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Counteracting the hematological toxicity of gossypol by using selenium supplementation in rams

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ABSTRACT

This study was conducted to evaluate the hematological toxicity of natural gossypol in ration and the ability of selenium supplementation to alleviate this toxicity in rams. Twenty-five mature crossbred (Barki × Rahmani) rams were randomly divided into five equal groups. The first group served as a control (received ration contained 0 gossypol). The second and third groups received rations containing 9 and 14 mg of free gossypol/kg Bw/day (low level and high level of gossypol), respectively. The fourth and fifth groups received the same rations given to the second and third groups, respectively, and an oral administration of 1 mg selenium (as sodium selenite) per day for each animal. Results showed that both levels of gossypol reduced ($P < 0.05$) hemoglobin concentration, packed cell volume, mean corpuscular volume and mean corpuscular hemoglobin compared to control. On the other hand, selenium supplementation showed improvements ($P < 0.05$) in hemoglobin concentration, red blood cells counts and packed cell volume compared to absence of selenium. Furthermore, selenium supplementation in ration containing low level of gossypol failed to alleviate the hematological toxicity of gossypol, but selenium supplementation in ration containing high level of gossypol successfully improved most of hematological parameters. In conclusion, natural gossypol in ration induced hematological toxicity in rams and this toxicity could be successfully alleviated by selenium supplementation.

1. Introduction

Even though whole cottonseed and cottonseed meal are important sources of protein for ruminants, they contain toxic polyphenolic pigment gossypol; the toxicity of gossypol is considered to be as free gossypol (Beradi and Goldblatt, 1980). Ruminants can exhibit similar pathological changes like monogastric species if dietary levels exceed the detoxification capacity of the rumen. Increasing dose of gossypol resulted in significant reduction in hemoglobin concentration and packed cell volume (PCV) in calves (Risco et al., 1992; Velasquez-Pereira et al., 1999) and increase in erythrocyte fragility in goats (Solamin et al., 2009; Menges, 1991) consuming gossypol. In addition, some gossypol toxicity signs have been related to a decrease in antioxidants concentrations (Lane and Stuart, 1990) and this toxicity can be reversed by feeding antioxidant materials (Velasquez-Pereira et al., 1998). So, the present study was designed to evaluate the hematological toxicity of feeding cottonseed meal and the effectiveness of selenium to counteract potential gossypol effects on hematological parameters of rams.

2. Materials and methods

This study was carried out at the Agricultural Experimental Station (31° 20′ N, 30° E), Department of Animal Reproduction, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Experiments were carried out after the Department approval, and were done without any commercial profit purposes for the Authors or the Department. All procedures and experimental protocols were conducted in accordance with the "Guide for the Care and Use of Agricultural Animals in Research

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Table 1
Composition of the experimental rations.

	Control ration (group 1)	Ration of LD gossypol[a] (groups 2 and 4)	Ration of HD gossypol[b] (groups 3 and 5)[c]
Free gossypol (mg)	0	9	14
Soybean meal	18%	–	–
Cottonseed meal	–	30%	48%
Wheat bran	40%	32%	12%
Corn	40%	36%	38%
Limestone + salt	2%	2%	2%
CP, %	16.04	15.9	16.5
TDN, %	68.4	67.6	68

[a] LD: low dose of gossypol formulated to supply 9 mg of free gossypol/kg BW/day.
[b] HD: high dose of gossypol formulated to supply 14 mg of free gossypol/kg BW/day. CP: crude protein, TDN: total digestible nutrients.
[c] Animals of groups 4 and 5 received 1 mg (Se) per day.

and Teaching", Federation of Animal Science Societies, 2010. The present study was conducted on crossbred Barki × Rahmani rams during the breeding season (from July to September).

2.1. Animals and management

Twenty-five mature crossbred (Barki × Rahamani) rams aged 1.5–2 years and weighing 73.92 ± 0.86 kg were used throughout the present study. Animals were kept outdoors with shelter during the day time and housed in a semi-open barn at night. They were offered roughage and concentrated supplement according to their body weight requirement (NRC, 1985). Animals were given Egyptian clover hay (*Trifolium alexandrinum*), and each animal also received 1 kg per day of a concentrated mixture that contained 68% total digestible nutrients (TDN) and 16% crude protein. Water was offered in access to animals at all times. Animals were free of any disease and were clinically normal with a healthy appearance.

2.2. Experimental design

The rams were randomly divided into five groups of five animals each. The first group served as a control (the animals were given a ration that contained (0 mg) natural gossypol and contained soybean meal as a source of protein). In the second group, animals were given a ration that contained cottonseed meal as a source of protein which was formulated to supply 9 mg of free gossypol/kg BW/day (low level of gossypol which is usually present in the commercial rations). In the third group, the animals were given a ration that contained cottonseed meal as a source of protein which was formulated to supply 14 mg of free gossypol/kg BW/day (high level of gossypol which was reported to cause hematological deterioration in ruminants (Velasquez-Pereira et al., 1998)). Both the second and third groups were used to study the hematological toxicity of gossypol in rams. The fourth and fifth groups received the same rations given to the second and third groups, respectively with an oral addition of 1 mg selenium as sodium selenite per day for each animal. Both fourth and fifth groups were used to study the ability of selenium to counteract the toxicity of low and high levels of natural gossypol on hematological parameters in rams. The free gossypol was determined in cottonseed meal and in the formulated rations containing the cottonseed meal as a protein source according to AOAC (1984). The compositions of the rations are presented in Table 1.

2.3. Data collected

Blood samples were collected biweekly for 8 weeks from the jugular vein of each animal in the early morning before access to feed and water using a clinical needle. Blood was withdrawn into heparinized vials to determine levels of hemoglobin (Hb) concentration, red blood cell (RBC) counts, packed cell volume (PCV), and white blood cell (WBC) counts. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formulas proposed by Schalm et al. (1975). Blood Hb concentration was determined using the cyanomethemoglobin procedure (Wintrobe, 1965). Red blood cells and white blood cells were counted on A0 Bright line hemocytometer using light microscope at 400× and 100× magnifications, respectively. Micro Wintrobe hematocrit tubes and hematocrit centrifuge were used to determine the PCV or hematocrit value.

2.4. Statistical analysis

To study the toxic effect of gossypol compared to control, data were analyzed using (GLM) generalized linear model procedure (SAS, 2000) by one way ANOVA. Comparisons among means within each trait were carried out using $LSD_{0.05}$. Experimental factorial design (2 × 2) was used to study the effect of level of gossypol (low and high levels); effect of addition of selenium (0, +) and the interaction between them. Significant differences among means within each classification were tested using $LSD_{0.05}$.

3. Results

3.1. Effect of level of gossypol on hematological parameters

Data of the effect of level of natural gossypol in ration compared to control on some hematological parameters is shown in Table 2 (first panel). Regardless of the level of natural gossypol in ration, the presence of gossypol in ration resulted in a significant ($P < 0.05$) decreases in hemoglobin concentration, packed cell volume, mean corpuscular volume and mean corpuscular hemoglobin compared to control group. On the other hand, white blood cell count was not affected significantly in the presence of gossypol compared to control group however, its mean value was higher ($P < 0.05$) in the high level gossypol group compared to the low level of gossypol group.

3.2. Effect of selenium on hematological parameters

Effects of level of gossypol in ration and selenium supplementation on hematological parameters are presented in Table 2 (second and third panels, respectively). Regardless of selenium supplementation, increasing level of gossypol in ration resulted in significant increases in mean values of hemoglobin concentration, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and white blood cell count compared to that of the low dose-treated group. Furthermore, regardless of gossypol level in ration, selenium supplementation revealed significant increases in hemoglobin concentration, red blood cell count and packed cell volume compared to absence of selenium supplementation.

The interaction between gossypol and selenium (Table 2, fourth panel) showed that high level of natural gossypol in the presence of selenium supplementation revealed significant increase ($P < 0.05$) in hemoglobin concentration, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and white blood cell counts. However, other groups showed no significant differences between each other for all parameters mentioned except for mean corpuscular hemoglobin concentration and white blood cell counts. In these two case, low level of gossypol (regardless presence or absence of selenium supplementation) revealed low significant ($P < 0.05$) values compared to other groups.