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EFFECT OF VITAMIN AD3EC INJECTION ON HEMATOLOGICAL INDICES AND SOME BIOCHEMICAL PARAMETERS IN BUFFALOES HEIFERS IN UPPER EGYPT

(With 5 Tables and 5 Figures)

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تأثير حقن فيتامين اد٣هـ جـ على الصوره الدمويه وبعض التغيرات البيوكيميائيه فى عجلات الجاموس فى صعيد مصر

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أستخدم فى هذه الدراسه ١٠ عجلات جاموس لدراسة تأثير حقن فيتامين اد٣هـ جـ على الصوره الدمويه وبعض التغيرات البيوكيميائيه فى سيرم الدم. ولقد أختيرت الحيوانات من مزرعة كلية الزراعه - جامعة أسيوط وقسمت الى مجموعتين متساويتين عشوائيا حسب الوزن والعمر. المجموعه الأولى (كضابط للتجربه) لم يتم حقنها بالفيتامين أما المجموعه الثانيه (المعامله) أعطيت ٢٠ مللى فيتامين اد٣هـ جـ لكل رأس كل إسبوعين. وقد تم أخذ عينات دم من كل الحيوانات المستخدمه فى التجربه شهريا لمدة ٣ شهور طوال فترة التجربه. ولقد أوضحت النتائج أن زيادة المتوسطات العامه لكل من تركيز الهيموجلوبين ، العدد الكلى لكرات الدم الحمراء والبيضاء ومتوسط تركيز الهيموجلوبين داخل كرات الدم الحمراء وبينما إنخفض تركيز كل من نسبة الخلايا المصمته ومتوسط حجم كرات الدم ومتوسط كرات الدم المحمله بالهيومجلوبين وذلك فى المجموعه المعامله بالمقارنه بالمجموعة الضابطة. و ذاد في المجموعه المعاملة متوسط كل من فيتامين ا بنسبة ١٩٠ ٪ وفيتامين هـ بنسبة ٣٢ ٪ فى سيرم الدم بينما ذاد متوسط بيتا كاروتين بنسبة ٤٥ ٪ بعد ٩٠ يوم من التجربه (الفتره الثالثه). بينما كان تركيز الجلوكوز فى سيرم المجموعه المعامله يميل الى الزياده فى حين كان تركيز الكوليستيرول يتناقص تدريجيا طوال فترة التجربه بالمقارنه بالمجموعه ألاخرى ، هذا وقد ذاد الجلوكوز بنسبة ٢٩ر٢٧ ٪ بينما إنخفض الكوليستيرول بنسبة ٦٠ر٥ ٪ وذلك فى الفتره الثالثه من التجربه. ولم يتأثر المتوسط العام لكل من البروتين الكلى والجلوبيولين بالمعامله ولكن بالرغم من إنخفاض نسبة الالبيومين فى سيرم المجموعه المعامله خلال فترة التجربه إلا أن المتوسط العام ذاد بنسبة ١٠ر٧٦ ٪ فى المجموعه المعامله بالمقارنه بالآخرى. بالرغم من إرتفاع نسبة الدهون الكليه فى المجموعه المعامله إلا أن هناك إرتباط سالب بين فيتامين هـ فى سيرم الدم وبنسبة الدهون الكليه فقط فى المجموعه المعامله. بينما كان تركيز نيتروجين اليوريا فى سيرم دم المجموعه المعامله يتجه الى الإرتفاع بنسبة ٢٣ ٪ إنخفض تركيز البليروبين بنسبة ٩ر٠٩ ٪ فى الفتره الثالثه من التجربه كما لوحظ زيادة تركيز كل من الكالسيوم والفوسفورالغير عضوى فى المجموعه المعامله بالمقارنه بالآخرى بنسبة ٧٣ر٨ ٪ ، ٤٣ ٪ على التوالى وذلك فى الفتره الثالثه من التجربه. هذا ولم يتأثر تركيز كل من الصوديوم والبوتاسيوم بالحقن ولكن إرتفع تركيز الكلوريد فى سيرم الدم بنسبة ٦٠ر٦ ٪ خلال الفتره الثالثه من التجربه فى المجموعه المعامله بالمقارنه بالمجموعه الضابطه.

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SUMMARY

This study was conducted on 10 females buffalo heifers, to evaluate the effects of vit AD3EC injection on the hemogram picture and some metabolic profiles in blood serum. The animals were selected from the herd of experimental farm of animal production department, Assiut University. They were devided into two groups randomly according to body weight and age. The first group was the control group which did not receive vit AD3EC injection and the second group (treated group) received an intramuscular injection of 20 ml vit AD3EC biweekly for a period of 3 months. Blood samples were collected from all animals monthly after two succesive injections at 9.00 A.M. The results showed that, injection of vitamin AD3EC caused an increase in the overall means of hemoglobin concentration ($P<0.07$), total red ($P<0.01$) and white blood corpuscles counts and mean corpuscular hemoglobin concentration with a decrease in the hematocrit value ($P<0.07$), mean corpuscular volume ($P<0.01$) and mean corpuscular hemoglobin ($P<0.01$) in the treated group when compared with the control one. Both of blood serum vitamins A and E levels appeared to be higher ($P<.01$) by 190% and 32, respectively in the treated group while serum beta-carotene level was elevated only by 45% at the third of experimental period in the treated group than the control one. Meanwhile, blood serum glucose was increased with a decrease in the level of cholesterol in the treated group when compared with the control one. At the third period serum glucose level was elevated by 29.27% while cholesterol level was decreased by 5.60% in the treated group than the control one. Blood serum total protein and globulin concentrations did not differ as a result of the given treatment. Even though serum albumin was decreased ($P<0.07$) through the experimental period, means of serum albumin tended to be higher by 10.76% ($P<0.07$) in the treated group when compared with the control one. Although serum total lipids was decreased in the treated group yet a negative correlation was observed between vit E and total lipids ($r= -0.34$, $P<0.21$). At the third period serum urea nitrogen (BUN) tended to increase by 23% ($P<0.01$) while serum total bilirubin decreased by 9.09% in the

treated group. Serum ca and p concentrations appeared to be increased in the treated group when compared with the control one. At the third period blood serum ca and p were higher by 8.73% and 43% ($P<0.01$) in the treated group than the control one. Serum Na and K concentrations did not differ during the treatment but serum Cl tended to be higher by 6.60% at the third period in the treated group when compared with the control one.

INTRODUCTION

Vitamins are fundamental factors in cellular metabolism and their requirements will generally be the natural feeds plus microbial synthesis in the rumin *(SIMONS and HAND, 1986)*. Some vitamins play an important role in the reproductive performance of dairy cows. The role of beta-carotene as a precursor of vitamin A in animal reproduction has been established by *LOTTHAMMER* et al. *(1976) and COOKE & COMBEN (1978). LOTTHAMMER (1979) and SIMONS & HAND (1986)* revealed that vitamin A deficiency in breeding cows may lead to the impairment of oestrus while conception rates are reduced due to delayed ovulation and secretion of poor quality colostrum. *WARD* et al. *(1971)* studied the effects of vitamin D supplementation on dairy cows fertility and they found that the animals receiving 300.000IU vitamin D3 weekly had shorter uterine involution times, more clearly marked oestrus and better fertility rates. At the beginning of lactation, calcium utilization was also improved. The authers cncluded that vitamin D has an oestrogenic effects. The main function of vitamin E in the body is as an antioxidant, and protecting factor in the cells against oxidative destruction *(GLAWISCHNIG, 1975)*. He added that in vitamin E deficiency, cell membranes are destroyed. Additionally vitamin E deficiency can have a negative effect on fertility indirectily through liver disorders which are in turn related to fertility disturbances *(LOTTHAMMER, 1975)*. The aim of the present study was to evaluate the metabolic role of vitamin ADɜEC* concentrations multidose on the hemogram picture and blood serum clinical profiles of buffaloes heifers as an attempt to improve reproductive performance in native buffalo cows.

* 1 ml multidose provides: 40.000 IU of vitamin A, 5000 IU of D3, 30 mg ofvitamin E, 100 mg of vitamin C, 30mg of vitamin E-acetate and 112.4mg of sodium ascorbate. Produced by Gebr-schaette KG pharmacentic biologicl products Bad-Waldess & Germany.

MATERIALS and METHODS

Animals and Management:

The experiment was carried out on ten buffalo heifers from the herd of experimental farm of Animal Production Department, Assiut University, Assiut, Egypt. Animals were devided randomly into two groups. The 1st group was the control group while the 2nd group was the treated one. Every animal of the treated group received an intramascular injection of 20 ml of vit AD3EC biweekly for a period of 3 months (6 successive injection). All animals were fed a pelleted commerical concentrate diet consisting of wheat bran, corn, cotton seed meal, soyabean meal, molasses, flax straw, rice hulls, limestone and salt. The concentration diet contained 10.04% moisture, 13.96% crude protein, 6.17% either extract, 14.12% crude fiber, 49.4% nitrogen free extract and 6.24% ash.

Blood sampling and procedures:

Three consecutive blood samples were collected from all animals from the jugular vein puncture at 0900 h. (before feeding and after two successive injection) for a period extended to three months at monthly interval throughout the experimental period. Each time two Blood samples were collected. The 1st blood sample was mixed with anticoaguiant (EDTA) while the 2nd sample was allowed to clot at room temperature and centrifuged at 3000 r.p.m. for 20 min. to obtain clear serum which was stored in the refrigerator at -20 C for at least two hours until analyzed. Whole blood was analyzed after the collection for total red blood corpusle (RBC), total white corpuscle blood (WBC) and hemoglobin concentration (Hb) using electronic cell counter and its diluter (Cell Dyne 300 Sequoia Turnor). Packed cell volume (PCV) was estimated according to the standard methods of hematology *(SCHALM, 1961 and COLES, 1986)*. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated as described by *HEPLER (1966)*. Blood serum glucose, cholesterol, total lipids, urea nitrogen (BUN) and total bilirubin were estimated using test kits supplied by Diamond Diagnostic, Cairo, Egypt. Serum albumin, inorganic phosphorus and calcium levels were estimated using test kits supplied by Biomerieux (Bains & France). Serum total protein was determined using test kit supplied by Bio-Dwic, Cairo, Egypt. Blood serum sodium and potassium concentrations were determined using flame photometer (Corning 400). Serum chloride conentrate was estimated using chloride analyser model 925. Serum vitamin A (vit A) and beta- carotene were estimated

according to the method of *CARR and PRICE (1926)* while vitamin E (vit E) in blood serum was determind according to the method of *HAWK* et al. *(1954)*.

Statistical Analysis:

Data were analyzed by least-squares analysis of variance (ANOVA) *SAS (1987)* for personal computers.

RESULTS

I- Hemogram picture:

Hemoglobin (Hb): The treated group had higher ($P<0.01$) Hb concentration (13.05 g/dl) when compared with the control one (11.26 g/dl) at the 1st period of the trail (Table 1 and Fig. 1). A more pronounced decrease of Hb concentration occured in the treated group til the minimum level at the 3rd period where both groups had similar means. The overall means of Hb level was slightly higher ($P <0.07$) in treated group when compared with the control one. The interaction between a treatment X experimental period ($P <0.01$) was noted for Hb concentration.

Red blood corpuscles cell (RBC): At the 1st period these cells in treated group tended to be higher ($P<0.01$) (7.14 x 10 cubic mm) when compared with the control one (5.47 x 10 cubic mm). A gradual increase was observed in both groups through the experimental period (Table 1 and Fig. 1). At the 3rd period RBC count was elevated by 32% ($P <0.01$) in the treated group than the control one. Differences of RBC count between periods of study were significantly at 5% levels while the overall mean of RBC count was hgiher ($P <0.01$) in the treated group when compared with the control one.

White blood corpuscles cell (WBC): The means of WBC count at the 1st period were similar in both groups. Changes in WBC count were in parallel with RBC count. At the 3rd period the means of WBC count were hgiher ($P <0.05$) in the treated group (12.44 x 10/mm) than the control (11.60 x 10/mm). Treatment did not affect WBC count (Table 1 and Fig. 1).

Packed cell volume (PCV %): The means of PCV were higher at the first period in the control group when compared with the treated one. Through the experimental period the control group was sharply declined ($P <0.05$) in PCV to the minimum level at the third period. Treatment did not affect PCV% but the overall means to be higher ($P<0.07$) in the control group when compared with the treaded one (Table 1 and Fig. 1).

Mean corpuscular volume (MCV u): Means of MCV were significantly different ($P <0.01$) between the treatment and the experimental period. Changes in MCV were paralled with PCV

(Table 1 and Fig. 1). The overall mean was higher (P <0.01) in the control group when compared with the treated one.

Mean corpuscular hemoglobin (MCH ug): Changes in MCH volumes during the experimental perids were not significantly effected but the overall means were higher (P <0.01) in the control group when compared with the treated one (Table 1 and Fig. 2).

Mean corpuscular hemoglobin concentration (MCHC%): No significant differences in MCHC concentration were observed between treatment but experimental period were affected (P <0.06) (Table 1 and Fig. 1). The overall means tended to be higher by 10% in the treated group and treatment X period interaction were differ (P <0.05) than the control one.

II- Serum chemical profiles:

1- Blood serum vit A & E and beta-carotene: A graduel increased was detected in the levels of vit A (P<0.01) in blood serum through the experimental period (Table 2 and Fig. 2). The overal means of serum vit A and E levels were higher (P<0.01) by 190% and 32% in the treated group when compared with the control one. A psoitive correlation was observed between the levels of serum vit A and E only in the treated group (r= + 0.46; P <0.05). A treatment X period interaction (P<0.06) and during the experimental period (P <0.07) were detected for the level of beta- carotene in blood serum. At the third period the level of beta-carotene was increased by 45% in the treated group when compared with the control one. A positive correlation was observed between the levels of serum beta - carotene and both of serum vit. E (r= +0.12; P <0.68) and vit A (r= +0.47; P<0.07).

2- Blood serum glucose, cholesterol, total protein and total lipids: No significant differences in serum glucose and cholesterol levels were detected between treatment (Table 3 and Fig. 3) but a gradual increase was observed through the experimental period. At the third period serum glucose was higher by 29% in the treated group when compared with control one. A positive correlation was observed between serum glucose and vit E (r= +0.37; P <0.17) only in the treated group. Serum cholesterol showed an opposite direction where it decreased gradually through the experimental period and reached a lower value at the end of trial (55.66 $\pm$ 7.99 mg/dl). The overall mean tends to be lower by 9% in the treated group when compared with the control one. A negative correlation was observed between the levels of serum vit A and serum cholesterol (r= -0.45; P <.07) only in the treated group. A highly significant differences in serum total protein and globulin were observed during the experimental period with interaction between the treatment X period (Table 3 and Fig. 3). A negative correlation

was found in the treated group between the levels of vit E and both total protein (r= -0.30; P <0.29) and globulin (r= -0.18; P<0.53), respectively. The overall mean of serum albumin was higher (P <0.07) in the treated group when compared with the control one and the level varied during the experimental period (P <0.07). A negative correlation was observed between serum albumin and vit E (r= -0.42; P<0.11) only in the treated group. The levels of serum total lipids did not differ between treatments (Table 3 and Fig. 3). The overall means tend to be higher by 17% in the treated group when compared with the control one but serum vit E was negatively correlated with serum total lipids in the treated group (r= -0.34; p<0.21).

3- Serum urea nitrogen and total bilirubin: Serum urea nitrogen (BUN) varid throughout the experimental period (P<0.01) with interaction between the treatment X period (P<0.05) but means tended to be higher by 7% in the treated group compared with the control one (Table 4 and Fig. 4). Serum vit E levels were significantly in postiive correlation with BUN (r= +0.70; P<0.004) only in the treated group. Serum total bilirubin did not differ between the treatment but a negative correlation was found between serum total bilirubin and the level of vit E in the treated group (r= - 0.10; P<0.70).

4- Serum Minerals: Serum inorganic P and Ca contents were increased with the advancing period of experiment to reach higher levels in the third period by 43% (P<0.01) and 8.7%, respectively in the treated group when compared with the control one (Table 5 and Fig. 5). The overall means of P and Ca contents were higher in the treated group than in the control one. No significant differences in serum Na, K and Cl concentrations were found during the treatment (Table 5 and Fig. 5). The interaction between a treatment X period (P<0.06) was noted for serum K. Concentration of serum Cl significantly differed (P<0.01) during experimental period. A treatment X period interaction were detected (P<0.05) for serum Cl. Levels of vit E were positively correlated with serum Cl (r= +0.66; P<0.007) only in the treated group.

DISCUSSION

I- Hemogram picture:

As shown in table (1) and Fig. (1) buffalo heifers given vitamins AD3EC (treated group) by intramuscular injection at weekly intervals showed high (P<0.01) vitamin E levels in blood serum (188.8 ug%) by 32% when compared with the control (142.5 ug%) which did not received vitamin injection (Table 2 and Fig. 2). The increase in vitamin E levels in blood serum of the treated group may lead to the elevation in the means values of

total red blood corpuscles (P<0.01) when compared with the control (Table 1 and Fig. 1). This can be attributed to the moderate level of vit E supplementation which enhanced the synthesis of red blood corpuscles as a result of the decrease in the oxidative demange of the red blood cell membrane by free radicals which increased the life spane of these cells. Defficiency of both vitamin E and selinum was associated by mild hemolytic anemia as reported by *COMBES (1981).* Moreover, andother explanation to the increase in the total count of RBCs in the treated group is that vitamin E is promoted release of FSH, ACTH and LH hormones *(BARNES and SMITH, 1975)* which affected the rate of RBC production from spleen as a reflection of metabolic stress.

In spite of the overall means of mean corpuscular volume (MCV ug%) and mean corpuscular hemoglobin (MCH ug%) whcih were significantly lower (P<0.01) in the treated group yet hemoglobin concentration (g/dl) was elevated (P<0.07) and mean corpuscular hemoglboin concentration (MCHC %) was increased by 10% in the overall means in treated heifers when compared with the control one. A significant positive correlation (r= +0.55; P<0.05) was observed between Hb concentration and MCHC only in the treated group. Meanwhile, the high level of vitamin E in blood serum of treated heifers may stimulates hemoglobin synthesis in these animals as reported by *CAASI* et al. (1972) who claimed that a direct effect of vitamin E on the biosynthesis of haeme. In this field, *CHRISTENSEN (1983)* observed that, vitamin E plays a specific role in the snthesis of haeme. He added also that anemia which occures in vitamin E deficiency may be due to hemolysis of RBCs.

In treated group, the increase in the total WBC count at the third period (P<0.05) and the overall mean (P<0.10) when compared with the control group may be due to the increase of lymphocytes cells in the treated group due to a high level of vitamine E in the blood serum. *REDDY* et al. *(1986)* observed that calves injected by vitamin E (1400 mg alphatocopherol/weekly) had significantly higher lymphocytes stimulation index. Meanwhile, *WATSON and PETRO (1982)* noted that lower serum corticosterone in mice fed a high vitamin E diet may explain some of the observations of enhanced lymphocyte activity.

II- Serum metabolic profiles:

Intramuscular injection of vit AD3EC caused a highly significant increase the overall means in the levels of vit A by 190% and vit E by 32% in the treated group when copmared with the control one (Table 2 & Fig. 2). These results are very important as vit A and E play an important role in the reproductive performance of dairy cows. This is because vit A

deficiency leads to short gestation period, delayed ovulation, retained placentae and early abortion. On the other hand, the fact that the pituitary gland has a high level of vit E in comparison with other organs *(ZINTZEN, 1976)* is thought that vit E may promote the relasea of FSH, ACTH and LH hormones *(BARNES and SMITH, 1975)*. In this aspect, the elevated level of beta-carotene by 45% at the third period of the experiment and the positive correlation which was observed between vit A and beta-carotene ($r= +0.47$; $P<0.07$) only in the treated group when compared with the control may give an explanation of the role of vit A precursor in prmoting the vital processes in the animals. Bata-carotene has not only an importance as a precursors of vit A but also has an additional specific action on the function of the reproductive process in female cattle *(LOTTHAMMER, 1979 and SIMONS & HAND, 1986)*. In this field *JACKSON* et al., 1981 showed that the synthesis of steroid hormones by the ovaries is reduced in cows with low beta-carotene plasma level.

The increase in serum glucose in the treated group during the experimental period and in the overall means may be due to the decrease in the rate of glucose uptake by cells. Our results agreed with those reported by *REDDY* et al. (1985) who noted that blood glucose level tended to be higher in vit E supplemented animals.

The decline in serum cholesterol in treated group when compared with the control one may be due to the fact that vit E increased activity of cholesterol 7- alfha-dehydroxylase (an enzyme involved in degratiation of cholesterol to bile acid in lvier) resulting in lowering of cholesterol level *(AGBOOLA* et al., *1988)*. In addition the supplementation of vit E had been proved to decrease serum and muscle cholesterol in Holstein bull calves. Our results did not agree with that reported by *CHUPUKCHAROEN* et al. *(1985)* and *LEHNINGER (1982)* who reported that supplemented vit E increase the production of cholesterol. In our study serum cholesterol was negatively correlated with the level of vit E in blood serum in treated group ($r= -0.45$; $P<0.09$) which agreed with *AGBOOLA* et al. *(1988)* who found that this correlation was ($r= -.30$, $P<0.10$). The possible explanation for decreased serum cholesterol in the treated group was that a high level of serum inorganic phosphorus was observed in the treated group (Table 5 and Fig. 5) which caused an inhibition of cholesterol synthesis by detoxification mechanism in the liver *(GIBBONS* et al., *1982)*. In our study serum cholesterol was negatively correlated with serum P ($r= -0.17$; $P<0.54$) in the treated group. Our results in this aspect agree with *AGBOOLA* et al., *(1988)* who found that $r= -0.14$.

Serum total lipids were negatively correlated with the level of vit E in blood serum in the treated group (r= -0.34; P<0.21) when compared with the control one. This observation could be explained by *PRITCHARD* et al. *(1986)* who reported that the possibility of dietary vit E causing a reduction in plasma triglycerides in diabetic rats by affecting the lipoprotein lipase activity. In this study serum total lipids were positively correlated with serum cholesterol in the treated group (r= +0.34; P<0.21). These results agreed with those reported by *MAYES (1975)* who noticed that decreased levels of cholesterol is consequently reflected upon the levels of total lipids.

The significant increase in serum BUN in the treated group during the last period of the experiment and in the overall means (23% higher than the control) may be attributed to that vit E could have decreased the efficiency of nitrogen retention by increasing gluconeogenesis from amino acids, which in turn increased BUN in the treated group when compared with the control one. This was furtherly emphasised by *DUNCAN and PRASSE (1986)* who reported that most urea is synthesised in the liver from ammonia, either formed from protein catabolism or absorbed from the gastrointestinal tract.

The decreased in serum total bilirubin in treated group during the last experimental period when compared with control group may be due to the results of decreased rates of erythrocytes breakdown, as vit E maintains the integrity of erythrocyte membrane by preventing peroxidation of lipids within the membrane *(SHETAEWI* et al. *1992).*

Serum inorganic P and Ca contents were higher during the experimental period and in the overall means in the treated group when compared with the control one. These results agreed with *REINHARDT and CONRAD (1980).* The higher Ca observed in the treated group agrees with the observation of *costanzo and WEINER (1976).* Concerning the role of Na in this aspest, it is clear that there is a competitive action betwwnboth Ca and Na especially in the process of retention of Na through the excretory processes where the increase of Ca will evantually cause an increase in the sodium retention in the addition blood *(AGBOOLA* et al., *1988).* Furthermore, the addition of vit E evantually improve the digestability of fat which will favour the Ca absorption *(ROY* et al., *1964).* For all these findings Ca was positively related to the level of vit E in the blood serum in the treated group (r= +0.29; p<0.30).

The significant increase in serum CI concentration in the treated group during the experimental period when compared with the control one may be due to that vit E could enhance absorption or decrease excretion of some macrominerales i.e. P, Ca amd Cl and consequently increases their concentrations in

the blood. Then vit E increased apparet absorption of Ca, P and Cl while serum Na and K concentrations were not affected by vitamin E. This may be probably because tissue uptake of these minerals was also increased.

CONCLUSIONS

It can be concluded from this study that administration of Vit. AD3EC lead to improvement in both haematological and same clincial profiles of buffalo heifers which are more related to fertility such as Vit A and E, glucose and inorganic phosphorus levels in blood serum. Then Vit AD3EC can be used as an attempt to overcome the reproduction problems facing buffalo heifers at such periods.

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Table (1): Some hemogram picture in control and treated buffalo heifers

Item	Group (G)	Period (P) I X±S.E.	II X±S.E.	III X±S.E.	Overall means X±S.E.	ANOVA G	P	G X P
Hb (g/dl)	Control	11.26±0.34	13.14±0.34	12.34±0.38	12.25±0.21	0.07	n.s	0.01
	Treated	13.05±0.38	12.64±0.34	12.58±0.34	12.74±0.21			
RBC (10^6/mm^3)	Control	5.47±0.32	6.34±0.32	6.39±0.36	6.07±0.19	0.01	0.05	n.s
	Treated	7.14±0.36	7.41±0.32	8.44±0.32	7.70±0.19			
WBC (10^3/mm^3)	Control	11.84±0.35	11.86±0.35	11.60±.039	11.77±0.21	n.s	n.s	n.s
	Treated	11.30±0.39	12.02±0.35	12.44±0.35	11.96±0.21			
PCV (%)	Control	50.00±3.14	44.20±3.14	35.00±3.50	43.07±1.89	0.07	0.05	n.s
	Treated	44.25±3.51	37.40±3.14	40.80±3.14	40.57±1.89			
MCV (U^3/cell)	Control	91.13±5.36	71.27±5.36	53.14±5.99	72.52±5.01	0.01	0.01	n.s
	Treated	62.29±5.99	51.12±5.36	48.54±5.36	53.39±3.03			
MCH (Ug)	Control	19.88±0.93	21.06±0.93	18.65±1.04	20.15±0.56	0.01	0.09	n.s
	Treated	18.56±1.04	17.18±0.93	14.92±0.93	16.76±0.56			
MCHC (%)	Control	22.68±2.46	30.43±2.46	35.05±2.27	29.52±1.48	n.s	0.06	0.05
	Treated	32.24±2.75	34.14±2.46	31.16±2.46	32.51±1.48			

Hb : Hemoglobin.
RBC: Red blood cells.
WBC: White blood cells.
PCV: Packed cell volume.
MCV : Mean corpuscular cells.
MCH : Mean corpuscular hemoglobin.
MCHC: Mean corpuscular hemoglobin conc.

Table (2): Serum vitamins A & E and beta - Carotene in control and treated buffalo heifers.

Item	Group (G)	Period (P) I X±S.E	II X±S.E	III X±S.E.	Overall means X±S.E.	ANOVA G	P	G X P
Vit A (Ug%)	Control	23.78±7.22	28.10±7.22	35.11±8.07	30.00±4.34	0.01	n.s.	n.s.
	Treated	75.67±8.08	97.30±2.21	101.3±1.35	91.44±3.68			
Vit E (mg%)	Control	133.8±5.60	140.4±6.05	156.1±3.76	142.5±3.83	0.01	0.01	0.08
	Treated	165.7±3.60	185.1±2.70	211.1±3.56	188.8±5.47			
Beta - Carotene (Ug%)	Control	84.70±23.6	70.58±8.16	30.88±5.52	64.34±10.41	n.s.	0.07	0.06
	Treated	34.73±5.03	70.76±12.5	44.88±2.82	51.22±6.11			

Table (3): Serum Glucose, Cholesterol, Total protein, Albumin, Globulin and Total lipids in control and treated buffalo heifers.

Item	Group (G)	Period (P) I X±S.E.	II X±S.E.	III X±S.E.	Overall means X±S.E.	ANOVA G	P	G X P
Glucose (mg/dl)	Control	57.76±9.54	65.95±9.54	58.45±10.7	60.95±4.64	n.s.	n.s.	n.s.
	Treated	48.61±5.00	62.78±9.54	75.56±16.5	62.37±7.21			
Cholesterol (mg/dl)	Control	68.49±7.99	71.22±21.4	58.76±8.94	66.70±7.83	n.s.	n.s.	n.s.
	Treated	69.45±16.4	60.06±7.99	55.66±7.99	61.17±5.63			
Total protein (g/dl)	Control	7.65±0.44	7.45±0.44	8.17±0.50	7.83±0.27	n.s.	0.01	0.01
	Treated	9.43±0.50	6.08±0.44	7.64±0.44	7.72±0.47			
Albumin (g/dl)	Control	2.99±0.21	3.03±0.21	2.61±0.23	2.88±0.13	0.07	0.07	n.s.
	Treated	3.44±0.23	3.26±0.21	2.87±0.22	3.19±0.13			
Globulin (g/dl)	Control	4.66±0.40	4.71±0.40	5.56±0.44	4.98±0.24	n.s.	0.01	0.01
	Treated	5.99±0.44	2.84±0.30	4.78±0.40	4.55±0.24			
Total lipids (mg/dl)	Control	877±96	948±96	872±108	901±57	n.s.	n.s.	n.s.
	Treated	1273±108	852±96	1027±96	1035±66			

Table (4): Serum nitrogen (BUN) and total bilirubin in control and treated buffalo heifers.

Item	Group (G)	Period(P) I X±S.E.	II X±S.E.	III X±S.E.	Overall means X±S.E.	ANOVA G	P	G X P
BUN (mg/dl)	Control	27.82±1.18	24.11±1.18	26.26±1.32	26.06±0.71	n.s.	0.01	0.05
	Treated	26.36±1.32	24.86±1.18	32.30±1.18	27.84±0.71			
Bilirubin (mg/dl)	Control	0.96±0.15	0.79±0.15	1.10±0.17	0.95±0.09	n.s.	n.s.	n.s.
	Treated	1.02±0.17	1.25±0.15	1.00±0.15	1.09±0.09			

Table (5): Serum Calcium, Phosphorus, Sodium, Potassium and Chloride in control and treared buffalo heifers.

Item	Group (G)	Period(P) I X±S.E.	II X±S.E.	III X±S.E.	Overall means X±S.E.	ANOVA G	P	G X P
Calcium (mg/dl)	Control	8.47±0.49	9.62±0.49	8.93±0.55	9.01±0.29	n.s.	n.s.	n.s.
	Treated	9.09±0.55	9.62±0.49	9.71±0.49	9.47±0.29			
Phosphorus (mg/dl)	Control	4.91±0.44	4.80±0.44	3.67±0.49	4.46±0.26	n.s.	n.s.	n.s.
	Treated	4.95±0.49	5.09±0.44	5.25±0.49	5.10±0.26			
Sodium (mmol/L)	Control	153.4±5.76	165.2±5.76	149.1±6.44	155.9±3.46	n.s	n.s.	n.s.
	Treated	151.9±6.44	170.2±5.76	150.6±5.76	157.6±3.46			
Potassium (mmol/L)	Control	5.70±0.16	5.34±0.16	5.30±0.18	5.44±0.10	n.s.	n.s.	0.06
	Treated	5.20±0.18	5.64±0.16	5.16±0.16	5.34±0.10			
Chloride (mmol/L)	Control	96.40±3.26	108.0±3.26	106.0±3.64	103.9±1.96	n.s.	0.01	0.08
	Treated	98.50±3.65	99.0±3.26	113.0±3.24	103.5±1.96			

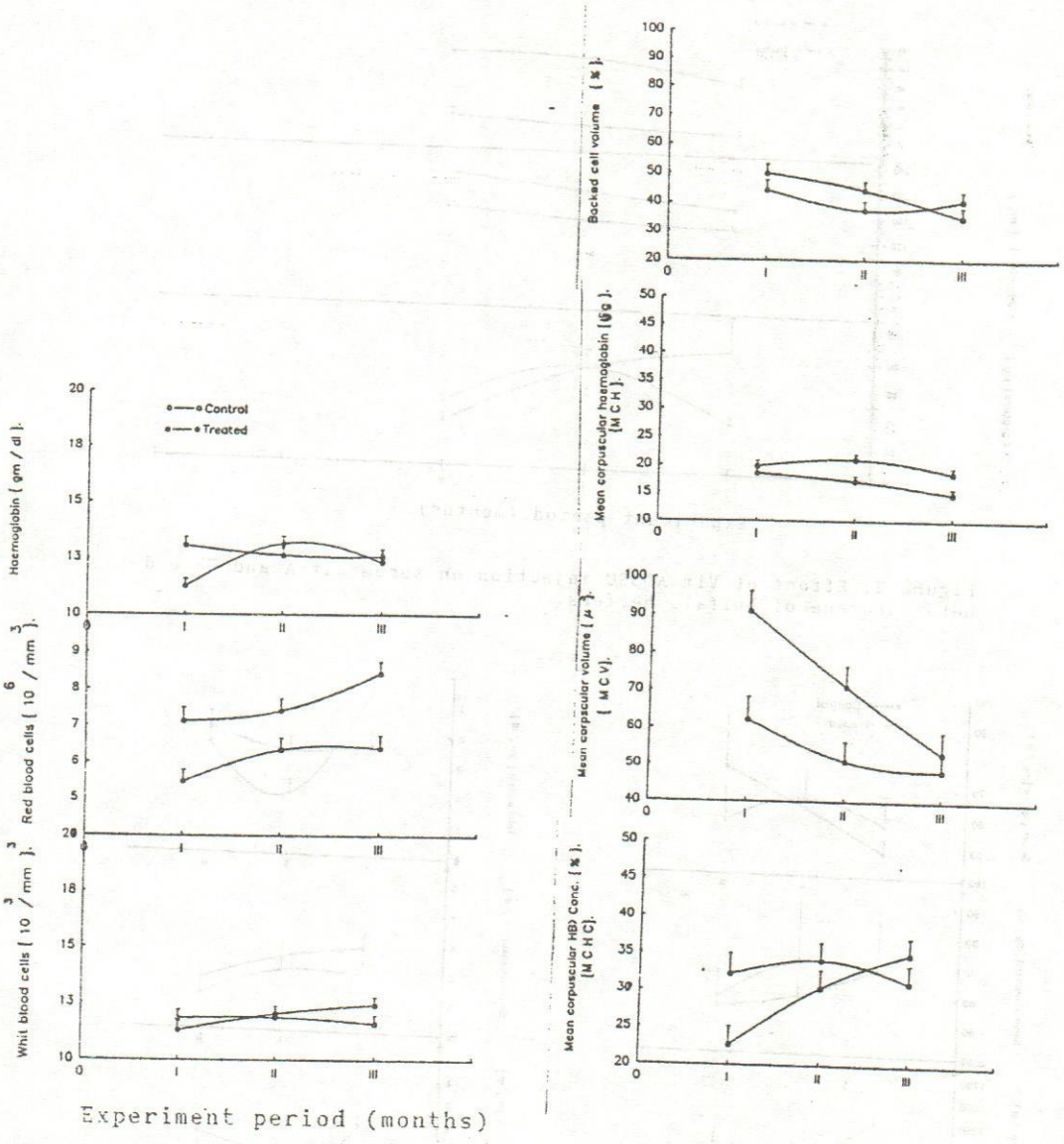

Figure 1. Effect of Vit AD3EC injection on haemogram picture of buffalo heifers.

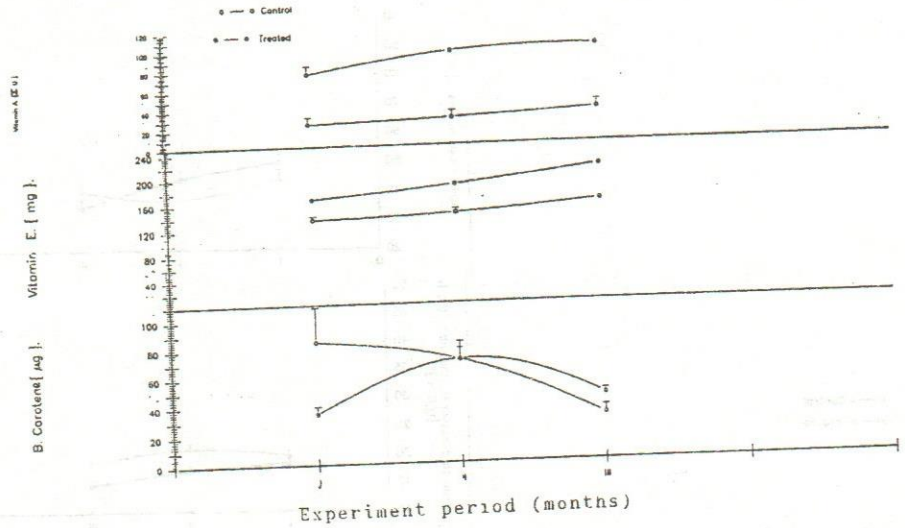

Figure 2. Effect of Vit AD3EC injection on serum vit A and E and beta carotene of buffalo heifers.

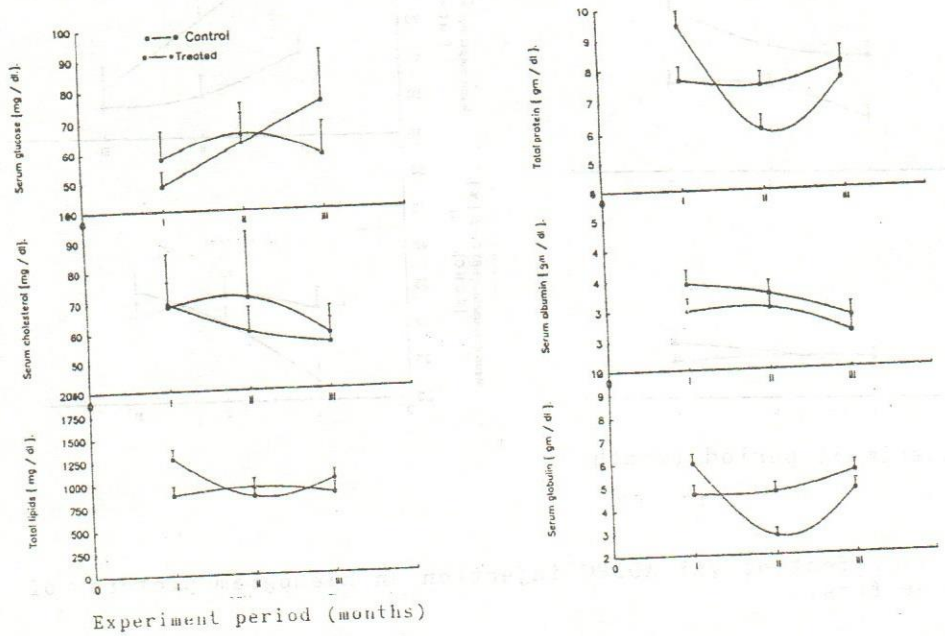

Figure 3. Effect of Vit AD3EC injection on serum glucose, cholesterol, total lipids, total protein and its fractions of buffalo heifers.

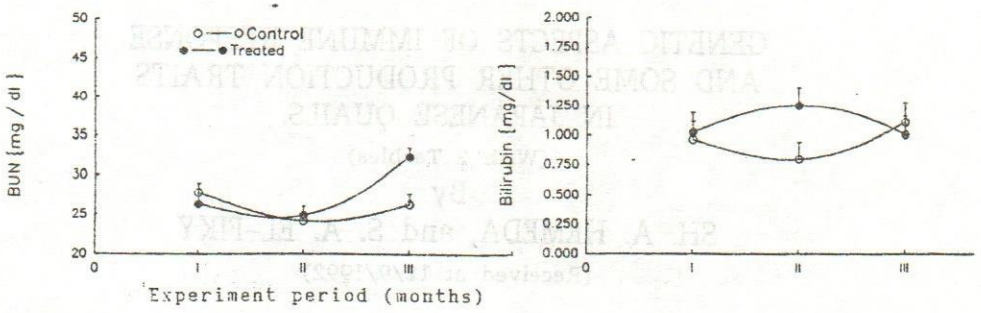

Figure 4. Effect of Vit AD3EC injection on serum Urea Nitrogen (BUN) and total bilirubin of buffalo heifers.

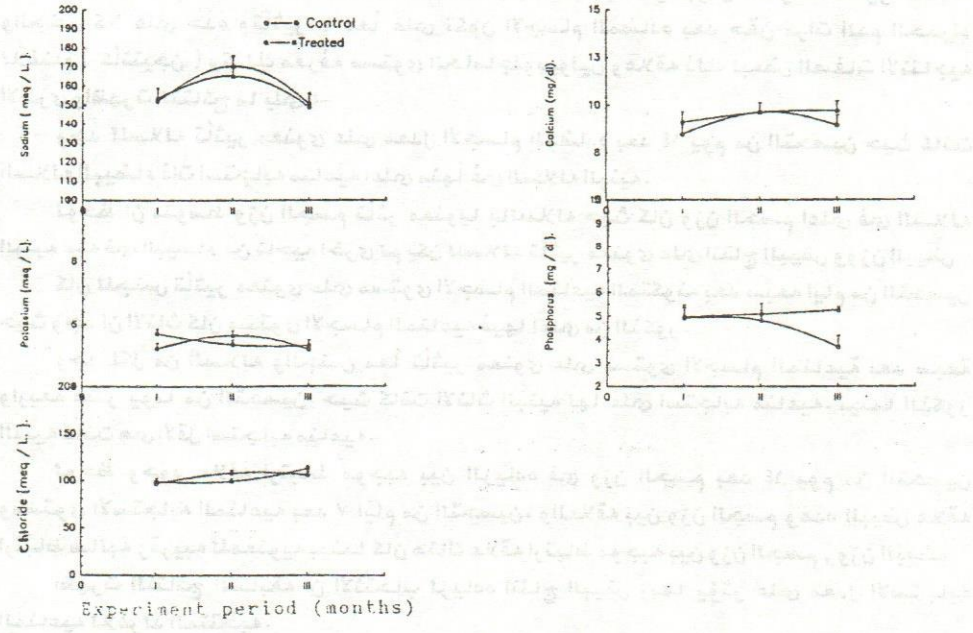

Figure 5. Effect of Vit AD3EC injection on serum sodium, potassium, chloride, calcium and inorganic phosphores of buffalo heifers.