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CLINICOHAEMATOLOGICAL CHANGES IN CATTLE FED CLOVER AND COTTON SEED MEAL

(With 2 Tables)

By

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التغيرات فى دم الأبقار بعد تغذيتها على البرسيم وكسب بذرة القطن

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أجريت الدراسة لمعرفة تأثير أحد الأستروجينات النباتية (بيتاسيتوستيرول) فى نبات البرسيم ومادة الجوسيبول فى كسب بذرة القطن على بعض مستوى الهرمونات ووظائف الكبد والكلية فى الأبقار الفريزيان. أستخدمت ٥٠ من الأبقار الفريزيان الحلاب بمزرعة ٦ أكتوبر بالقوات المسلحة قسمت إلى ثلاث مجموعات. المجموعة الأولى ١٠ أبقار وهى المجموعة الضابطة وتم تغذيتها بصورة طبيعية. والمجموعة الثانية ٢٠ حيوانا تم تغذيتها على العليقة المركزة (٣٠٪ كسب بذرة القطن). وأسفرت هذه الدراسة على زيادة فى الأسبارتات أمينو ترانسفيراز والألانين أمينو ترانسفيرازوالألكالين فوسفاتيز والبللـيروبين الكلى فى المجموعتين الثانية والثالثة، ونقص فى البروتين الكلى والألبيومين والجلوبيولينات فى المجموعة الثانية وزيادة غير معنوية فيهم فى المجموعة الثالثة، وأيضا زيادة فى نسبة اليوريا والكرياتينين فى الفوسفور والبوتاسيوم ونقص غير معنوى فى الصوديوم فى المجموعتين بالإضافة إلى نقص الكالسيوم فى المجموعة الثانية وزيادته فى المجموعة الثالثة. وأيضا نقص فى مستوى الأستروجين والبروجستيرون فى المجموعتين فيما عدا زيادة غير معنوية فى البروجستيرون بعد التغذية على البرسيم.

SUMMARY

The present study was carried out to investigate the changes in the blood serum constituents associated with some plants producing infertility in friesian cows. A total of 50 dairy cows at 6 th October farm of army forces were studied. The experimental animals were divided into 3 groups. The first group (10 cows) was fed normal ration & used as control. The second group (20 cows) was fed clover. The third group (20 cows) was fed concentrated ration (30% cotton seed meal). The results were increased blood serum

activities of AST,ALT, ALP and level of total, direct and indirect bilirubin in the two tested groups. Hypoproteinemia, hypoalbuminemia and hypoglobulinemia in the 2 nd group with insignificant increase in these parameters in the 3 rd group were noticed. Increase in blood urea, creatinine, hypophosphatemia, hypokalemia in addition to insignificant decrease in serum sodium level in the two tested groups was seen. Also, hypocalcemia in the 2 nd group and hypercalcemia in the 3rd group were noticed. A decrease in oestrogen and progesterone levels in the two groups was seen, with insignificant increase in progesterone in the 2nd group.

Key words: Cattle-serum parameters-clover- cotton seed.

INTRODUCTION

Fertility in farm animals has a close relationship with nutrition consumed by these animals. Certain materials-extracted from plants like phyto-estrogen which is extracted from clover (Barseem) and gossypol which is extracted from cotton seed meal-showed serious breeding problems, like infertility and other reproductive disorders *(GIHAD, 1971 and RANDAL et al., 1992).*

The administration of oestrogen to friesian cows caused a fall of blood urea, creatinine and bilirubin *(SOLIMAN et al., 1966).* An increase in serum AST and ALT activities was observed in cows suffering from uterine and ovarian disorders *(WATTKE and JAHN, 1971). CHRISTOPHER (1974)* noticed an increase in serum urea level in the low fertile and infertile dairy cows than in the normal ones. The serum albumin and potassium levels of cattle need more than 4 services to concieve-were significantly decreased, but the globulin level was significantly increased and there was no significant relationship between fertility of dairy cows and blood concentration of sodium *(ROLANDS et al., 1977). EL-BELEly (1993)* noticed lower values of total proteins, calcium and inorganic phosphorus levels in repeat breeder buffalo-cows as a result of grazing highly estrogenic pasture of clover.

An elevation in plasma total protein levels, AST and ALT activities was noticed in mature dairy cows fed excessive amounts of cotton seed meal *(LINDSEY et al., 1980).COPPOCK et al., (1985)* reported that dairy cows received 35% and 55% whole cotton seed for 3 weeks; revealed high levels of urea, creatinine, total bilirubin, calcium, AST, ALP, total proteins and albumin; in addition to low level of serum phosphorus. An elevated plasma concentration of the liver enzymes in addition to lower potassium ion

concentration were noticed in beef heifers fed on diets containing gossypol for 62 days *(GRAY et al., 1990)*.

The present study was aimed to provide more informations on serum biochemical changes associated with clover and cotton seed meal in friesian cows.

MATERIAL and METHOS

Fifty healthy friesian cows (primiparus), four years old from Military farm were divided into three groups. A control group of 10 cows (1 st group) and two equal groups each of 20 cows (2 nd and 3rd groups). The control group was fed on balanced ration which consists of 30 kgs barseem, 6 kgs concentrated ration and 2 kgs wheat straw in green season, 5 kgs drees, 30 kgs darawa, 6 kgs concentrated ration and 5 kgs wheat straw in dry season *(AHMED KHANAME, 1958)*. The second group was daily supplied by 50 kgs clover/ animal for 3 months, then subdivided into two equal subgroups (I and II). Subgroup I was intramuscularly injected with 5 ml of receptal (LH.RH and FSH.RH) for cows with cystic ovaries and intramuscularly injected with foligon {Pregnant mare serum gonadotrophin (PMSG)} 1000 I.U. for cows with smooth inactive ovaries (hormonal treatment). Clinical diagnosis of cystic ovaries was based upon the finding of a single or multiple formation of smooth fluctuant, rounded structures of 2.5 cm in diameter or large of one or both ovaries *(BIERSCHWAL et al., 1975)*. Cows on rectal palpation showing smooth ovaries without any structures were classified as inactive ovary *(SHARAWY, 1980)*. Subgroup II was treated by changing the ration by balanced one till the signs of heat appear on the animals from 6-8 weeks (nutritional treatment). The third group was daily supplied by 12 kgs concentrated ration/ animal for 3 months which consists of 30% cotton seed meal; 2% calcium carbonate; 1% sodium chloride; 1% mineral mixture; 26% rice straw and 40% maize *(AHMED KHANAME, 1958)*, then subdivided into two equal subgroups (III and IV) which exactly similar to subgroups I and II in the treatments. All animals in the second and third groups were inseminated artificially after treatments.

Blood samples of about 15 ml were drawn from the jugular vein from all cows after 3 months from the begining of the experiment and also after 48 hours from artificial insemination. The serum was separated and then used for the determination of liver function test which are aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activites, total proteins (T.P.), albumin, globulins, total bilirubin (T.B.), direct bilirubin (D.B.) and indirect bilirubin (Ind.B.). Also,

renal function tests which are urea, creatinine, calcium (ca), phosphorus (P), sodium (Na) and potassium (K) levels using test kits of bio-Merieux-France. Serum estrogen and progesterone hormones were measured by using kits from gamma trade.

All these parameters were statistically analyzed according to *SNEDECOR and COCHRAN (1967).* Using the "MSTAT" computer program. Also, L.S.D. was applied to compare the treatment averages.

RESULTS

The results are shown in tables 1&2. Increase blood serum activities of AST, ALT, ALP and level of total, direct and indirect bilirubin in the two tested groups was seen. Hypoproteinemia, hypoalbuminemia and hypoglobulinemia in the 2 nd group with insignificant increase in these parameters in the third group were noticed (Table. 1). Increase blood urea, creatinine, hypophosphatemia, hypokalemia in addition to insignificant decrease in serum sodium in the two tested groups was seen. Also, hypocalcemia in the 2nd group and hypercalcemia in the 3rd group were noticed. A decrease in oestrogen and progesterone levels in the two groups was seen with insignificant increase in progesterone in the 2nd group.

DISCUSSION

The increase in AST, ALT and ALP enzymes indicated that a liver damage was produced either by phyto-estrogen or gossypol. The increase in these enzymes was previously reported by *WATTKE and JAHN (1971), LINDSEY et al., (1980), COPPOCK et al., (1985) and GRAY et al., (1990).*

Hypoproteinemia, hypoalbuminemia and hypoglobulinemia seen in the present work, after feeding clover, may be due to liver damage. Our results agreed with *EL-BELELY (1993)* and partially agreed with *ROLANDS et al., (1977).*

The increase in total proteins, albumin and globulins after feeding cotton seed meal may be due to the high level of proteins in ration. The same results were previously reported by *LINDSEY et al., (1980) and COPPOCK et al., (1985).*

The increase in bilirubin after feeding the two plants may be attributed to the effect of phyto-estrogen and gossypol on the liver. Our result was in agreement with those reported by *COPPOCK et al., (1985)* and differed from the result obtained by SOLIMAN et al., (1966). The difference may be

due to the difference in the amount of feeding plant and/or the duration of the experiment.

The increase in urea and creatinine in the present work may be due to the effect of phyto-estrogen and gossypol on the kidney. Our data were similar to those of other studies *(CHRISTOPHER, 1974) and COPPOCK et al., 1985)* and in contrary to *SOLIMAN et al., (1966).*

The hypocalcemia after feeding clover may be due to the decrease in calcium reabsorption from damaged renal tubules or associated with hypoalbuminemia *(AWAD and HANNA, 1974).* While hypophosphatemia may be due to low phosphorus content in clover as reported by *MORROW (1969).* The same result was obtained by *EL-BELELY (1993).*

The hypercalcemia after feeding cotton seed meal may be due to feeding of concentrated ration (contains calcium carbonate) or the metabolism of calcium and phosphorus is closely linked in the body. Hypophosphatemia is always accompanied by hypercalcemia. The same result was obtained by *COPPOC et al., (1985).*

The insignificant change in serum sodium was previously reported by *ROLANDS et al., (1977).*

The hypokalemia may be due to nutritional influence (low potassium content in ration) as reported by *ANON (1978). Our data was similar to that obtained by GRAY et al., (1990).*

The decrease in oestrogen hormone after feeding the two plants may be due to negative feed back mechanism as a result of phyto-estrogen administration or due to inhibition of ovarian steriogenesis as a result of gossypol administration. The same result was obtained by *KELLY et al., (1976)* in ewes. They stated that grazing estrogenic pasture; lead to an inhibition in the production of endogenous oestrogen, and by *NEWSOME and KITTS (1979)* in ewes. They reported that ewes consuming forage containing phyto-estrogen had plasma level of oestrogen lower than those of control ewes.

The increase in progesterone as a result of feeding clover may be due to the fact that lower oestrogen level is alwaays accompanied by higher progesterone. The same result was obtained by *OBST and SEAMARK (1970).* They proved that the progesterone concentration of ewes grazing estrogenic and non estrogenic pasture was similar up to the day 12-13 of oestrous cycle, after which progesterone concentration in the estrogenic group continued to fall.

The progesterone level was decreased after feeding cotton seed meal as a result of inhibited conversion of cholesterol and pregnenalone to

progesterone or due to the effect of gossypol on the ovary. The same result was obtained by *GU et al., (1990)* in bovine.

CONCLUSION

Feeding of clover or cotton seed meal for long time could cause liver and kidney damage inaddition to hormonal changes in friesian cattle. After hormonal and nutritional treatments, most of these changes returned nearly to the normal. Some parameters which still changed may need longer time to return to the control level. Nearly the results of hormonal treatment were similar to the result of nutritional treatment.

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Table (1): Liver function tests in friesian cows feeding clover and cotton seed meal before and after treatments.

Groups	AST U/ ml	ALT U/ ml	ALP U/ ml	T. P. gm/ dl	Albumin gm/ dl	Globulins gm/ dl	T . B . mg/ dl	D . B . mg/ dl	Ind. B . mg/ dl
1 st group (Control)	60. 61 ± 1. 17 b	15. 12 ± 0. 67 b	12.34 ± 0. 43 d	7. 46 ± 0. 70 b	3.86 ± 0. 10 a	3. 60 ± 0. 70 b	0. 32 ± 0. 01 c	0.21 ± 0. 01 bc	0. 11 ± 0. 01 b
2 nd group (feeding clover for 3 months)	76.42 ± 1. 14 a	20. 94 ± 0. 65 a	21. 96 ± 0.42 a	5. 12 ± 0. 68 c	2. 32 ± 0. 10 b	2. 80 ± 0. 69 c	0. 37 ± 0.01 ab	0. 25 ± 0. 01 a	0. 12 ± 0. 01 ab
Subgroup I (After hormonal treatment)	59. 53 ± 1. 70 b	15. 08 ± 0.97 b	22. 70 ± 0.62 a	7. 24 ± 1. 02 bc	3. 87 ± 0. 14 a	3. 37 ± 1. 02 b	0. 35 ± 0. 01 b	0. 24 ± 0. 01 a	0. 11 ± 0. 01 b
Subgroup II (After nutritional treatment)	57. 62 ± 1. 61 b	14. 90 ± 0.92 b	16. 63 ± 0. 59 c	7. 55 ± 0.97 b	3. 89 ± 0. 13 a	3. 66 ± 0. 97 b	0. 35 ± 0.01 b	0. 23 ± 0. 01 ab	0. 12 ± 0. 01 ab
3 rd group (feeding cotton seed meal for 3 months)	75. 73 ± 1. 14 a	20. 94 ± 0. 65 a	18. 62 ± 0. 42 b	7. 91 ± 0.68 b	3. 95 ± 0. 10 a	3. 96 ± 0. 69 b	0.38 ± 0.01 a	0. 25 ± 0. 01 a	0. 13 ± 0. 01 ab
Subgroup III (After hormonal treatment)	59. 53 ± 1. 70 b	15. 08 ± 0. 97 b	17. 70 ± 0.62 bc	10. 57 ± 1. 02 a	3. 86 ± 0. 14 a	6.71 ± 1. 02 a	0. 35 ± 0. 01 b	0. 23 ± 0.01 ab	0. 12 ± 0. 01 ab
Subgroup IV (After nutritional treatment)	57. 63 ± 1. 61 b	14. 91 ± 0.92 b	16. 63 ± 0.59 c	7. 81 ± 0.97 b	3. 89 ± 0. 13 a	3. 92 ± 0. 97 b	0. 35 ± 0. 01 b	0. 20 ± 0. 01 c	0. 15 ± 0. 01 a
F - test	**	**	**	**	**	**	*	*	*
L. S. D.	3. 82	2. 18	1. 40	2. 29	0. 32	0. 56	0. 02	0. 02	0. 03

- Means followed by different or partially different letters were statistically significant and the highest values were represented with the letter a
- * : Significant at 0. 05 Probability.
- **: Significant at 0. 01 Probability.

Table (2): Renal function tests and some hormonal changes in friesian cows feeding clover and cotton seed meal before and after treatments.

Groups	Urea mg%	Creatinine mg%	Ca mg%	P mg%	Na mEq/ L	K m Eq/ L	Oestrogen Pg/ 100 ml	Progesterone ng/ ml
1 st group	23. 34 ± 0. 85^{c}	1. 45 ± 0. 06^{c}	10. 59 ± 0. 26^{bc}	5. 31 ± 0. 14^{a}	143. 91 ± 1. 12	4. 62 ± 0. 11^{b}	7. 21 ± 0. 33^{a}	1. 59 ± 0. 32^{ab}
2 nd group	4 1. 37 ± 0. 83^{a}	1. 96 ± 0. 06^{b}	8 . 89 ± 0. 26^{d}	3. 56 ± 0. 14^{b}	133. 53 ± 1. 09	2. 66 ± 0. 11^{c}	3. 70 ± 0. 32^{b}	1. 89 ± 0. 31^{a}
Subgroup I	23. 85 ± 1. 24^{c}	1. 49 ± 0. 09^{c}	10. 13 ± 0. 38^{c}	5. 28 ± 0. 21^{a}	147. 47 ± 1. 63	4. 89 ± 0. 17^{ab}	7. 21 ± 0. 48^{a}	1.82 ± 0. 46^{a}
Subgroup II	23. 35 ± 1. 17^{c}	1. 55 ± 0. 08^{c}	10. 78 ± 0.36^{bc}	5. 73 ± 0. 19^{a}	146. 80 ± 1.54	5. 17 ± 0.16^{a}	7. 40 ± 0. 46^{a}	1. 74 ± 0. 44^{a}
3 rd group	34. 87 ± 0. 83^{b}	2. 63 ± 0. 06^{a}	12. 91 ± 0.26^{a}	3. 66 ± 0. 14^{b}	133. 53 ± 1. 09	2. 60 ± 0. 11^{c}	3. 70 ± 0.32^{b}	0. 63 ± 0. 31^{c}
Subgroup III	23. 85 ± 1. 24^{c}	1. 88 ± 0. 09^{b}	11. 08 ± 0. 38^{b}	5. 61 ± 0. 21^{a}	147. 47 ± 1. 63	4. 99 ± 0. 17^{ab}	7. 21 ± 0. 48^{a}	0. 93 ± 0. 46^{bc}
Subgroup IV	23. 36 ± 1. 17^{c}	1. 62 ± 0. 08^{c}	10. 98 ± 0. 36^{bc}	5. 73 ± 0. 19^{a}	146. 80 ± 1. 54	5. 17 ± 0. 16^{a}	7. 40 ±0. 46^{a}	0. 37 ± 0. 44^{c}
F - test	**	**	**	**	N. S.	**	**	**
L. S. D.	2. 79	0. 19	0. 86	0. 46	____	0. 37	1. 08	0. 95

- Means followed by different or partially different letters were statistically significant and the highest values were represented with the letter a
- N. S. Non significant
- * : Significant at 0. 05 Probability.
- **: Significant at 0. 01 Probability.