

## Toxicological Effects of Feeding First Cut Sorghum Vegetation and Stalks to Rabbits

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**Abstract.-** We assessed toxic effects associated with sorghum feeding to rabbits. For this purpose 18 rabbits of almost same body weight and age were randomly divided into three groups (six animals per group) designated as A, B and C. Animals of each group were caged separately providing similar and standard environmental conditions. Group A (control) was fed on grass; group B was fed on sorghum stalks and group C was fed on sorghum leaves. Hematological studies revealed erythrocytopenia, leukocytopenia, decreased hemoglobin and lowered erythrocyte sedimentation rate (ESR) in group B as compared to group A and C from day 10 to 30 of the experiment. Biochemical analysis revealed methemoglobinemia and high level of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of group B as compared to group A and C from day 10 to 30 of the experiment. This indicates that these hematological abnormalities may be associated with sorghum stalk feeding in experimental rabbits. Further we analyzed the nitrate level of sorghum stalk, leaves and grass which we used in our experiments. We determined its highest level (1480ppm) in sorghum stalk as compared to sorghum leaves (850ppm) and grass (736ppm). We can not rule out that nitrate in sorghum stalk led to the observed toxicity, but this needs further investigation. Taken together, our observations show that sorghum stalks are more toxic than sorghum leaves when fed to laboratory animals like rabbits.

**Key words :** Alanine aminotransferase, erythrocytopenia, leukocytopenia, nitrates, sorghum toxicity.

### INTRODUCTION

Sorghum species are an important food crop in Africa, Central America, and South Asia and is the "fifth most important cereal crop grown in the world". Being tolerant to the arid conditions, sorghum is more commonly cultivated for animal fodder in Pakistan. Sorghum species and their hybrids are widely grown for warm season grazing and for hay fodder. There has been reports of poisoning and mortality by feeding sorghum in various species of animals, especially for sorghum grown early in the season, during low temperatures, high humidity and after nitrogen fertilization (Allmucatt, 1989). The economic losses associated with sorghum poisoning are significant. It may causes frequent urination, urinary incontinence that leads to haematuria, followed by a serous nasal

discharge, erratically increased body temperature,, depression, reduced appetite and ultimate weight loss (Varshney and Gupta, 1996).

Micronutrients such as minerals are important constituents of animal food and livestock fodder. These minerals, especially the nitrates, are essential for the normal metabolic functions of the animals (Booth and Macdonald, 1977). Sorghum vegetation is considered to be a rich source of nutrients and routinely used in many parts of Pakistan, but indiscriminate heavy use of fertilizers in agriculture and livestock farming made it an apparent cause of chemicals toxicity such as nitrate toxicity. Maximum recommended safe levels of nitrate in forage for livestock is 3000 ppm; however concentrations of more than 5000 to 10,000 ppm nitrate is required to produce acute toxicity and poisoning. Mortality has been reported from 15,000 ppm nitrate (Louis *et al.*, 1975). The nitrate level in plants is higher in the vegetative tissues (leaves and stalks), being the highest in the stalk. These may contain a variety of chemicals, which although less toxic by themselves, can be transformed into more

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toxic compounds when eaten. Some of these chemicals or their derivatives are not only mutagenic but can also be powerful carcinogens (Roberts *et al.*, 1990). Nitrate toxicity is mainly a problem in herbivores especially ruminants. Acute poisoning may occur when the level of nitrate in feed (dry matter basis) exceeds 1% (National Academy of Sciences, 1972). The oral administration of nitrite-polluted water to Rabbits causes a decrease in weight gain and clinical signs of diarrhea, frequent urination and vomiting (Shahid *et al.* 2001). Nitrate itself although not toxic but it can be reduced metabolically to nitrite, which is carcinogenic in nature (Butler and Jones, 1973). There is great concern about the mutagenicity of deaminating agents, particularly nitrites resulting in deamination of DNA (Roberts *et al.*, 1990). Environmental and physiological conditions that influence nitrate assimilation in higher plants are light, drought, minerals nutrition, hormonal treatment and genetic composition. Nitrate accumulation is associated with deficiencies of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{K}^{2+}$ . Nitrate also induces the expression of the enzymes nitrate and nitrite reductase (Butler and Jones, 1973). In the present study we evaluated the effects of sorghum stalks and vegetation feeding to rabbits using hematological and biochemical parameters. We found that sorghum stalks are more toxic than sorghum leaves. We measured the nitrate values in sorghum leaves, stalk and compared it to grass that were fed to the experimental rabbits. In the course of our trials, we found accumulation of nitrate in stalks.

## MATERIALS AND METHODS

A total number of 18 rabbits of almost six months of age were randomly divided into three groups (six animals per group) designated as A, B and C. Animals of each group were caged separately in experimental shed of University of Veterinary and Animal Sciences, Lahore, providing similar standard environmental conditions, and experimental approach as recommended by Canadian Council on Animal Care. At the start of the experiment all the rabbits were weighted separately (Table I). The animals of group "A" served as control and were fed on grass through out

the experiment. The group "B" was fed on sorghum stalks for the period of 30 days. Group "C" was fed sorghum leaves for the period of 30 days through out the experiment. We obtained the sorghum leaves and grass in one go from the selected fields and offered at *ad libitum* to experimental rabbits during the course of our experiment.

**Table I-** Body weight of rabbits of groups A, B and C before feeding.

Group	Body weight (g)
Group A	
M <sub>1</sub>	1250.3
M <sub>2</sub>	1270.5
M <sub>3</sub>	1390.6
M <sub>4</sub>	1338.2
M <sub>5</sub>	1312.3
M <sub>6</sub>	1215.9
Group B	
M <sub>1</sub>	1160.4
M <sub>2</sub>	1348.2
M <sub>3</sub>	1326.0
F <sub>1</sub>	1278.9
F <sub>2</sub>	1256.6
F <sub>3</sub>	1346.2
Group C	
M <sub>1</sub>	1289.3
M <sub>2</sub>	1225.9
M <sub>3</sub>	1346.8
M <sub>4</sub>	1374.2
M <sub>5</sub>	1298.0
F <sub>1</sub>	1325.2

### *Quantitative analysis of nitrates in sorghum*

Sorghum samples were collected from different fields, near Bund Road Lahore, Pakistan. From each field 10 samples were collected randomly and each sample was analyzed in duplicate as mentioned earlier (Ranjhan and Gopal, 1981). One gram of air-dried sorghum leaves and stalks from each sample were mixed up in 125 ml of 0.1N HCl in round flask through orbital shaking. The samples were kept at room temperature with occasional shaking for one hour to extract nitrate. The extract was filtered through filter paper. To this filtrate 0.5 gm of Bray's indicator was added and then it was shaken for one minute. The solution was kept away from light and centrifuged at 4000 x g

until the supernatant liquid was cleared. Any film at the top of the solution was removed and the clear red solution was poured into spectrophotometer cuvettes and measured at a wavelength of 520 nm. We compared the observations to standard nitrate solutions ranging from 0.5 to 1.9  $\mu\text{g NaNO}_3/\text{ml}$  that were measured at 520  $\mu\text{m}$ , after setting the instrument at zero absorbance with a blank solution. We calculated standard factor (SF) (absorbance/ $\mu\text{g}$  of  $\text{NaNO}_3$ ) and multiplied SF by absorbance of each sample to get the quantity of nitrate in sorghum and grass. The animals were examined daily for any abnormality in their behavior throughout the experimental period.

#### *Hematological studies*

Total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin estimation (Hb) and erythrocyte sedimentation rate (ESR) of each blood sample were performed for each animal separately at day 0, 10, 20 and 30 as mentioned earlier (Benjamin, 1985).

#### *Biochemical analysis*

##### *Methemoglobin concentration*

Blood samples were taken from marginal ear vein of all rabbits aseptically with the help of disposable syringes and analysed for methemoglobin (MethHb) concentration at day 0, 10, 20 and 30 as reported earlier (Richard and Henry, 1966).

##### *Estimation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST)*

ALT and AST were determined at day 0, 10, 20 and 30 of the experiment following the procedure mentioned by International Federation of Clinical Chemistry, 1980. The data from hematological study as well as biochemical analysis in various groups were analyzed statistically using one-way analysis (Steel and Torrie, 1984). The P value (0.05) was considered as standard. Means with differences ( $P < 0.05$ ) was considered as significantly different.

## RESULTS

#### *Nitrates in sorghum*

We obtained random samples of several

fields (field A to D) and selected field A as a source of sorghum for the experimental rabbits (Table II). Grass from the grounds of the University of Veterinary and Animal Sciences, Lahore, was fed to control group A.

**Table II.- Concentration (ppm) of nitrates in different fields of sorghum**

Source	Field A	Field B	Field C	Field D
Leaves	850	812	741	778
Stalks	1480	1100	1115	1105

Each value represents the mean of the duplicate samples of one of the two repeated independent experiments.

#### *Hematological parameters*

Statistical analysis showed that there was no significant difference ( $P > 0.05$ ) in the mean value of TEC of group A, B and C at day 0 (Table III). We noted significant difference ( $P < 0.05$ ) in the mean values of TEC of group B as compared to group A and C but there was no significant difference ( $P > 0.05$ ) in the mean values of TEC of group A and C at day 10, 20 and 30. There was significant difference ( $P < 0.05$ ) within the mean values of TEC of group B on day 10, 20 and 30 but there was no significant difference within the mean values of TEC on day 10, 20 and 30 in group A and C on same days.

At day 0 of the experiment we observed no significant difference ( $P > 0.05$ ) in the mean values of ESR of groups A, B and C, but we noted a significant difference ( $P < 0.05$ ) within the mean values of the ESR of group B on day 20 and 30. At day zero of the experiment we observed no significant difference ( $P > 0.05$ ) in the mean values of Hb in all our experimental groups. Anyhow we observed significant difference ( $P < 0.05$ ) in the mean values of Hb of group B as compared to group A and C but there was no significant difference ( $P > 0.05$ ) between the mean values of Hb of groups A and C. There was significant difference ( $P < 0.05$ ) within the mean values of Hb of group B on day 10, 20 and 30 (Table III).

#### *Biochemical analysis*

There was no significant difference ( $P > 0.05$ ) in the mean values of MetHb of groups A, B and C

**Table III.- Different hematological and biochemical parameters (Mean±SEM) in experimental and control groups of rabbits.**

Hematological parameters	Groups	Days post feeding			
		0	10	20	30
Total erythrocyte count ( $10^6/\text{mm}^3$ )	A	5.93 ± .371 <sup>a</sup>	5.80 ± .264 <sup>a</sup>	5.72 ± .472 <sup>a</sup>	6 ± .422 <sup>a</sup>
	B	6.55 ± .387 <sup>a</sup>	4.72 ± .217 <sup>b*</sup>	4.28 ± .147 <sup>b*</sup>	4.53 ± .194 <sup>b*</sup>
	C	6.47 ± .406 <sup>a</sup>	6.44 ± .358 <sup>a</sup>	6.38 ± .446 <sup>a</sup>	6.25 ± .407 <sup>a</sup>
Leukocyte count ( $10^3/\text{mm}^3$ )	A	7.93±765 <sup>a</sup>	8.06±.649 <sup>a</sup>	7.89 ± .831 <sup>a</sup>	7.94±.720 <sup>a</sup>
	B	7.91±.984 <sup>a</sup>	7.01 ± .865 <sup>b*</sup>	6.80 ± .701 <sup>b*</sup>	7.03 ± 0727 <sup>b*</sup>
	C	7.79 ± .845 <sup>a</sup>	7.67 ± .706 <sup>a</sup>	7.78 ± .559 <sup>a</sup>	7.75 ± .607 <sup>a</sup>
Erythrocyte sedimentation rate (mm/hr)	A	1.80 ± .486 <sup>a</sup>	2.1 ± .276 <sup>a</sup>	1.96 ± .122 <sup>a</sup>	1.8 ± .278 <sup>a</sup>
	B	2.5 ± .227 <sup>a</sup>	2.11 ± .157 <sup>a</sup>	1.90 ± .150 <sup>a*</sup>	1.61 ± .215 <sup>a*</sup>
	C	2.05 ± .319 <sup>a</sup>	1.96 ± .233 <sup>a</sup>	1.81 ± .199 <sup>a</sup>	1.41 ± .302 <sup>a</sup>
Hemoglobin estimation (gm/dl)	A	13.9 ± 1.01 <sup>a</sup>	14.1 ± 1.20 <sup>a</sup>	14.02 ± 1.02 <sup>a</sup>	13.5 ± .741 <sup>a</sup>
	B	15.6 ± .834 <sup>a</sup>	13.1 ± .75 <sup>a*</sup>	10.95 ± .499 <sup>b*</sup>	9.35 ± .52 <sup>b*</sup>
	C	12.8 ± 1.22 <sup>a</sup>	12.9 ± .55 <sup>a</sup>	13.1 ± 2.10 <sup>a</sup>	12.98 ± .82 <sup>a</sup>
Methemoglobin concentration (gm/100dl)	A	0.227 ± .026 <sup>a</sup>	0.228 ± .051 <sup>a</sup>	0.237 ± .044 <sup>a</sup>	0.232 ± .045 <sup>a</sup>
	B	0.151 ± .019 <sup>a</sup>	0.287 ± .017 <sup>a*</sup>	0.507 ± .018 <sup>b*</sup>	0.729 ± .037 <sup>b*</sup>
	C	0.221 ± .054 <sup>a</sup>	0.227 ± .057 <sup>a</sup>	0.246 ± .058 <sup>a</sup>	0.252 ± .054 <sup>a</sup>
Alanine aminotransferase (i.u/l)	A	55.6 ± 2.10 <sup>a</sup>	55.9 ± 2.32 <sup>a</sup>	55.6 ± 1.78 <sup>a</sup>	55.08 ± 1.85 <sup>a</sup>
	B	57.5 ± 2.4 <sup>a</sup>	70.17 ± 2.33 <sup>b*</sup>	94.2 ± 2.21 <sup>b*</sup>	97.4 ± 2.03 <sup>b*</sup>
	C	56.2 ± 2.9 <sup>a</sup>	62.8 ± 3.26 <sup>a</sup>	65.2 ± 2.94 <sup>c*</sup>	66.4 ± 2.18 <sup>c*</sup>
Aspartate aminotransferase (i.u/l)	A	51.8 ± 2.33 <sup>a</sup>	52.5 ± 2.17 <sup>a</sup>	52.8 ± 2.93 <sup>a</sup>	50.9 ± 1.77 <sup>a</sup>
	B	50.7 ± 2.56 <sup>a</sup>	85.9 ± 3.09 <sup>b*</sup>	90.5 ± 3.16 <sup>b*</sup>	104.1 ± 1.68 <sup>b*</sup>
	C	54.7 ± 2.66 <sup>a</sup>	60.9 ± 2.90 <sup>c</sup>	64.06 ± 2.81 <sup>c*</sup>	65.8 ± 2.81 <sup>c*</sup>

Figures with different super scripts (a, b & c) in a column are significantly different ( $P < 0.05$ ).

The values having super script (\*) in the same row are significantly different then zero day of experiment.

SEM = standard error mean. A, control group; B, rabbits fed on sorghum stalks; C, rabbits fed on sorghum leaves.

Above results are representative of one of the three similar values of independent experiments.

at day zero of the experiment. Statistical analysis showed that there was significant difference ( $P < 0.05$ ) in the mean values of MetHb of group B as compared to group A and C but there was no significant difference ( $P > 0.05$ ) in the mean values of MetHb of group A and C at day 20 and 30. There was significant difference ( $P < 0.05$ ) with in the mean values of MetHb of group B on day10, 20 and 30. Similarly at day zero of the experiment there was no significant difference ( $P > 0.05$ ) in the mean values of ALT in all groups. But we noted significant difference ( $P < 0.05$ ) in the mean values of ALT of group B as compared to group A and C but there was no significant difference ( $P > 0.05$ ) in the mean values of ALT of group A and C at day 10. Our statistical analysis shows that there was significant difference ( $P < 0.05$ ) in the mean values of ALT of group A, B and C on day 20 and 30 of experiment and there was significant difference ( $P < 0.05$ ) within the mean values of ALT of group B on day 10, 20, 30 and group C on day 20 and 30 of experiment.

There was no significant difference ( $P > 0.05$ ) in the mean values of ALT of groups A, B and C at day 0 of the experiment. Similarly significant difference ( $P < 0.05$ ) in the mean values of AST of group A, B and C on day 10, 20 and 30 of experiment was seen. There was significant difference ( $P < 0.05$ ) with in the mean values of AST of group B on day 10, 20, 30 and group C on day 20 and 30 (Table III).

## DISCUSSION

Minerals are important constituents of the fodder and some of them are essential for the normal metabolic functions of the organism, but they can be present up to toxic level in the fodders. Nitrate is one of the elements that may be present in high concentrations in fodder especially, sorghum and is known as a dietary toxicant. To cope with increasing demand of food for human beings, pesticides and fertilizers are heavily used by farmers resulting in an increase of nitrate and nitrite levels in

crops like sorghum (Booth and Macdonald, 1977). Maximum safe level of nitrate in forage for livestock is up to 3000 ppm although more than 5000 to 10,000 ppm nitrate may be required to produce acute poisoning (Louis *et al.*, 1975). Environmental and physiological conditions, which influence nitrate assimilation in higher plants, include light, drought, mineral and hormonal treatments (Butler and Jones, 1973). We showed that nitrate in sorghum accumulated in stalks and leaves and our quantitative data shows that the accumulated concentration in stalks was higher than in leaves. This correlates with earlier findings of (Osweiler, 1985) who also reported that nitrates accumulated more in stalks than leaves. The nitrate contents of stalks and leaves were higher in field A, when compared to field B, C and D. This high level of nitrate in sorghum may be due to excessive use of nitrogen containing fertilizers by the farmers, which was verified by a questionnaire. In these experiments, animals of group "B" and "C" were fed on sorghum stalks and leaves of field A for 30 days. Here we assessed the hematological and biochemical abnormalities associated with sorghum toxicity. A similar approach has been published to measure sodium nitrate toxicity in ewes and ruminants, respectively (Shalaby, 1990; Koss, 1992).

TEC, TLC, ESR and Hb estimation of each blood sample were performed separately. The hematological and biochemical values recorded at day 0 were similar as reported by Mitruka and Howard (1997) in normal rabbits. The results showed that TEC values of group B were significantly lower than the TEC values of groups A and C on day 10, 20 and 30. Similar pattern of erythrocyte decrease due to nitrate toxicity was also noted by others (Jiri *et al.*, 1983; Shahid *et al.*, 2001; Nikolov, 2001). Jiri *et al.* (1983) found that the oral administration of nitrate and nitrite resulted an acute decrease of red blood cells (RBCs) in first two weeks; later on causing gradual increased nitrate feeding to animals resulted in the formation of methemoglobin due to which the oxygen carrying capacity of RBCs was tremendously impaired. Shahid *et al.* (2001) reported that the oral administration of nitrite, nitrate and polluted water to rabbits caused a decrease in abdominal stress and

convulsions. Hematological studies in rabbits fed on nitrate/nitrite revealed erythrocytopenia; leukocytopenia, decreased hemoglobin and lowered erythrocytic sedimentation rate up to 20 to 30 days post feeding and slowly increased thereafter. Nikolov (2001) reported that nitrate poisoning due to forages rich in nitrates caused considerable economical losses in ruminants (sheep) in cases of simultaneous ingestion. We hypothesize that decrease in erythrocytes could be due to nitrate present in sorghum, which has an effect on the cellular Ca, Mg levels and ATPase activity of the cell membrane of RBCs, since it has been reported that nitrates altered the cell membrane components of red blood cell and components of microsomal electron transport system of liver resulting in decreased ATPase activity (Manabu *et al.*, 1983; Koss *et al.*, 1992).

The TLC of group B was dropped significantly in animals fed on sorghum stalks on day 10, 20 and 30. Similar finding was observed by Shahid *et al.* (2001) who reported that the oral administration of nitrite, nitrate and polluted water to rabbits caused erythrocytopenia; leukocytopenia, decreased hemoglobin and lowered erythrocytic sedimentation rate up to 20 to 30 days post feeding and slowly increased thereafter. The hemoglobin estimation showed significantly decreased hemoglobin level in group B on days 10, 20 and 30. This is in agreement with Jiri *et al.* (1983) and Shalaby (1990). Shalaby (1990) reported decreased ESR, RBCs count, hemoglobin concentration, plasma total protein, albumin, cholesterol, calcium and inorganic phosphorus levels in barki, ewes after feeding 60mg/kg body weight sodium nitrate during late stages of pregnancy. We noticed that ESR of group B was significantly decreased at day 20 and 30. This decrease in ESR might be due to erythrocytopenia and methemoglobin formation as observed by Jiri *et al.* (1983) and Shahid *et al.* (2001). Present results show that in group B there was increased level of methemoglobin on day 10 to 30. This increase in methemoglobin concentration may be due to the binding of nitrates with hemoglobin as reported by Ellis *et al.* (1992) and Gaworski (1992). Gaworski (1992) reported that ingestion of isobutyl nitrite at 300 ppm reduced the body weight gains in mice. Isobutyl nitrite related

clinical pathology changes included reduced RBC counts accompanied by moderate increases in mean corpuscular volume and erythrocyte counts, increased WBC counts, and mildly increased methemoglobin concentration.

Strikingly, the nitrate contents in sorghum stalks are in the range of what is considered a safe level generally (Louis *et al.*, 1975). We, therefore, speculate that the toxicity may be due to derivatives of nitrates in combination with other elements associated with sorghum stalks which augmented its toxicity for rabbits. Alternatively, rabbits are more sensitive to nitrate and nitrate derivatives. This opens a new area of research which should be investigated further. We showed that there was significant increase in the mean values of ALT and AST of group B on day 10, 20 and 30 as compared to group A and C. This increase in AST and ALT values are associated with sorghum stalks feeding. Similar findings were reported by Gufrii (1993) and Mondal and Pandeny (1999) who observed high level of ALT and AST in nitrate toxicity in ruminants. Taken together, in our experiments, we noticed that accumulations of nitrates are greater in sorghum stalks than in leaves. Rabbits fed with sorghum stalks exhibited erythrocytopenia, leukocytopenia, hemoglobinemia and increased level of methemoglobin. Serum AST and ALT levels were high in sorghum stalks fed rabbits. Together our data suppose that sorghum stalks feeding to rabbits is associated with toxicity.

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