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BLOOD SERUM-BIOCHEMICAL, HEMATOLOGICAL AND CLINICAL STUDIES ON GOSSYPOL TOXICITY IN FATTENING FRIESIAN CALVES FED ON WHOLE COTTONSEED

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SUMMARY

Gossypol toxicity was diagnosed in eighteen fattening friesian calves, of six months old, fed for three weeks on concentrated diet contained 20% ground cottonseed. Clinicaly, calves showed anorexia, intermittent diarrhoea and abdominal pain. Later on cough, polypnea, signs of dyspnea and hemoglobinuria were observed in four calves. These calves were emergency slaughtered. Serous fluid in thoracic and abdominal cavities and congestion of parenchymatous organs were inspected. Significant decrease in erythrocytes, hemoglobin and hematocrit values was obtained during hematological examination. Leukocytosis with neutrophilia, lymphopenia and monocytosis was also significant. Serum biochemical studies revealed significant decrease in total protein and albumin and significant increase in AST and ALT activities, creatinine and BUN values.

All recorded clinical signs disappeared and serum biochemical and hematological parameters were significantly improved after the change of diet to low concentrate one.

INTRODUCTION

Whole cottonseed is commonly fed to ruminant animals as a source of energy , crude protein and fiber. However, a major constraint to feeding high dietary levels of cottonseed is possible gossypol toxicity (Calhoun et al. 1995 and Blauwiekel et al. 1997). Gossypol is a polyphenolic pigment contains aromatic rings with hydroxyl group. It occurs in pigment glands scattered throughout the seed and it is produced by the cotton plant as a natural defense mechanism against

insect pests (Berardi and Goldbaltt, 1980). It is detoxified in ruminants through binding to soluble proteins forming gossypol-protein complex in the rumen (Nikokayris et al. 1991).

Although ruminant animals are less susceptible to gossypol toxicity than non ruminant species, toxicity has been reported in calves (Holmberg et al. 1988 and Risco et al. 1992). The highest mortality losses have been reported in calves which were fed on start ration, where cottonseed meal was their protein source (Ogilvie, 1998).

The clinical signs of gossypol toxicity have been reviewed by Hudson et al. (1988). Laboured breathing, anorexia, diarrhoea and hemoglobinuria were the most common signs of chronic toxicity, however sudden death was reported in calves fed on high concentration of cottonseed meal (Morgan, 1989). Pulmonary congestion, excessive abdominal, pericardial and pleural fluids and chronic passive congestion of the liver " nutmeg liver " were the most pathological lesions (Ogilvie, 1998).

The present study aimed to investigate gossypol toxicity in fattening friesian young calves fed concentrated diet contained whole cotton seed regarding to serum-biochemical, hematological, clinical and pathological alterations.

MATERIAL AND METHODS

Anorexia, intermittent diarrhoea and abdominal pains were the farm's owner complain in eighteen fattening friesian calves of about six months old at Kafr El Sheikh Province. These calves had been fed for 3 weeks on concentrated diet contained 20% ground cottonseed.

Later on, cough, polypnea, signs of dispense and hemoglobinuria were observed in four calves. These calves were emergency slaughtered. Serous fluid in the thoracic and abdominal cavities, congestion of the parenchymatous organs were recorded. Gossypol toxicity was the tentative diagnosis. Therefore the diet was completely apolished and low concentrate diet and mineral mixture fed for 3 weeks. Diseased calves were examined clinically according to Rosenberger (1979).

Serum biochemical, hematological and urinalysis were conducted on diseased calves and 3 weeks after receiving low concentrate diet. Two blood samples were collected from each diseased calve as well as from other ten calves of the same age and locality proved to be healthy used as control. Heparinized blood samples were used for determination of complete blood picture by using Automatic Hematology Cell Counter (MS9 Melet Schoesing laboratories, France). Blood serum samples were used for determination of total protein (Peters, 1968), albumin (Doumas et al.

1971), total globulins (by substraction of albumin from total proteins), albumin : globulin ratio, AST and ALT activities (Reitman and Frankel, 1957), Creatinine (Giorio, 1974),and BUN (Richterich, 1968).

Urine samples were collected during normal urination and were examined according to Benjamin (1979). Obtained results were designed as before and after experimentation.

Statistical analysis of data was performed according to Snedecor and Cochran (1982) by using MSTAT-C computer program.

RESULTS

On clinical examination the diseased calves showed diarrhoea and signs of abdominal pains mainfested by grunting, arched back and restlessness. Increased respiratory (60 ± 2.0 / min) and pulse rates (110 ± 5.0/min) were recorded. Body temperature was slightly eleveated (39.8 ± 0.5 C). decrease intensity of heart beat, muffled heart sound and moist rales were detected during examination of the heart and chest.

Hematological results revealed significant decrease in erythrocytes, hemoglobin and hematocrit, leukocytosis with neutrophilia lymphopenia and monocytosis (Table 1).

Serum biochemical analysis showed significant decrease in total protein and, albumin, and significant increase in AST and ALT activities, creatinine and BUN values (Table 2).

Urine examination revealed proteinuria (30-100 mg/dl), hemoglobinuria and high specific gravity (1.030 - 1.040).

Hematological and serum biochemical values of diseased calves after 3 weeks from receiving low concentrate diet were improved towards normal (Tables 1and 2).

Table (1): Hematological values of diseased calves before and 3 weeks after change of diet .

Parameters	Unit	Healthy (n = 10)	Diseased calves in (n : 14)	
			Before	After
RBCs	10^6/ul	7.20 ± 0.61^{a}	$4.40 \pm 0.45^{***b}$	$5.60 \pm 0.32^{*c}$
Hb	g/dl	11.50 ± 0.55^{a}	$8.90 \pm 0.50^{***b}$	$9.70 \pm 0.45^{*bc}$
PCV	%	37.80 ± 2.20^{a}	$28.50 \pm 1.85^{***b}$	32.60 ± 1.80^{ab}
WBCs	10^3/ul	9.25 ± 0.25^{a}	$10.30 \pm 0.40^{*b}$	9.50 ± 0.30^{ab}
Granulocytes	%	53.70 ± 1.50^{a}	$60.00 \pm 1.80^{**b}$	$54.50 \pm 1.60^{*ac}$
Lymphocytes	%	24.10 ± 1.70^{a}	$35.7 \pm 1.3^{**b}$	$41.90 \pm 1.50^{*ac}$
Monocytes	%	3.30 ± 1.60^{a}	$4.3 \pm 0.2^{**b}$	$3.60 \pm 0.30^{*ab}$

* Significant at P<0.05 ** Significant at P<0.01 *** Significant at <0.001
Means followed by different letters were statistically significant and the highest mean value was represented with the letter (a) followed by letters (b&C)

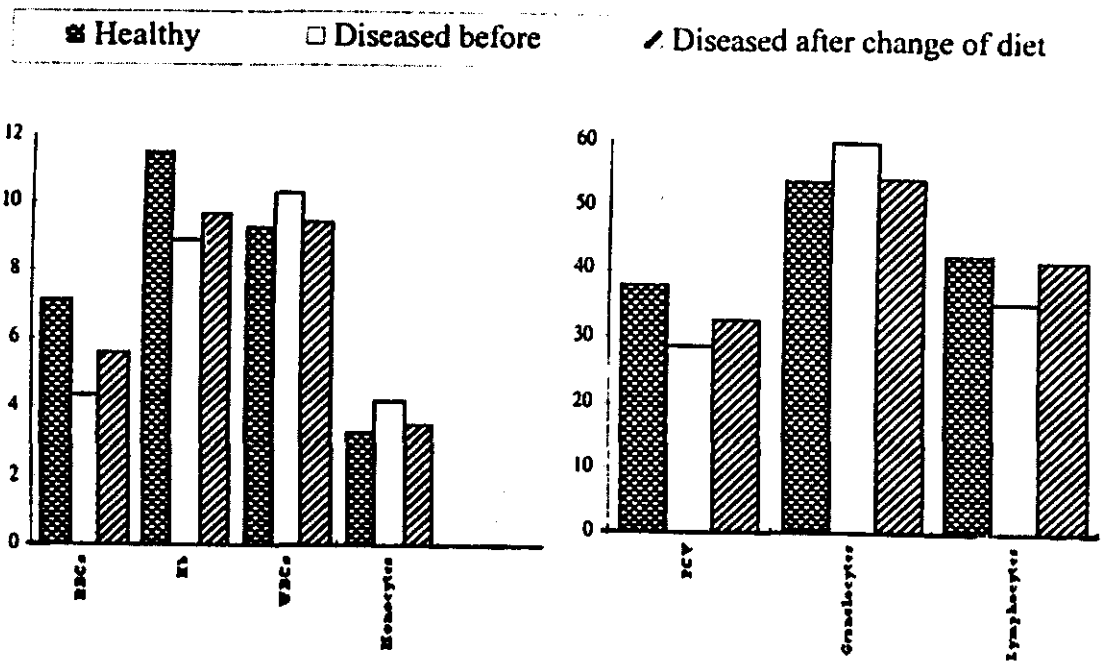

Fig. (1): Serum biochemical values of diseased calves before and 3 week after change of diet

Table (2): Serum biochemical values of diseased calves before and 3 weeks after change of diet after

Parameters	Unit	Healthy (n = 10)	Diseased calves in (n : 14)	
			Before	After
Total protein	gm/dl	7.60 ± 0.50^{a}	$5.90 \pm 0.20^{**b}$	6.50 ± 0.20^{ab}
ALbumin	gm/dl	4.30 ± 0.30^{a}	$3.10 \pm 0.15^{**b}$	3.70 ± 0.20^{ab}
Globulin	gm/dl	3.35 ± 0.2^{a}	2.95 ± 0.2^{a}	2.70 ± 0.15^{a}
A/G	Ratio	1.30 ± 0.07^{a}	$1.05 \pm 0.08^{**b}$	1.35 ± 0.07^{ab}
AST	u/l	31.40 ± 2.50^{a}	$67.00 \pm 3.30^{***b}$	$50.50 \pm 2.90^{****c}$
ALT	u/l	14.10 ± 1.60^{a}	$26.90 \pm 2.20^{***b}$	$19.80 \pm 1.30^{**c}$
Greatinine	mg/dl	1.90 ± 0.10^{a}	$3.10 \pm 0.30^{***b}$	$2.30 \pm 0.20^{*ac}$
BUN	mg/dl	20.30 ± 1.60^{a}	$30.70 \pm 2.50^{***b}$	$24.20 \pm 1.60^{*ac}$

* Significant at P<0.05 ** Significant at P<0.01 *** Significant at <0.001
Means followed by different letters were statistically significant and the highest mean value was represented with the letter (a) followed by letters (b&C)

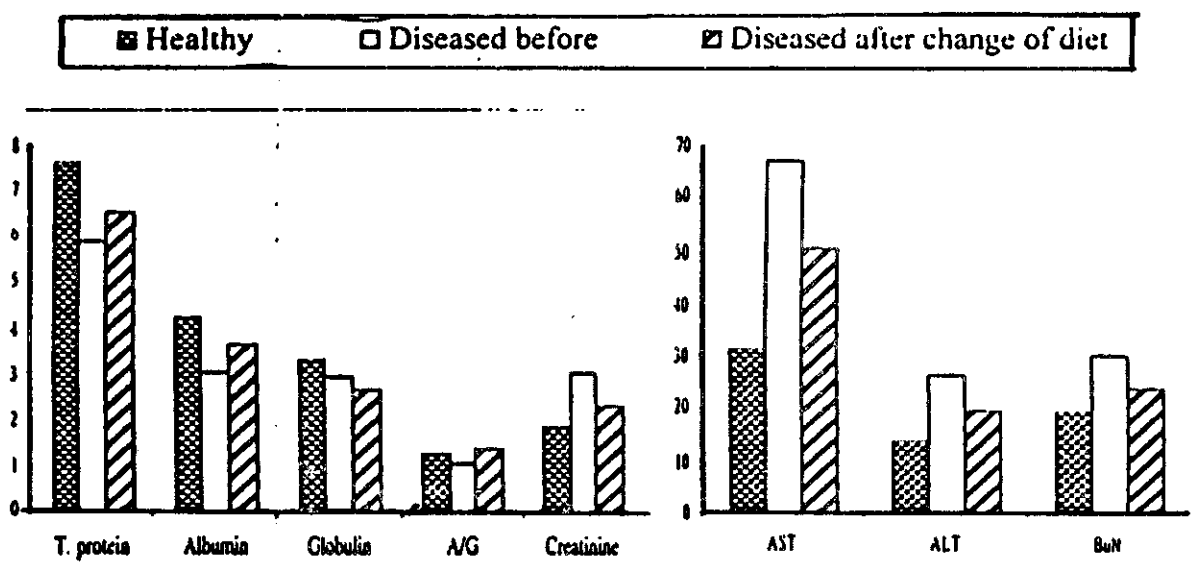

Fig. (2): Serum biochemical values of diseased calves before and 3 weeks after change of diet

DISCUSSION

Most Literature show that introducing cottonseed meal into rehabilitation diet is not nutritionally harmful in ruminants if it is given in the recommended dose. Ruminants have the ability to detoxify gossypol because the ruminal flora bind free gossypol to protein forming a digestable gossypol - protein complex (Nikokayris et al. 1991, Poore 1995 and Blauwiekel et al., 1997). Although ruminants are more tolerant to gossypol, prolonged feeding of whole cottonseed causes gossypol toxicity. The toxicity indicates that some gossypol escapes from detoxification in the rumen and is absorbed in the liver (Reiser and Fu, 1962).

The diagnosis of toxicity in our study depended on the nature of the diet, calve's age, clinical signs, mortality losses as well as clinicopathological alterations. Holmberg et al. (1988) reported that feeding high concentration of cottonseed to young calves resulted in death with lesions compatible with gossypol toxicity. Also, Morgan (1989) mentioned that gossypol toxicity must be considered at any time if there is mortality losses in young calves that have been fed on cottonseed for several weeks.

The previously mentioned clinical signs and necropsy findings in emergency slaughtered calves were suggestive of gossypol toxicity, these were similar to those mentioned by Hudson et al. (1988), Morgan et al. (1988) and Poore (1995). Laboured breathing and cough were observed in critical cases due to heart failure and the fill up of the lungs with fluid. Pericardial effusion and pulmonary edema explain the decrease intensity of heart beat, muffled heart sound and moist rales. The present results are compatible with those previously reported by Cheeke (1991), who mentioned that gossypol caused damage to the heart and lungs resulting in cardiac irregularity, heart attack and pulmonary edema. It is note worthy to mention that these calves didn't respond to therapeutic drugs (Bronchodilator and heart tonic) and they were exposed for emergency slaughter for salvage.

Hematological studies revealed a significant decrease in erythrocytes, hemoglobin and hematocrit values. This may be attributed to increased erythrocytes fragility under effect of gossypol (Hawkins et al. 1985 and Calhoun et al. 1995) causing intravascular hemolysis, hemoglobinemia and hemoglobinuria (Christopher and Margaret 1998). Dark colored urine is produced as a result of hemoglobinemia which exceeds renal threshold (Kaneko, 1989). Positive hemoglobinuria during chemical examination of urine confirmed this observation. On the other hand, significant leukocytosis with neutrophilia, lymphopenia and monocytosis were obtained which suggest gossypol as stress factor. Coles

sue destruction and irrespective of its cause will produce an increase in the number of circulating neutrophils. Also monocytosis occurs to phagocytose the cellular debris that accumulate in damaged tissues.

Serum biochemical analysis showed significant decrease in total protein and albumin. which may be attributed to several causes; Gossypol react with protein to produce bound protein resulting in a lowered lysine availability; Gossypol causes intestinal necrosis and nutrient malabsorption; Imperfect synthetic function of the liver cells as a result of liver damage (Blauwiekel et al., 1997 and Kaneko, 1989).

Significant increases ($P< 0.001$) in AST and ALT activities were detected. These results coincide with those reported by Duo et al. (1988). Elevation of AST and ALT activities indicate liver and heart damage (Kaneko, 1989). The levels of them remained significantly high in the serum of calves after diet change. The magnitude of increase in both enzymes is proportional to the amount of tissue damage. Significant increase in both creatinine and blood urea nitrogen were obtained. Renal damage, impaired renal perfusion and loss of glomerular filteration associated with cardiovascular insufficiency are the main cause of elevation of creatinine and blood urea nitrogen. These results correlate with Coles (1986).

There is no treatment currently available for gossypol toxicity but therapeutic plan depends on removal of gossypol from the diet and the ability of animals to recover depends on the severity of the heart damage.

It could be concluded that cotton seed is highly nutritious substance for dairy and beef cattle but some certain precautions should be applied: Cottonseed should be treated by heat to produce gossypol bound protein; iron salts are added to the diet to react with free gossypol and thus becomes physiologically inactive; Diet should be tested for gossypol concentration; Avoid feeding cottonseed to calves till rumen function is completed, finally the percentage of cottonseed in the diet should not exceed 13% (Cheek, 1991 and Ogilvie, 1998).

REFERENCES

Benjamin M.M. (1979): Outline of Veterinary Clinical Pathology. 3 rd Ed. Iowa Univ. Press Ames Iowa USA.

Berardi L.C. and Goldblatt L.A. (1980): Gossypol P.183 in Toxic Constituents of Plant Foodstuffs I.E. Liener, ed. Acad. Press, New York, NY.

Blauwiekel R; Xu S; Harrison J.H; Loney K.A; Riley R.E. and Calhoun M.C. (1997): Effect of whole cottonseed, gossypol and ruminally protected lysine supplementation on milk yield and composition. J. Dairy. Sci. 80:7, 1358-1365.

Calhoun M.C; Kuhlmann S.W. and Baldwin Jr. (1995): Cotton feed product composition and gossypol availability and toxicity Proc. 2nd National Symposium on Alternative Feeds for Dairy and Beef Cattle. St. Louis, Mo. September 24-26.

Cheeke P.R. (1991): Applied Animal Nutrition. 1st Ed. Macmillan Publishing Company, New York P. 91.

Christopher C and Margaret C (1998): Hematopoietic and hemolymphatic disorders In Large Animal Internal Medicine by Ogilive. 1st Ed. Williams and Wilkins Awaverly Company London P. 321-356.

Coles E.H. (1986): Veterinary Clinical Pathology. 4th Ed. Baillier. Tindal, London. PP: 10 - 79.

Doumas B., Watson W. and Biggs H. (1971): Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acta, 31: 87- 93

Duo X; Cai WJ; Zhu BH; Dong CJ; Zheng ZC. and Gao ZW. (1988): Clinical safety of longterm administration of gossypol in 32 Cases contraception. 37, 2: 129 - 135.

Giorio J.D. (1974): Clinical Chemistry, Prinicples and Techniques. Harper and Row. Hagerstown.

Hawkins G.E; Cummins K.A; Silverio M. and Jilek J.J. (1985): Physiological effects of whole cottonseed in the diet of lactating dairy cows. J. of Dairy Science 68: 2608- 2614 .

Holmberg CA; Weaver LD; Guterbock WM; Genes J. and Montgomery P. (1988): Pathological and toxicological studies of calves fed a high concentration seed meal diet. Vet. Pathology, 25: 2, 147-153.

Hudson LM; Karr LA. and Maslin WR. (1988): Gossypol toxicosis in a herd of beef calves. J. of the Am. Vet. Med. Association 192 : 9, 1303 - 1305.

Kaneko J.J. (1989): Clinical Biochemistry of Domestic Animals 3rd Ed. Academic Press, New York. P. 543.

Morgan S. (1989): Gossypol as a toxicant in livestock. In Hurrows GE (ed): The Veterinary Clinics of North America: Food Animal Practice. Philadelphia W.B. Saunders PP: 251-263.

Morgan S; Stair EL; Martin T; Edwards WC. And Morgan GI. (1988): Clinical, clinicopathologic, pathologic and toxicologic alterations associated with gossypol toxicosis in feeder lambs. Am. J. Vet. Res. 49: 4, 493 - 499.

Nikokayris P; Kandylis K; Deligiannis K. and Liamadis D. (1991): Effect of gossypol content of cotton seed cake on blood constituents in growing- fattening lambs J. Dairy Sci. 74:12, 4305-4313.

Ogilvie T.H. (1998): Large Animal Internal Medicine. 1st Ed. Williams and Wilkins, Awaverly Company. London. P.204.

Peters T. (1968): Coloremtric determination of total protein in serum based on the principle of the biuret reaction. Clin. Chem., 14:1147.

Poore M. (1995): Potential for gossypol toxicity when feeding whole cottonseed. Animal Husbandry Newsletter june /july.

Reiser R. and Fu H.C. (1962): The mechanism of gossypol detoxification by ruminants. J. Nutr. 76:215.

Reitman S. and Frankel S. (1957): Colorimetric method for determination of serum glutamic pyruvic and glutamic oxaloacetic transaminase. Am. J. Clin. Path., 28 : 56-59

Richterich E. (1968): Estimation of urea by Berthholet reaction, Klinische Chemie, Akademiche. Ver - Lag - Sgesllschaft, Frankfurt. Main: 254.

Risco CA; Holmberg CA. and Kutches A. (1992): Effect of graded concentrations of gossypol on calf performance:

toxicological and pathological considerations. J. Dairy. Sci. 75(10): 2787-2798.

Rosenberger G; Dirksen G; Grander H.D; Grunert E; Krause D. and Stober M. (1979): Clinical Examination of Cattle. 3rd Ed. Verlag Paul Parey, Berlin and Hamburg P. 68.

Snedecor G.W. and Cochran W.G. (1982): Statistical Methods. 8th Ed., Iowa State University Press, U.S.A.