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HE IMMUNO-POTENTIATING IMPACT OF VITAMIN E AND ZINC UPPLEMENTATION ON BUFFALO CALVES VACCINATED AGAINST INDERPEST*.

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SUMMARY

Rinderpest is a highly contagious and fatal disease of ruminants. Complains from unsatisfactory immune response to vaccines are quite frequently raised. A study was carried out to evaluate the potential of long te m feeding ofigh levels of vitamin E and Zinc on buffalo calves immune response vaccinated with live attenuated tissue culture Rinderpest vaccine. Two groups of buffalo calves were used. The calves of the treated group were supplemented with a combination of 1500 IU of dl-alpha-tocopherol and 7 g. zinc oxide per animal at weekly intervals 7 weeks prior to vaccination and continued for further 4 weeks post vaccination. The ingredients of the ration were analyzed for moisture, crude protein anz zinc. Heparinized and non-heparinized blood samples were collected at vaccination time ,1,2,3 and 4 weeks post vaccination. Serum neutralization test and lymphocyte blastogenic response to phytohaemagglutinin mitogen were used as humoral and cell mediated immune measurements. The results showed that the serum neutralizing antibody titre as well as the blastogenic response of the supplemented gr up (56.0± 8.0 and 2.35 ± 0.16 respectively), were significantly ($P<0.01$) higher as compared to that of the unsupplemented group (26.69 ± 5.33 and 1.577 ± 0.06 respectively). The trial confirmed that benefits from vitamin E and zinc supplementation might favor542y modulate the immune competence under infectious stressful conditions.

Key work: Buffalo calves, vitamin E, zinc, Rinderpest vaccine

INTRODUCTION

The buffalo is considered the main dairy animal in Egypt since about 40% of the total milk production is obtained from this species . Buffalo's milk contains nearly twice as much fat as cow's milk and butter fat fetches a good price in the Egyptian market. Transboundary epizootic

highly contagious with the potential for very serious and rapid spread, irrespective of the national borders. These diseases are of serious socio-economic and public health concerns and also of utmost significance in the international trade of animals and animal products. Rinderpest is one of the six major epizootic diseases of transboundary importance. Dhar et al. (1995) reported that Rinderpest specific antibodies could be first detected 6 days after infection. A gradual increase in antibody titer from 7 days after infection was observed in animals which ultimately recovered.

Complaints from unsatisfactory immune response to vaccines or immunization failure are quite frequently raised in dairy and beef industry. The causes such immunization failures are seldomely investigated. Specific nutrient imbalances vary in the ability to influence the immune response. Morrill and Reddy (1988) reported that serum alpha tocopherol concentrations greater than 200 ug/ 100 ml in calves are considered desirable. Calves fed on natural feeds had concentrations less than this, but their growth rate and immune responses were improved when they were improved when they were supplemented with alpha tocopherol (125 IU./calf daily). It has been demonstrated that vitamin E supplementation beyond the physiological requirements could improve and potentiate the immune response in many animal species; among one of them is the buffalo (Mohamed et al., 1995). Ballarini et al. (1981) studied Vitamin E as a modifier of the immune response of calves vaccinated with strain 19 brucella vaccine. They indicated that supplementary vitamin E had a stimulatory effect. Moreover, zinc plays very srucial metabolic and immunogenic roles inside the animal body. Zinc deficiency has been shown to cause suppression of cell mediated immunity as measured by Lymphocyte blastogenesis to phytohaemagglutinin as well as thymic atrophy (Allen et al., 1981, Flynn, 1984). On the other hand, zinc supplementation enhanced both cell mediated immunity as well as humoral immunity (Mohamed, 1991; Bires et al. 1993 and Mohamed et al. 1995). The only available data regarding the relation between zinc and immuno-potentiation in vaccinated calves is that reported by Paulik et al. (1990) who monitored serum immunoglobulin and albumin concentration after parentral zinc administration combined with vaccination against Trichophytosis. They found that during the following 30 post-vaccination days, calves given zinc had higher immunoglobulin levels lower albumin levels than those given the vaccine alone. A combination of vitamin E and zinc was demonstrated to potentially improve the immune response (Mohamed, 1991 and Mohamed et al. 1995). Thus, the objective of the current study was to assess the combined effect (s) of vitamin E and zinc on the humo al and cell mediated immune responses of bufalo calves vaccinated with live attenuated tissue culture Rinderpest vaccine.

MATERIAL AND METHODS

Seven native breed yearling buffalo calves free from external, internal and blood parasites, had received neither medication nor vaccines, except Haemorrhagic septicemia vaccine, were on trial

for 11 weeks and were allotted to two groups. Group (1) as a control, where no vitamin E or zinc supplementation with a combination of 1500mg. of dl-alpha-tocopherol (Rhone-Poulenc, France) and 7 g. zinc oxide at weekly intervals for seven weeks prior to vaccination and continued for further four weeks post vaccination . Each animal of the two groups was fed on 5kg. concentrate mixture, 3.0kg. be s 547547m hay and 1.5kg. wheat straw, while water was supplied ad-libitium. The concentrate mixt 547 was made of g ound co wheat bran, horse bran, horse bean straw, peanut straw, rice polish, calcium carbonates, sodium chloride. Samples of the concentrate, berseem hay and wheat straw were analyzed for Dm and CP (AOAC, 1980). Furthermore, zinc contents and serum zinc levels were determined according to Butrimovitz and Purdy (1977). The samples and the standards were read using atomic absorption spectrophotometer (Perkin Elmer, U.S.A.). The chemical analysis of the used feeds revealed 91.5%, 10.2%, 8.6%, 2.1%, 10.91%, 59.69% and 40 ppm for the concentrate mixture and 88.8%, 10.9%, 25.9%, 3.2%, 10.7%, 39.9% and 11ppm for hay and 93.64%, 0.0%, 34.28%, 1.09%, 18.76%, 37.51% and 0.0ppm for wheat straw respectively for MD, CP,CF,EE, Ash, NFE and zinc. Live attenuated tissue cultuy e R inderpest vaccine (obtained from Serum and Vaccines Research Institute in Abassia, Cairo, Egypt) was given (1ml S/C in the dewlap region). Lymphocyte blastogenic response to Phytohaemagglutinin mitogen (PHA) was used as a measue of cell mediated immunity (Boyum, 1978), while serum neutralization test (SN; according to Singh et al. 1967) was used as humoral immune parameter. Analysis of Variance of the obtained results was performed using Minitab Data Analysis Sofware (1986).

RESULTS AND DISCUSSION

Table (1). Nutrients Balance Sheet

	DMI (kg / d)	CP (g / d)	Ca (g / d)	P (g / d)	Zn (ppm)
Requirements	8.40	740	20	20	40
Supplies	8.45	837	35	23	23
Surplus	0.05	+97	+ 15	+ 3	-
Deficit	-	-	-	-	17

* According to Kearl, (1982)

The average nutrient's requirements and intakes are calculated and the differences are reported in

the nutrient balance sheet (Table 1). The sheet identified large deficit in zinc intake.

Serum zinc level at the time of vaccination (zero point) showed no significant difference between the treated and the control group, whereas four weeks post vaccination with Rinderpest vaccine the serum zinc level was significantly higher ($P<0.05$) in the vitamin E and zinc supplemented group than that in the control one (Table 2). Similar evidenced observations were obtained by Mohamed, (1991); Chirase et al. (1994) and Mohamed et al (1995). Homeostatic control of body zinc in accordance with needs is achieved, in part, through regulation of zince absorption (Hahn and Evans, 1975) or possibly through mobilization. Thus, under the cu ent experimental setting the calves showed a corresponding demand for the supplemented zinc. The latter could be accounted for and "suggested as an extra requirement" for zinc in buffalo calves; specially under vaccination / disease stresses.

Effect of vitamin E and zinc supplementation on serum neutralization test and on blastogenic response of peripheral blood lymphocytes of buffalo calves vaccinated with live attenuated tissue culture Rinderpest vaccine is reported.

Results in Table 3 indicate that at time of and one week post vaccination there was a zero level serum neutralizing antibodies in both the treated (vitamin E and zince supplemented) and the control group. However, two weks post vaccination and throughout the study there were substantially increased levels of the neutralizing antibodies titer in the treated group than in the control one. The increase was statistically significant ($P<0.05$) in the third week post vacination (Table 3). Such significant increase in humoral immune response is in general agreement with the results obtained by Mohamed (1991) in rabbits.

Table (2). Mean (± SEM) Serum Zinc Levels (ug %) at Time of and Periodically after Vaccination.

Time of vaccination	Control	Treated
Vaccination point	0.2163±0.04	0.234±0.02
4 Weeks post vaccination	0.191± 0.01	0.279± 0.015*

Values are mean ± standard error of the mean.
* $P< 0.05$

The effect of supplementation of vitamin E and zinc on the responsiveness of lymphocytes to T-cell mitogen PHA was repeatedly investigated. Previous studies evaluated the effect of vitamin E deficiency on lymphocyte responsiveness and most agreed on the fact that vitamin E deficiency suppresses the lymphocyte response to different mitogens in a variety of specied (Langweiler et al. 1981 and Lessard et al 1991), whereas vitamin E supplementation enhances the blastogenic response of peripheral blood lymphocytes (Cipraino et al. 1982, Watson and Petro 1982, Reddy et al., 1986, Mohamed , 1991 and Mohamed et al. 1985). Reports on zinc supplementation and its possible effects on cell mediated immune response suggested that supplementation with zinc increases lymphocye blastogenic response to mitogens (Duchateau et al. 1981, Antonious and Shalhoub 1985 and Mohamed et al. 1995).

Findings of the present study demonstarte that a combination of vitamin E and zinc significantly ($P<0.01$) increased lymphocyte blastogenesis to PHA four weeks after vaccination with live attenuated tissue culture Rinderpest vaccine in the supplemented group as compared to the non-supplemented control group (Table 4). The same results were achieved by Ballarini et al. (1981) in calves supplemented with vitamin E and vaccinated with strain 19 beucella vaccine. From the experiment carried out by Morril and Reddy (1988) it appeared that calves fed on natural feeds

Table (3). Mean Serum Neutralization (SN) Levels at Time of and Periodically after Vaccination against Rinderpest

Sampling time	Control	Treated
Vaccination point	zero	zero
1 Week post vaccination	zero	zero
2 Weeks post vaccination	18.0± 7.04	28.0± 12.0
3 Weeks post vaccination	26.67± 5.33	56.0± 8.0*
4 Weeks post vaccination	42.0± 10.68	80.0± 16.0

Values are mean ± standard deviation of the mean.
* $P < 0.05$

Table (4) Effect of vitamin E and zinc supplementation on the blastogenic response of peripheral blood lymphocytes (as measured by stimulation index using PHA mitogen).

Time of vaccination	Control	Vitamin E & zinc
Vaccination point	1.733 ± 0.047	1.695 ± 0.078
4 weeks post vaccination	1.577 ± 0.064	2.350 ± 0.159 **

Values are means ± standard error of the mean.
** $P<0.01$

had concentrations of vitamin E less than that desired for optimal health performance; and the immune response were improved by alpha tocopherol supplementation; an observation which would confirm the present results. Moreover, Paulik et al. (1990) showed that calves given zinc had higher immunoglobulin levels than those given the Trichophytosis vaccine alone.

However, perusal of the recently available literature revealed no more accessible data regar ding the role of vitamin E and zinc on the immune responses of vaccinated calves.

Several possible mechanisms for the higher blastogenic response to PHA in vitamin E and zinc supplemented calves, which include the natural antioxidant effect as well as prevention of proxidative damage to the membranes of the cells and sub cellular organelles (such as mitochondria, microcosms and lysozymes) might be involved (Reddy et al., 1986).

Conclusions:

Dietary vitamin E and zinc supplementation showed stimulating effect on the serum neutralizing antibody titer as wll as on the blastogenic response of peripheral blood lymphocytes to PHA mitogen of vaccinated buffalo calves. From this study it could be safely concluded that the synergistic interaction between vitamin E and zinc is one of the most important immuno-potentiating factors through which the complaints of unsatisfactory immune response to vaccines and/or immunization failure could well be overcome.

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