (seed coat) rather than their inner parts. Tannin contents appeared to be related to the color of the seed coat (Serna-Saldivar and Rooney, 1995).

Tannins present in sorghum grains have been identified as condensed tannins. Strumeyer and Malin (1975) described sorghum tannins as "condensed tannins". High concentration of tannins has been found in sorghum grains. Different concentrations of tannins have been recorded ranging from 0.028 to 1.195% in various categories of sorghum (Dykes and Rooney, 2006). It is generally agreed that hydrolysable tannins, if present, are found in traces (William, 1983), who found that hydrolysable tannin in sorghum varied from 2 to 40 monomeric units.

Protein digestion in birds using sorghum tannins was found to be lower than that of control group (0% tannin). A

marked decrease in the protein digestibility was observed when tannin containing sorghum grains were fed to commercial layers (Diao *et al.,* 1990) and caecectomized roosters (Vasan *et al.,* 2008; Mahmood *et al.,* 2006).

The reduction in nitrogen digestibility has been linked with the formation of stable complexes with dietary protein (Selle *et al.,* 2010). Tannins also form stable complexes with digestive enzymes (Griffith, 1989; Mahmood *et al.,* 1997) and inhibit their activity. Tannins exhibited more affinity for proteins than starch (Hagerman, 1989) due to strong hydrogen bindings formed by carboxyl oxygen of peptides group. However, reduction in nitrogen digestibility cannot be explained clearly because it is still difficult to say whether it is due to reduced digestibility of dietary protein or to an increased secretion of endogenous protein due to tannin-endogenous enzyme complexes (Horigome *et al.,* 1988) or mucosal hyper secretion of gut Mitjavilla *et al.* (1977) or both.

Tannins are known to form complexes with carbohydrates, particularly with starch. The birds fed diets containing sorghum tannins in this experiment showed significantly lower digestibility of starch. A probable explanation of lower digestibility of starch may be due either to the formation of tannin-enzymes (Mahmood *et al.,* 2008) or to tannin-carbohydrates (Selle *et al.,* 2010) complexes or both, rendering starch indigestible. Longstaff and McNab (1991) observed a significant decrease in starch digestibility in chicks fed tannin from faba bean hulls.

The severity of effects in the birds also depended upon the level of dietary tannins. The decrease was severe in the birds consuming high amount of tannins. The negative effect of tannins on starch digestibility in poultry depends on the quantity of tannins ingested (Flores *et al.,* 1994; Mahmood *et al.,* 2006). Overall, the presence of different levels of sorghum tannins in layer diet markedly depressed protein digestibility but showed a moderate effect on the digestibility of starch.

In contrast to the reduction in overall digestibility of nitrogen and starch observed in layers consuming diets containing sorghum tannins, however, no adverse effect on the digestibility of ether extract was observed. Lipase is known to play a pivotal role in the digestibility of fat contents of ingredients. An increased activity of lipase in the intestinal contents of rats due to dietary inclusion of tannins has been reported by Horigome *et al.* (1988). These results indicate that tannins have less affinity for lipase. Therefore, it is possible that presence of sorghum tannins in the gut did not adversely affect the activity of lipase and subsequently no adverse effect on the digestibility of ether extract. Studies involving salseed meal tannin (Mahmood *et al.,* 1997; Mahmood *et al.,* 2006) also reported that tannins may not exhibit any effect on digestibility of ether extract in broiler cockerels.

Dietary sorghum tannins significantly ($P<0.05$) lowered the mineral absorption in experimental birds. A possible explanation of reduction in mineral absorption in the birds fed tannins may be either due to less availability of minerals as a result of reduced feed intake (Karimian *et al.,* 2004) or to tannin-minerals interaction in the gut (Hassan *et al.,* 2003) as tannins are known to form insoluble complexes with divalent metal ions thus reducing their availability in the gut for absorption (Rao and Prabavathi, 1982) or both. The minerals which are ionised in the stomach (iron, calcium, magnesium, sodium and potassium) are prone to variety of absorption interferences (Arigator and Samman, 1994). Tannins also interact with minerals such as iron (Hassan *et al.,* 2003), calcium (Mitjavilla *et al.,* 1977) and sodium (Freeland *et al.,* 1985), thus ultimately resulting in decreased availability of these minerals for absorption. Mehansho *et al.* (1987) and Chang and Fuller (1993) also observed similar reduction in calcium and iron absorption in animal feeds as in present investigation. The tannin-mineral interaction resulted due to presence of dietary sorghum tannins, which caused reduced absorption in layer birds.

In conclusion, making a final assessment of the study, it could be concluded that dietary sorghum tannins markedly reduce the digestibility of crude protein and starch in caged White Leghorn layers. The absorption of minerals i.e. calcium, magnesium, iron, sodium and potassium is also adversely affected. These effects have a linear increase with increase in the level of the dietary sorghum tannins.

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