PROPHYLAXIS OF ANTIOXIDANT INSUFFICIENCY IN NEWBORN CALVES

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ABSTRACT

Forty newborn calves were divided into four equal groups. The experimental groups were treated with the antioxidants vitamins A, E, C and rutin, as well as a new preparation placenta denatured emulsion (PDE). The results showed the immaturity of the antioxidant system in newborn calves. Neonatal calves have low antioxidant activity at birth and during the first two weeks of life. Calves are born with a high erythrocyte peroxidation index. The stabilization of erythrocyte membranes take place by one month of age. This is indicated by the level of erythrocyte peroxidation and the concentration of malondialdehyde (MDA) found in the blood serum. All treatments had a significant effect on the antioxidant activity of the newborn calves. Erythrocyte peroxidation was decreased during the first three weeks. Combinations of vitamins A, E, C and rutin gave more conclusive results.

INTRODUCTION

The use of antioxidants in the animal feed industry for protecting fat, carotene, vitamin A and other organic material from the process of lipid peroxidation, improves the quality of feed stuff (Emanuil and Lyskovskaia, 1961). Vitamin E (α-tocopherol), several selenium containing compounds and animal tissues extracts are known to have antioxidant properties (Holban *et al.*, 1989; Holban and Reiliani, 1990; Al-Qudah, 1990b). Santochin, diludin and ionol are examples of generic antioxidants.

The use of antioxidants in veterinary practice has been largely empirical until recently. Some attempts have been made to determine the status of the antioxidant systems depending on the physiological situation of the animal. There is also inadequate information about the pathology of free radical oxidation in the living animal. It is known that stress intensifies the process of free radical oxidation in the living organism and leads to exhaustion of reserves of vitamin E in the lipid composition and the structure of cellular membranes is changed as well (Burlkova and Archipova, 1982).

The purpose of this study was to evaluate the therapeutic benefits of several antioxidants including vitamins A, C, E and rutin, as well as a new preparation placenta denatured emulsion (PDE; Kishinev Agricultural Institute, Str. Gribova 44, Kishinev, Maldova) prepared by Holban and Reiliani (1990). The antioxidant properties of PDE have been reported by Al-Qudah (1990a). The effects of same pharmaceutical agents on the status of antioxidant systems of calves during the first month of life was also determined by measuring erythrocyte peroxidation, malondialdehyde (MDA) concentration and catalase activity in blood serum.

MATERIALS AND METHODS

Forty newborn calves were equally divided into four groups. Calves in the first group were injected subcutaneously 1 mL of PDE on day 3, 7 and 12 of life. Calves in group 2 were treated orally with 0.2 gm of a vitamin A and E combination (1.0 gm vitamin A and 0.1 gm vitamin E) every other day for three weeks in addition to 1 mL of PDE on day 3, 7 and 12. Calves in group 3 were treated orally with a combination of ascorbic acid and rutin (0.05 gm each of ascorbic acid and rutin) every other day for three weeks in addition to the treatment received by group two. Calves in the control group did not receive any medication.

All animals were monitored clinically and clinical signs were recorded. Body weight was determined at the age of one day through 30 and growth rate was calculated daily. Blood samples were collected on the 1, 3, 5, 15 and 30 days after birth. All blood samples were tested for erythrocyte resistance to peroxides (ERP) by measuring erythrocyte peroxidation (EP) (Garanov *et al.*, 1987), catalase activity (Koroluk and Ivanova, 1988) and malondialdehyde (MDA) (Gavrelov and Gavrelova, 1987) level in blood serum. All samples were tested for erythrocyte count, hemoglobin and heamatocrit values.

RESULTS

Clinical Findings

No clinical changes were noted following any of the three treatments administered to the calves. Body temperature, heart rate and respiratory rates were within normal range.

Significant difference were noted in this experiment in the daily body growth rate of the calves (Fig. 1). There were also significant differences in measured blood parameters. The control animals had a significant decrease in all measured blood parameters ($P<0.05$) by third day of experiment. By the 15 and 30 days the decrease had become more noticeable. The animals of the first group which were injected with 1 mL of PDE, did not have significant differences from the control group at the end of the 30 days of experiment. The most significant effect on hematopoiesis was in the group treated with vitamin A and E (Table 1). In these animals erythron findings on the first day of life were lower in comparison with the control group, but higher than the animals of group one. By the third day of life the values of hemoglobin, haematocrit, and erythrocyte counts had decreased. However, these findings stabilized, and at the age of one month, the calves in group two had the highest levels of haemoglobin and haematocrit.

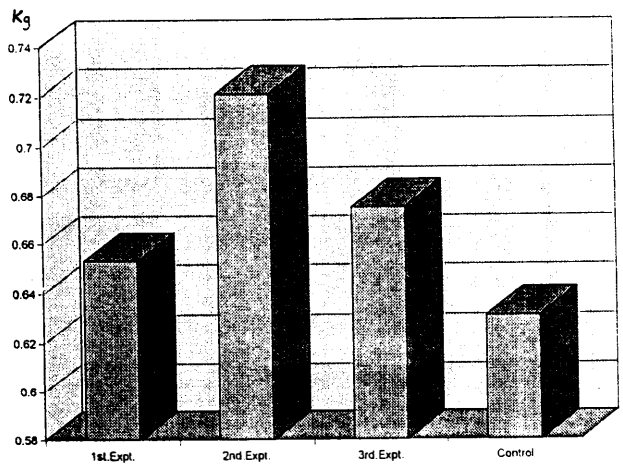

Fig. 1: Growth performance of calves treated with placenta denatured emulsion (PDE) (Exp. 1), Vit. A & E, (Exp. 2), Vit. C and rutin (Exp. 3), and not treated (control, Exp. 4).

In group that was given vitamins A & E, ascorbic acid and rutin, and PDE, ascorbic acid and rutin did not add an effect to the erythron findings beyond that of administration of vitamin A & E.

Antioxidant Activity

At birth the calves of group one had the highest erythrocyte peroxidation and the lowest erythrocyte counts. Calves of the control group had no change in erythrocyte peroxidation through the first two weeks of life. By one month of age this finding had decreased significantly ($P<0.01$) as shown in Table 1., indicating an increase in the resistance of the erythrocyte membrane to the peroxidation process. The same change in the erythrocyte peroxidation was noticed in the first group treated with PDE. After the second week this value had decreased significantly ($P<0.05$). Vitamin A & E and ascorbic acid and rutin had the most significant effects on antioxidants activity.

Vitamins A & E and PDE given to the calves of the second group, promoted a lowering of the erythrocytes peroxidation (EP). At day 15 and at one month of age this finding was decreased significantly ($P < 0.05$).

More conclusive results were obtained when vitamins A & E and ascorbic acid and rutin were combined. The EP on day 7, 15 and 30 of age was decreased significantly ($P<0.05$). These results show that calves are born with a high erythrocytes peroxidation index. The stabilization of erythrocytes membranes takes place by on month of age in calves.

Catalase actively destroys hydrogen peroxide, and stabilizes cellular membrane performance. This enzyme exists in erythrocytes in high concentration, catalase is a membrane-linked enzyme. Amy increase in catalase activity in the blood serum has a diagnostic significance. In this study the highest catalase was present at birth. At three days of age catalase activity had increased significantly than the control group ($P<0.05$) as shown in Table 1.

The highest concentration of MDA were found at birth. By day three the level of MDA in plasma had decreased significantly ($P<0.05$) as shown in Table 1. Through days 7 and 15 the level of MDA was relatively stable but, by the age of one month it had begun to increase again.

There appears to be a correlation between MDA in the blood serum and the daily growth rates. Calves in group two had the highest daily growth rate (0.721 Kg) and the highest amount of MDA in blood serum (3.048 μmol/mL). Calves in the control group had the lowest daily growth rate and the MDA concentration was less than that of group two 0.379 μmol/mL. Animals in the first and third groups had intermediate values.

Table 1: Effect of vitamin A, E, C and rutin on some haematologicl parameters and lipid peroxidation index in newborn calves.

Parameters	Exp. Days	Experiment			
		1st	2nd	3rd	Control
Hb: Conc. (g/dL)	1	$10.0\pm0.71^{a,A}$	$11.5\pm0.28^{a,A}$	$11.9\pm0.95^{b,A}$	$13.3\pm1.13^{b,A}$
	3	$9.32\pm1.09^{a,B}$	$10.5\pm1.23^{b,A}$	$10.5\pm1.23^{a,A}$	$11.5\pm1.25^{b,A}$
	7	$9.50\pm6.57^{a,B}$	$11.5\pm0.53^{b,A}$	$11.5\pm0.53^{b,A}$	$12.8\pm1.30^{b,A}$
	15	$8.90\pm0.57^{a,B}$	$11.4\pm1.39^{b,A}$	$11.1\pm1.25^{b,A}$	$10.9\pm0.98^{b,A}$
	30	$8.90\pm0.39^{a,B}$	$10.6\pm0.89^{b,A}$	$09.8\pm0.80^{a,A}$	$09.8\pm0.81^{a,B}$
PCV (%)	1	$23.2\pm2.04^{a,A}$	$41.3\pm2.39^{b,A}$	$41.2\pm3.32^{b,A}$	$43.0\pm0.70^{b,A}$
	3	$30.6\pm1.93^{a,B}$	$36.5\pm1.93^{b,A}$	$33.2\pm2.56^{b,A}$	$38.5\pm3.86^{b,A}$
	7	$24.7\pm1.70^{a,A}$	$31.8\pm3.01^{b,A}$	$35.0\pm2.04^{b,A}$	$36.8\pm4.80^{b,A}$
	15	$27.2\pm2.99^{a,A}$	$35.0\pm5.14^{b,A}$	$35.0\pm4.54^{b,A}$	$36.2\pm2.57^{b,A}$
	30	$30.7\pm1.18^{a,B}$	$35.5\pm2.62^{b,A}$	$33.0\pm1.29^{b,A}$	$32.6\pm1.96^{b,A}$
Erythrocyte counts ($10^6/\mu L$)	1	$5.18\pm0.40^{a,A}$	$6.42\pm0.22^{b,A}$	$6.20\pm0.16^{b,A}$	$6.70\pm0.31^{b,A}$
	3	$4.94\pm0.32^{a,A}$	$5.32\pm0.50^{a,A}$	$5.32\pm0.14^{a,A}$	$5.90\pm0.44^{a,A}$
	7	$4.35\pm0.40^{a,A}$	$5.17\pm0.45^{b,A}$	$5.17\pm0.30^{b,A}$	$5.92\pm0.76^{b,A}$
	15	$4.55\pm0.36^{a,A}$	$4.82\pm0.57^{a,A}$	$5.22\pm0.52^{a,A}$	$5.20\pm0.47^{a,A}$
	30	$4.80\pm0.26^{a,A}$	$5.00\pm0.39^{a,A}$	$5.08\pm0.46^{a,A}$	$5.99\pm0.67^{b,A}$
EPR (unit)	1	$78.5\pm10.6^{a,A}$	$62.5\pm08.6^{a,A}$	$67.5\pm10.4^{a,A}$	$73.2\pm10.4^{a,A}$
	3	$76.1\pm07.4^{a,A}$	$54.5\pm03.4^{b,A}$	$60.4\pm05.3^{b,A}$	$75.2\pm04.7^{a,A}$
	7	$77.5\pm08.5^{a,A}$	$73.6\pm05.3^{a,A}$	$60.0\pm15.7^{b,A}$	$75.6\pm07.8^{a,A}$
	15	$71.9\pm05.1^{a,A}$	$66.3\pm06.9^{a,A}$	$53.5\pm07.6^{a,A}$	$75.0\pm12.4^{a,A}$
	30	$56.2\pm09.5^{a,B}$	$49.6\pm03.9^{a,B}$	$36.8\pm02.3^{b,B}$	$49.2\pm04.1^{b,B}$
Catalase activity (mol.cata/L)	1	$170.0\pm41.0^{a,A}$	$282.0\pm42.0^{b,A}$	$169.0\pm42.0^{a,A}$	$259.0\pm46.0^{a,A}$
	3	$73.0\pm42.0^{a,B}$	$67.0\pm40.0^{a,B}$	$48.0\pm21.0^{b,B}$	$061.0\pm33.0^{a,B}$
	7	$115.0\pm27.0^{a,A}$	$86.0\pm54.0^{b,B}$	$100.0\pm49.0^{a,A}$	$129.0\pm86.0^{a,A}$
	15	$128.0\pm41.0^{a,A}$	$163.0\pm20.0^{b,A}$	$148.0\pm27.0^{b,A}$	$090.0\pm31.0^{a,A}$
	30	$196.0\pm23.0^{a,A}$	$177.0\pm26.0^{a,A}$	$186.0\pm31.0^{a,A}$	$151.0\pm37.0^{a,A}$
MDA Conc. (μ mol/mL)	1	3.17 ± 0.14	3.67 ± 0.49	3.01 ± 0.19	3.39 ± 0.17
	3	2.39 ± 0.18	2.45 ± 0.45	2.36 ± 0.19	2.69 ± 0.26
	7	2.49 ± 0.24	2.29 ± 0.15	2.61 ± 0.37	2.38 ± 0.30
	15	2.40 ± 0.11	2.23 ± 0.12	2.51 ± 0.51	2.54 ± 0.19
	30	2.84 ± 0.22	2.69 ± 0.27	2.69 ± 0.27	2.67 ± 0.12

^{a,b} Denote difference ($P<0.05$) in rows; ^{A,B} Denote difference ($P<0.05$) in columns

DISCUSSION

This study was done on healthy, physiologically normal calves during their first month of life. The body weight at birth, daily growth rate, physiological findings including temperature, pulse and respiration rates were within normal range. Erythron findings were also normal (Table 1).

The study demonstrate the immaturity of the antioxidant system in newborn calves. This is indicated by the level of erythrocyte peroxidation, catalase activity and MDA concentration found in the blood of the experimental animals. This agrees with the findings of other researchers (Safford and Swingle, 1955; Vrzgula and Kovac, 1979).

Apparently intrauterine transfer of maternal antioxidants, especially vitamin E, does not occur in cattle. Calves are born with extremely low antioxidant activity. The concentration of vitamin E in the blood serum of newborn calves is only 10 per cent of that in the blood serum the cows during late gestation (Safford and Swingle, 1955). Cow's milk is also known to be low in vitamin E (Thompson, 1968).

The results of this study showed that calves have low antioxidant activity at birth and during the first two weeks of life. The placenta retains significant amounts of vitamin E to prevent peroxidation that might be caused to placental tissue by oxygen circulating through the fetal tissues. No significant amount of vitamin E passes through the placenta to the fetus.

The low vitamin E level in neonatal calves is also due in part to the feeding of milk alone during that time. During the third week of life and if the animals start consuming other feeds the antioxidant activity improves significantly (Table 1).

PDE alone or in combination with vitamin A & E shows a positive effect on erythrocyte peroxidation (Table 1). The decrease in EP in the experimental group took place after the first week of life. The greatest effect occurred in group three in which all three treatments were given.

The effect of the three treatments (Vitamins A & E, ascorbic acid and rutin, and PDE) on catalase activity and the level of MDA (Table 1) in blood serum was slight. Catalase activity and MDA concentration are age-dependent (Table 1). The values are highest on day one, drop off rapidly and then gradually increase. At the age one month, the levels in all groups had increased but, remained below the level that was present at birth. The experimental groups had higher catalase activity and MDA level than the control group but there were not significant differences between the three treatments used in this study.

The EP test reflects the functional status of the antioxidant system to a greater than does the measurement of catalase activity and the MDA concentration in blood serum of calves during the first month of life.

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