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THE EFFECT OF LIVERIA® ON SOME HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF TREATED BUFFALO CALVES

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ABSTRACT

The effect of intramuscular injection of Liveria® at a dose of 1ml/20kg body weight on hematological and biochemical parameters was investigated in fifteen apparently healthy buffalo calves of our local breed, 2-3 months age old, with average body weight 53-72 Kg in especial farm at Dakahlia Governorate. Buffalo were divided into three groups, each group five animals. The first group was injected with saline I/M (1ml/20kg) and lefted without any drug (the controlled animals). The second group was injected intramuscular by Liveria® (1ml/20 kg body weight). The third group was injected three successive intramuscular doses by Liveria (1ml/20 kg body weight) for three days. Hematological (RBCs- WBCs-Hb), biochemical aspects (total protein- albumin- globulin- serum: ALT, AST and ALP) and some minerals concentration (Ca, P and Na) were evaluated. The results showed that the administration of Liveria in calves caused a significant decrease in total erythrocytic count, haemoglobin concentration, total leukocytic count, total protein, albumin and serum AST with a significant increase also in serum calcium and inorganic phosphorus. Moreover, no changes in ALT, ALP activities and globulin level. Therefore, it was concluded that a single dose of Liveria® at dose rate (1ml /20 kg body weight) induced a significant improvement in some heamatological and biochemical parameters of treated calves.

INTRODUCTION

During the last decade, many feed additives were available as antibiotics, enzymes and probiotics that were added to the grower buffalos and cattle, milking and fattening diet for farm animals in Egypt to improve the growth performance, nutritional parameters and carcass traits. (El-Ashry *et al.*, 1993; El-Basiony, 1994; El Hassan *et al.*, 1996; Allam *et al.*, 2001; Hassan, 2009)

Multivitamin-minerals have been used for improving the growth as well as feed utilization theraby helps in realization of better return of production as well as

economy. (Sahin *et al.*, 2003; Upadhayay andVishwa, 2014).

Researchers reported that L-carnitine regulates metabolic processes in high yielding lactating cows and also ewes in an advanced stage of pregnancy. Recent studies indicate that while supplemental L-carnitine in the diet is not required, its use is recommended in domestic animals especially in cattle to increase performance and to support medical treatment. (Citil *et al.*, 2009; Pirestani *et al.*, 2011)

Our study was delinated to study the effect of Liveria® on the Heamatological

parameters, some biochemical parameters and serum mineral concentration of treated calves.

MATERIAL AND METHODS

I. Drug:

Liverea® ready to use preparation for intramuscular injection produced by Provet Co.Turkey. It used in a **Dose** rate 1ml / 20 kg body weight. (Heiko Scholz, 2014)

II. Experimental animals:

Fifteen apparently healthy buffalo calves of our local breed, 2-3 months age old, with average body weight 53-72 Kg in especial farm at Dakahlia Governorate were used in this study. They were fed on concentrated ration 21% protein (2.25% of animal weight) + milk (3kg / head decreased gradually every week) and water was provided ad-libidum. They were kept under complete hygienic conditions during the experimental period.

III. Experimental design

Buffalo were divided into three groups, each group five animals. The first group was injected with saline I/M without any drug (the controlled animals). The second group was injected intramuscular by Liveria® (1ml/20 kg body weight). The third group was injected three successive intramuscular doses by Liveria (1ml/20 kg body weight) for three days.

IV. Sampling

Blood samples were collected from each animal in the three groups at zero day and after 1 week, two weeks and three weeks post the

drugs administration. Blood samples divided into two parts:

- 1- The first part was collected on Wassermann tube containing EDTA (0.5 mg ml blood) from tail vein of all calves for hematological parameters studies (erythrocytic count, leukocytic count, Hb, PCV, MCV, MCH, MCHC and thrombocyte count.)
- 2- The second part was collected in Wassermann tube without anticoagulant from tail veins of all buffalo calves and allowed to clot at room temperature. The serum was separated by centrifugation at 3000rpm for 15 minutes the sera were collected in 1.5 ml Eppendroff tubes and kept frozen at -20° c for biochemical studies (total proteins, albumin, ALT, AST, ALP, calcium, phoshporus and sodium). (Stoffregen *et al.*, 1997)

V. Hematological parameters (Schalm *et al.* 1986), (Cohen 1967), blood indices (Feldman *et al.* 2000), Total Protein (Doumas, 1975), Albumin (Doumas and Biggs, 1972), globulin calculated (Doumas and Biggs, 1972). Liver enzymes activities AST, ALT (Reitman and Frankel, 1957), ALP activity (Rosalki, 1993). Calcium level (Gindler and King, 1972), phosphorus (El-Merzabani.M.M., 1977) and serum sodium level (Henry *et al.* 1974).

VI.Statistical analysis: as described by Snedecor and Cochran (1967).

Table (1): The effect of single intramuscular dose and three successive intramuscular doses of Liveria® (1ml/20kg body weight) on blood parameters in buffalo calves: (Mean ± S.E) (n = 10)

Parameter	Group	Time/ week			
		First	Second	Third	Fourth
Hb (gm/dl)	Control	11.8 ± 0.37 ^a	11.4 ± 0.24 ^a	11.6 ± 0.24 ^a	11.6 ± 0.24 ^a
	One	11.4 ± 0.24 ^a	11 ± 0.44 ^a	11.2 ± 0.20 ^a	11.6 ± 0.24 ^a
	Three	11.6 ± 0.24 ^a	10.8 ± 0.37 ^b	11.4 ± 0.24 ^a	11.8 ± 0.20 ^a
RBCs (n×10 ⁶)	Control	7.46 ± 0.2 ^a	7.46 ± 0.22 ^a	6.20 ± 0.37 ^a	6.20 ± 0.37 ^a
	One	7.22 ± 0.2 ^a	6.30 ± 0.43 ^b	6.32 ± 0.28 ^a	6.74 ± 0.37 ^a
	Three	7.60 ± 0.24 ^a	6.50 ± 0.44 ^b	5.80 ± 0.37 ^b	6.44 ± 0.24 ^a
PCV (%)	Control	33.8 ± 0.37 ^b	31.4 ± 0.6 ^a	32.4 ± 0.67 ^b	33 ± 0.31 ^b
	One	33.6 ± 0.4 ^b	31.4 ± 0.87 ^a	31.8 ± 0.8 ^c	31.2 ± 0.73 ^c
	Three	34 ± 0.31 ^a	31.4 ± 0.67 ^a	33.6 ± 0.4 ^a	35.8 ± 0.73 ^a
WBCS (n×10 ⁶)	Control	8 ± 0.31 ^a	7.4 ± 0.4 ^a	8.8 ± 0.58 ^a	8.6 ± 0.5 ^a
	One	8.2 ± 0.58 ^a	6.6 ± 0.67 ^a	8.4 ± 0.67 ^b	8.8 ± 0.37 ^a
	Three	7.8 ± 0.74 ^b	6.8 ± 0.37 ^a	8.8 ± 0.58 ^a	8.4 ± 0.67 ^a

The different litters in the same column mean significance at (p< 0.05)

Table (2): The effect of single intramuscular dose and three successive intramuscular doses of Liveria® (1ml/20kg body weight) on differential leukocytic count in buffalo calves: (Mean ± S.E) (n = 10)

Parameter	Group	Time/ week			
		First	Second	Third	Fourth
Neutrophils (%)	Control	38 ± 0.96 ^a	33.2 ± 1.56 ^a	33.2 ± 0.73 ^b	32 ± 0.74 ^a
	One	33.6 ± 1.63 ^b	30.4 ± 2.55 ^{ab}	35.6 ± 1.28 ^a	31.8 ± 1.32 ^a
	Three	29.6 ± 1.2 ^c	28.2 ± 0.8 ^b	28.9 ± 1.88 ^c	32.6 ± 0.87 ^a
Lymphocytes (%)	Control	60 ± 1.01 ^c	64.8 ± 1.5 ^a	65.8 ± 0.92 ^b	66 ± 1.46 ^a
	One	64.8 ± 1.21 ^b	68.6 ± 1.5 ^a	62.8 ± 1.15 ^c	67 ± 0.63 ^a
	Three	68 ± 0.7 ^a	69.8 ± 3.42 ^a	69.9 ± 2.8 ^a	65.6 ± 1.2 ^a
Monocytes (%)	Control	2 ± 0 ^a	2 ± 0.01 ^a	1 ± 0 ^b	2 ± 0.1 ^a
	One	1.2 ± 0.44 ^a	1 ± 0.01 ^b	1.6 ± 0.24 ^a	1.2 ± 0.2 ^b
	Three	2.2 ± 0.58 ^a	2 ± 0.01 ^a	1.2 ± 0.2 ^{ab}	1.8 ± 0.2 ^a
Eosinophils (%)	Control	0 ± 0 ^c	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a
	One	0.4 ± 0.02 ^a	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a
	Three	0.2 ± 0.02 ^b	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a
Basophils (%)	Control	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a
	One	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a
	Three	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a

The different litters in the same column mean significance at (p< 0.05)

Table (3): The effect of single intramuscular dose and three successive intramuscular doses of Liveria® (1ml/20kg body weight) on liver function in buffalo calves: (Mean ± S.E) (n = 10)

Parameter	Group	Time/ week			
		First	Second	Third	Fourth
Total protein level (gm/dl)	Control	7.88 ± 0.47 ^a	8.64 ± 0.32 ^a	6.7 ± 0.33 ^a	6.9 ± 0.39 ^b
	One	7.88 ± 0.45 ^a	7.68 ± 0.39 ^b	6.18 ± 0.61 ^a	7.26 ± 0.77 ^a
	Three	6.66 ± 0.6 ^a	7.78 ± 0.59 ^b	6.66 ± 0.36 ^a	8.02 ± 0.22 ^a
Albumin level (gm/dl)	Control	4.54 ± 0.2 ^a	4.32 ± 0.5 ^a	3.02 ± 0.16 ^a	3.04 ± 0.14 ^a
	One	4.16 ± 0.51 ^a	3.5 ± 0.25 ^b	3.18 ± 0.44 ^a	3.84 ± 0.25 ^a
	Three	4.04 ± 0.37 ^a	3.68 ± 0.62 ^b	2.78 ± 0.29 ^b	3.36 ± 0.37 ^b
Globulin level (gm/dl)	Control	3.32 ± 0.49 ^a	4.32 ± 0.62 ^a	3.7 ± 0.3 ^a	3.86 ± 0.29 ^a
	One	3.72 ± 0.42 ^a	4.18 ± 0.14 ^a	3.0 ± 0.73 ^a	3.22 ± 0.77 ^a
	Three	2.62 ± 0.34 ^a	4.1 ± 0.48 ^a	3.92 ± 0.54 ^a	4.66 ± 0.5 ^a
ALT activity (u/l)	Control	55.32 ± 1.24 ^a	58.74 ± 2.10 ^a	53.37 ± 2.03 ^a	56.28 ± 0.72 ^a
	One	56.73 ± 1.89 ^a	54.76 ± 1.46 ^a	57.63 ± 2.01 ^a	55.78 ± 1.27 ^a
	Three	58.62 ± 1.07 ^a	56.58 ± 1.32 ^a	58.39 ± 2.53 ^a	59.3 ± 0.73 ^a
AST activity (u/l)	Control	92.65 ± 4.14 ^a	92.6 ± 4.03 ^a	90.46 ± 4.03 ^a	88.06 ± 0.57 ^b
	One	94.07 ± 6.54 ^a	97.97 ± 6.08 ^a	94.81 ± 7.51 ^a	96.5 ± 4.38 ^a
	Three	94.66 ± 2.58 ^a	94.6 ± 6.17 ^a	86.11 ± 2.77 ^a	102.92 ± 4.68 ^a
ALP activity (u/l)	Control	144.5 ± 4.31 ^a	161.77 ± 6.7 ^a	177.26 ± 4.62 ^a	179.8 ± 4.97 ^a
	One	159.81 ± 4.83 ^a	152.44 ± 5.52 ^a	156.08 ± 6.02 ^a	153.05 ± 6.42 ^b
	Three	153.08 ± 4.9 ^a	151.84 ± 3.52 ^a	156.81 ± 5.29 ^b	152.84 ± 6 ^b

The different letters in the same column mean significance at (p < 0.05)

Table (4): The effect of single intramuscular dose and three successive intramuscular doses of Liveria® (1ml/20kg body weight) on some minerals in buffalo calves: (Mean ± S.E) (n = 10)

Parameter	Group	Time/ week			
		First	Second	Third	Fourth
Calcium (mg/dl)	Control	8.31 ± 0.72 ^b	9.63 ± 0.75 ^a	10.06 ± 0.61 ^a	9.55 ± 0.66 ^a
	One	11.07 ± 0.59 ^a	10.55 ± 1.16 ^a	11.51 ± 1.14 ^a	10.46 ± 0.88 ^a
	Three	11.31 ± 1.25 ^a	10.96 ± 0.76 ^a	10.38 ± 1.14 ^a	10.1 ± 0.79 ^a
Phosphorus (mg/dl)	Control	5.98 ± 1.42 ^a	6.84 ± 1.7 ^a	6.42 ± 1.52 ^a	6.06 ± 0.2 ^a
	One	6.2 ± 2.1 ^a	7.14 ± 2.9 ^a	4.7 ± 1.15 ^b	5.58 ± 0.57 ^b
	Three	6.82 ± 2.07 ^a	5.42 ± 0.93 ^b	6.18 ± 1.19 ^a	4.34 ± 1.24 ^c
Sodium (mmol/l)	Control	143.44 ± 3.56 ^a	147.25 ± 2.45 ^a	145.35 ± 2.31 ^a	143.52 ± 0.35 ^a
	One	141.41 ± 4.2 ^a	142.38 ± 3.21 ^{ab}	147.35 ± 9.27 ^a	144.67 ± 3.13 ^a
	Three	146.33 ± 3.77 ^a	139.94 ± 1.49 ^a	141.57 ± 4.54 ^a	145.14 ± 3.36 ^a

The different letters in the same column mean significance at (p < 0.05)

RESULTS & DISCUSSION

The effect of single intramuscular dose and three successive intramuscular doses of Liveria® (1ml/20kg body weight) on some hematological picture in buffalo calves:

The present study showed a significant decrease in total erythrocytic count at second and third weeks post treatment on third group compared to the control group and also at second week post treatment on second group while at fourth week it is return back to its normal value. (Table 1)

The present work reflected that a significant decrease in hemoglobin content at second week post treatment on third group compared to the control group then at third and fourth week became normal. While a significant increase on packed cell volume at first, third and fourth weeks was recorded on third group but, a significant decrease at third and fourth weeks was occur on second group. (Table 1)

Our data was in agreement with that of **Thiernel and Jelínek (2004)** who recorded no difference in total erythrocytes. However the hemoglobin showed a significant decrease in experimental animals in comparison with the control. Moreover **Akbari et al. (2010)** stated that no significant effect of L-carnitine on hemoglobin, RBC, MCV, MCH and MCHC. Also **Caruso et al. (1983)** did not observe any effect of L-carnitine on hemoglobin concentrations even after six months of treatment.

In contrast, **Karadeniz et al. (2008)** showed a significant increase in RBC, hemoglobin, PCV and MCHC of broilers when L-carnitine was added to diet.

The effect of single intramuscular dose and three successive intramuscular doses of Liveria® (1ml/20kg body weight) on total and differential leucocytic counts in buffalo calves:

The present data mirrored a significant decrease at the first week post treatment on third group and at third week in second group. Data showed a significant decrease in neutrophil at first and second weeks in second and third groups then an increase at third week on second group occurred while third one still decreased at third week. All groups returned to normal at fourth week. Also results showed a significant increase in lymphocyte at first and third week on second and third groups post treatment compared to control. (Table 1, 2)

Our results evaluated a significant decrease in monocyte at second and fourth weeks in second group while results detected a non significant changes in eosinophil and basophil. (Table 2)

These data disagree with the that of **Karadeniz et al. (2008)** who stated that L-carnitine treatment has induced significant increases on the WBC, heterophile and lymphocyte counts. While **Thiernel and Jelínek (2004)** recorded that leukocytes showed no differences between control and experimental groups.

The effect of single intramuscular dose and three successive intramuscular doses of Liveria® (1ml/20kg body weight) on alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activities in buffalo calves:

The study reflected no significant changes in alanine transaminase (ALT) activity while on aspartate transaminase (AST) activity showed a significant increase at fourth

week post treatment on second and third groups compared to control group. (Table 3)

The present data agree with **Yalçin et al. (2005)**; (**Yalcin et al., 2006**) who showed that there were no differences among groups in serum activities of ALT, AST. In the same ground the obtained results were supported by the results of (**Mansour, 2006**; **Yapar et al., 2007**); **Ercan Keskin (2015)** confirmed that L-carnitine supplementation prevents hepatic injury and enzyme leakage from hepatocytes.

On the other side of view these results were disagree with those recorded by (**Thiemel and Jelínek, 2004**); **Citil et al. (2009)** as they showed that L-carnitine revealed lower levels of liver enzymes (AST, ALT) activities.

The effect of single intramuscular dose and three successive intramuscular doses of Liveria® (1ml/20kg body weight) on Total Protein, Albumin and Globulin in buffalo calves:

Our results showed a significant decrease in total protein at the second week post treatment and on the third group compared to the control group while there is a significant increase at fourth week post treatment on second and third groups. Also the work detected a significant decrease in albumin and no changes in globulin post treatment. (Table 3)

The obtained results were supported by the result of **Thiemel and Jelínek (2004)** who found that the administration of L-carnitine lowered the level of total protein.

On the other side of view our results were disagree with those of **Cetin et al. (2003)** who found no differences were found for total protein concentrations. **Citil et al. (2009)** recorded that total serum protein was not affected by carnitine and an increased amount of albumin in blood samples of carnitine. Also

Parsaeimehr et al. (2014) showed that treatments had no significant ($P>0.05$) effect on total protein, albumin and globulin.

The effect of single intramuscular dose and three successive intramuscular doses of Liveria® (1ml/20kg body weight) on Calcium, Phosphorus and Sodium in buffalo calves:

Our results showed a significant increase in calcium at first week post treatment on second and third groups compared to the control group. (Table 4)

Keeping with this line our data showed that a significant increase in phosphorus at second week post treatment on second group compared to the control group then at third and fourth week showed significant decrease. Also the third group revealed a significant decrease at second and fourth weeks post treatment. While no significant changes on sodium was recorded all over the experiment.

These data disagree with these of **Thiemel and Jelínek (2004)** who found that administration of carnitine lowered the level of calcium and phosphorus. **Kaçar et al. (2010)** also, showed that L-carnitine did not have important difference for serum calcium concentration.

On the other side of view our results were disagree with those of **Elgazzar et al. (2012)** who recorded that L-carnitine administration significantly increase serum electrolytes (Na^+ , K^+ and Cl^-).

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المخلص العربى تأثير عقار الليفيريا على القياسات الدموية و البيوكيميائية فى عجول الجاموس

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تم دراسة تأثير الحقن العضلى لعقار الليفيريا على عدد ١٥ عجل جاموسى تتراوح اعمارهم بين ٢-٣ اشهر و اوزانهم بين ٥٣-٧٢ كجم بجرعة قدرها ٢٠/مل كجم باحدى المزارع الخاصة بمحافظة الدقهلية . تم تقسيم الحيوانات محل الدراسة الى ثلاث مجموعات (٥ حيوانات لكل مجموعة) المجموعة الاولى تم التعامل معها كمجموعة ضابطة بينما المجموعة الثانية و الثالثة تم التعامل معها بحقن الليفيريا بجرعة قدرها ١ مل / ٢٠ كجم من وزن الحيوان الحى عن طريق الحقن فى العضل بحيث كانت المجموعة الثانية تتلقى الجرعات لمدة ٣ اسابيع متتالية بين الجرعة و الجرعة أسبوع بينما المجموعة الثالثة تم التعامل معها بالحقن العضلى لمدة ٣ أيام متتالية و تم تكرار الحقن لمدة ٣ أسابيع أخرى بين الجرعة و الجرعة أسبوع. أوضحت نتائج الدراسة ان اعطاء الليفيريا ادى الى نقص معنوى فى عدد كرات الدم الحمراء و تركيز الهيموجلوبين و عدد كرات الدم البيضاء و البروتين الكلى و الزلال و انزيم الاسبرتات امينو ترانسفيراز (AST) مع زيادة معنوية فى املاح الكالسيوم و الفوسفور. بينما لم يؤثر العقار على الجلوبيولين و انزيم الألاتين امينو ترانسفيراز(ALT) و انزيم الفوسفاتاز القاعدى(ALP). و من هذه الدراسة نستخلص ان عقار الليفيريا أثبت كفاءة عالية على صورة الدم ووظائف الكبد و الوظائف الحيوية.