Vet Res Commun (2009) 33:711–721
DOI 10.1007/s11259-009-9220-9

ORIGINAL ARTICLE

Investigation of Immunmodulatory effects of levamisole and vitamin E on Immunity and some blood parameters in newborn Jersey calves

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Accepted: 2 April 2009 / Published online: 23 April 2009

Abstract The immunmodulatory effects of dl-α tocopherol (vitamin E) and levamisole on the immune system and some blood parameters of newborn Jersey calves were investigated with the present study. Treatment groups 1, 2 and 3 were injected 13,3 ml isotonic saline solution (0,9% NaCl), 3 mg/kg levamisole HCl and 2000 IU vitamin E weekly, starting at birth until the age of two weeks. Average serum IgM levels of the control, levamisole and vitamin E calves were 111,7±9,3 mg/100 ml, 251,9±27,6 mg/100 ml, 202,2±43,3 mg/100 ml respectively on day 22. Average serum IgG levels of the levamisole and vitamin E groups elevated, compared to the control group on days 1, 8, 15 and 22. However, there were stastistically differences in treatment and control groups for serum total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride and cortisol values and whole blood counts. All differences were in the reference ranges. Levamisole and vitamin E could be used as an alternative way for their beneficial effects such as improving the humoral immune responses of calves and their safety and practical use against the neonatal period infections in the field.

Keywords Levamisole · Vitamin E · Immunmodulatory · Newborn · Jersey calf

Introduction

Neonatal calves are highly susceptible to bacterial and viral pathogens (Roy 1980; Bryson et al. 1987). The effectiveness of passive immunity depends on the ability of the calf to ingest colostral antibodies immediately following birth, although as many as 20 to 30% of calves ingesting colostrum remain hypogammaglobulinaemic or agammaglobulinaemic (Brignole and Stott 1980). Variation in neonatal bovine serum immunoglobulin (Ig) concentrations has been associated with morbidity and mortality and disease prevalence is generally inversely related with this concentration (Corbeil et al. 1985). Risk of either disease will be reduced by effective passive immunity at birth and perhaps efficient onset of

This manuscript was preapared from the PhD. Dissertation of Didem Pekmezci.

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endogenous Ig production between 2 and 4 weeks of age (Burton et al. 1989). The high mortality of calf losses is mostly caused by infectious diseases. Intensive studies have recently been initiated to assess methods of preventing and treating infectious diseases. One of these methods has been non-specific immunoprophylaxis with natural or synthetic immunomodulators (Mohri et al. 2005).

Although treatment and prevention of infectious diseases are the most common reasons to use immunomodulators, other conditions, such as amelioration of stress-induced immunosuppression, maturation of the neonate's developing immune response, and strategies to reduce the metabolic cost of eliciting an immune response also are well suited for immunomodulation (Blecha 2001). It is known that vitamin E can stimulate the immune defense mechanisms in laboratory animals (Sheffy and Schultz 1979), and such beneficial effects also have been recorded in cattle (Cipriano et al. 1982; Reddy et al. 1986). Vitamin E has been associated with increased immune functions in calves (Reddy et al. 1986; 1987) and in dairy cows (Hogan et al. 1992; Politis et al. 1995). Dairy (Hogan et al. 1992) and beef cows showed marked decreases in plasma vitamin E around parturition, but calves begin life with deficient or nearly deficient plasma vitamin E concentrations (Cipriano et al. 1982; Reddy et al. 1986; 1992). Therefore, an increase in plasma and tissue vitamin E concentrations during certain periods may be beneficial to the health and well-being of many animals.

On the other hand, levamisole was originally developed as an anthelmentic and has been widely used in human and animal medicine (Mohri et al. 2005). It was subsequently found to be an active immunomodulator (Symoens and Rosenthal 1977) and it has also been widely used for this purpose. Most previous reports studied the effects of levamisole administration on immunocompromised or vaccinated animals but there are limited data concerning the effects of administered levamisole in normal immunocompetent neonatal calves (Mohri et al. 2005).

It was, therefore, our interest to determine whether levamisole might have an effect on the course of increasing the Ig levels as well as vitamin E on the humoral immune response in newborn Jersey calves. This research was designed to investigate the effects of high doses of vitamin E and immunstimulation dose of levamisole, could reduce the incidence of diseases by improving the immune competence in newborn calves.

Materials and methods

Animals and husbandry

The experimental procedures were approved with the certificated number (0145) by the Local Ethical Committee for Animal Studies (University of Ondokuz Mayıs, Turkey). The study was conducted in a dairy herd with approximately 282 calves per year at The Directorship of Karaköy Agricultural Enterprises in Samsun, Turkey. Dams were vaccinated with (ScourGuard 4(K)-Pfizer) in the last 50th and 20th day of the parturition intramuscularly by the veterinarians. The study subjects were consisted of 30 (16 male and 14 female) clinically healthy newborn Jersey calves, born between June and July 2007. All calves received 1st colostrum from their dams after the delivery and nursed by their dams until the 4th day of age. They were separated at the 4th day of birth, and housed individually in Fiberglass calf hutches. Then mature milk was given up to three months of age, and calves fed with a conventional calf starter diet after ten days of age, and ad libitum with free access to water.

Health status

The umbilicus was disinfected with povidine iodine after the delivery to protect calves from infections. All calves were evaluated daily for heart and respiration rates, rectal temperatures, behavior, nasal and ocular discharges, respiratory sounds, cough, fecal consistency for every sampling time. The health status of the calves was controlled by the veterinarians if there were any needs for supportive and medical treatments to the calves during the trial.

Groups, study period and test-scheme

Calves were divided into three groups of ten calves each. Calves, based on their birth dates were assigned randomly to one of control and treatment groups. The control group was administered 13,3 ml isotonic saline solution (% 0,9 NaCl-Eczacıbaşı Baxter), the levamisole group was received 3 mg/kg levamisole HCl (Actipar-injectable, 100 mg/ml, Alke Drugs) and the vitamin E group was given 2000 IU vitamin E (Evigen-injectable, 300 mg/2 ml, Aksu Pharma) intramuscularly, within the first hour after birth before the colostrum intake and on days 7 and 14 with same route for all three groups.

Sample collection

Blood samples were obtained just before colostrum intake (day 0) and on days 1, 8, 15 and 22 of the experiment from a jugular vein using the evacuated K_3EDTA (7.5% 0.040 ml) additive tubes 2 ml for whole blood counts and 5 ml for evacuated non-additive tubes for serum IgM, IgG, serum total cholesterol, LDL, HDL, triglyceride and cortisol values.

Blood sample analysis

Blood samples were taken for WBC (White Blood Cells), LYM (Lymphocytes), MONO (Monocytes), GRA (Granulocytes), LYM% (Lymphocytes%), MONO% (Monocytes%), GRA% (Granulocytes%), RBC (Red Blood Cells), HCT (Hematocrit), MCV (Mean Corpuscular Volume), RDWc (Red Cell Distribution Width), HGB (Hemoglobin Concentration), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), PLT (Platelet), PCT (Thrombocrit), MPV (Mean Platelet Volume), PDWc (Platelet Distribution Width) values and analyzed with Abacus Vet Junior hematology analyzer. Following the centrifugation of blood samples, serum samples were evacuated in non-additive tubes and frozen at −20ºC until analyzed. Total serum cholesterol, LDL, HDL, triglyceride values were determined with the Tomas Tokyo Boeki autoanalyser. Serum cortisol levels were analyzed with Chemilumine Immuno Assay (Immunolyte 2000 analyzer). Serum IgM and IgG levels were analyzed with Enzyme-Linked ImmunoSorbent Assay with using Bovine IgM and IgG ELISA Quantitation kits (Bethyl laboratories, Inc.). Tests were performed using the standards provided by the manufacturer and according the guidelines and all samples were calculated with spectrophotometer (Digital and Analog Systems S.R.L.), at 450 nm.

Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA) followed by Duncan's multiple range test was performed to compare the 0., 1., 8., 15. and 22nd day IgG and IgM values indices between the control and experiment groups. A logarithmic transformation

Table 1 Mean serum IgM levels in control and treatment groups from birth to 22 day of age (mg/100 ml), *p<0.05

Days	Groups				
	Control	Levamisole	Vitamin E	Maximum ± SEM	F
0	80,1	43,6	81,5	62,8	0,18
1	526,1	798,4	725,3	170,4	0,84
8	285,6	415,6	476,3	108,1	1,42
15	248,7	329,5	246,0	83,8	0,68
22	111,7	251,9	202,2	43,3	5,55*

was performed to obtain a normal IgG and IgM distribution but non-transformed means are reported. Variance analysis was applied for the separately daily comparision of the 0., 1., 8.,15. and 22nd day serum cortisol, total cholesterol, LDL, HDL and triglyceride values in addition to whole blood values including WBC, LYM, MONO, GRA, %LY, %MONO, % GR, RBC, HGB, HCT, MCV, MCH, MCHC, RDWc, PLT, PCT, MPW, PDWc of the control and experimental groups. Duncan's multiple range test was performed in the significance control of differences between groups in the comparision of the groups regarding the mentioned peculiarities (John, 1971). Repeated measure with the treatment, time and interaction by time were performed. Duncan's multiple range test was benefited for the significance control of difference between group averages and orthogonal polinomes were used in the determination of difference between times (John, 1971). A value of $P< 0.05$ was considered statistically significant. Data were expressed as the mean ± SEM.

Results

Serum Immunoglobulin levels

Mean serum IgM and IgG concentrations for day 0, 1, 8, 15 and 22 are presented in Tables 1 and 2. There were no differences on day 0, 1, 8 and 15 among treatments in IgM levels, but there was a trend towards higher Ig concentration in levamisole and vitamin E calves. Mean serum IgM levels of treatment groups on day 22 were elevated, compared to the control group (Table 1). Average serum IgG levels of the levamisole and vitamin E groups were elevated, compared to the control group on days 1, 8, 15 and 22 (Table 2).

Table 2 Mean serum IgG levels in control and treatment groups from birth to 22 day of age (mg/100 ml), *p<0.05

Days	Groups				
	Control	Levamisole	Vitamin E	Maximum ± SEM	F
0	1611,8	1342,6	1595,1	762,4	0,06
1	4206,7	45224,4	17552,4	16378,8	4,77*
8	3580,56	12886,3	16803,9	2421,7	12,29*
15	2416,6	7200,2	13987,6	2826,5	11,72*
22	1647,8	5043,6	14472,9	2371,5	20,97*

Serum total cholesterol, LDL, HDL, triglyceride and cortisol values

There was only a difference ($p<0.05$) on the first day between control and vitamin E group regarding the serum total cholesterol levels. On the other days of the experiment, there were no differences for the serum total cholesterol among the treatments and control group (Table 3). There was only a difference ($p<0.05$) on day 22 between control and levamisole group for serum LDL values and again there were no differences on the other days of the experiment among the treatments and control group (Table 3). No differences were observed between the treatments and control group of serum HDL (Table 3). Serum triglyceride levels on day 0 differed ($p<0.05$) among calves administered levamisole and control calves. However, on the other days of the experiment there were no differences among the treatments and control calves (Table 3). Serum cortisol concentration was statistically different ($p<0.05$) on day 0 among the control and vitamin E groups, and again between control and levamisole groups on day 22 (Table 3).

Whole blood counts

There was only a difference ($p<0.05$) on day 0 between control and vitamin E groups for the LYM value (Table 4). When compared with the control group, 15th day MONO value

Table 3 Total cholesterol, Triglyceride, LDL, HDL and Cortisol values in control and treatment groups from birth to 22 day of age, *P<0.05, n.s. non-significant

	Days														
	0			1			8			15			22		
	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P
Total Cholesterol (mg/dl) Groups															
Control	26	2,9	ns	28	3,3	*	88	7,9	ns	81	13,1	ns	106	15,0	ns
Levamisole	27	1,6	ns	33	2,4	ns	90	8,1	ns	84	8,7	ns	106	6,6	ns
Vitamin E	28	3	ns	38	2,1	*	86	4,3	ns	86	6,9	ns	106	8,6	ns
Triglyceride (mg/dl) Groups															
Control	22	3,9	*	41	8,1	ns	24	4,4	ns	18	2,6	ns	19	3,3	ns
Levamisole	31	2,9	*	32	3,5	ns	24	3,1	ns	23	2,7	ns	26	2,7	ns
Vitamin E	28	2,2	ns	45	7,4	ns	22	3,3	ns	19	1,8	ns	19	2,4	ns
LDL (mg/dl) Groups															
Control	7	1,0	ns	6	1,6	ns	29	5,0	ns	29	3,5	ns	43	7,1	*
Levamisole	10	1,6	ns	9	1,4	ns	25	3,5	ns	20	3,2	ns	23	1,8	*
Vitamin E	8	1,5	ns	9	1,1	ns	26	2,8	ns	26	2,8	ns	33	4,2	ns
HDL (mg/dl) Groups															
Control	15	1,7	ns	14	1,7	ns	54	4,4	ns	50	9,7	ns	60	8,7	ns
Levamisole	11	1,1	ns	19	2,4	ns	60	5,7	ns	61	6,7	ns	78	7,0	ns
Vitamin E	14	2,1	ns	20	2,1	ns	55	4,0	ns	58	4,8	ns	66	5,7	ns
Cortisol (ug/dl) Groups															
Control	12	1,8	*	3	0,4	ns	2	0,2	ns	1	0,1	ns	1	0,0	ns
Levamisole	10	1,0	ns	3	0,4	ns	2	0,2	ns	1	0,1	ns	1	0,0	*
Vitamin E	8	0,9	*	3	0,5	ns	3	0,4	ns	2	0,5	ns	1	0,0	ns

Table 4 WBC, LYM, MONO, GRA, LY% values in control and treatment groups from birth to 22 day of age, *P<0.05, n.s. non-significant

	Days														
	0			1			8			15			22		
	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P
WBC (10ˆ3/µl) Groups															
Control	9	0,5	ns	9	0,7	ns	8	0,8	ns	8	0,5	ns	9	0,8	ns
Levamisole	10	0,9	ns	9	0,7	ns	9	0,8	ns	11	1,3	ns	9	0,4	ns
Vitamin E	8	0,9	ns	10	1,1	ns	8	0,9	ns	8	0,7	ns	8	0,6	ns
LYM (10ˆ3/µl) Groups															
Control	4	0,6	*	4	0,2	ns	5	0,4	ns	6	0,6	ns	7	0,5	ns
Levamisole	4	0,4	ns	4	0,3	ns	6	0,5	ns	6	0,8	ns	6	0,6	ns
Vitamin E	3	0,3	*	4	0,3	ns	5	0,5	ns	5	0,3	ns	6	0,5	ns
MONO (10ˆ3/µl) Groups															
Control	0,3	0,1	ns	0,3	0,0	ns	0,1	0,1	ns	0,1	0,0	*	0,2	0,1	ns
Levamisole	0,2	0,0	ns	0,2	0,0	ns	0,1	0,0	ns	0,4	0,0	*	0,4	0,0	ns
Vitamin E	0,3	0,0	ns	0,2	0,0	ns	0,1	0,0	ns	0,3	0,0	ns	0,4	0,0	ns
GRA (10ˆ3/µl) Groups															
Control	4	0,4	ns	5	0,6	ns	3	0,5	ns	2	0,4	ns	2	0,3	ns
Levamisole	6	0,7	ns	5	0,5	ns	3	0,6	ns	3	0,7	ns	2	0,2	ns
Vitamin E	5	0,8	ns	6	0,8	ns	3	0,6	ns	3	0,7	ns	2	0,2	ns
LY% Groups															
Control	48	5,5	*	43	2,9	ns	66	4,5	ns	68	5,7	ns	72	2,3	ns
Levamisole	40	2,7	ns	45	2,8	ns	67	3,9	ns	67	2,8	ns	73	2,6	ns
Vitamin E	35	3,1	*	41	2,6	ns	62	4,3	ns	64	5,4	ns	73	2,7	ns

of the levamisole group was higher ($p<0.05$). There was only a difference ($p<0.05$) on day 0 for the LY% and GR% values and on day 22 for the MONO% value between control and vitamin E groups and again there were no differences within these two parameters on the other days of the experiment among the treatments and control groups (Tables 4 and 5). First day MCV value was different ($p<0.05$) in the control and vitamin E calves on day 0 and in the levamisole and control groups on day 1. Only on the first day of the trial, MCH and PDWc values were different ($p<0.05$) in control and vitamin E groups. And also a difference was observed on day 22 for PDWc value among the levamisole and vitamin E calves. No differences were observed between the treatments and control group for WBC, GRA, RBC, HGB, HCT, MCHC, RDWc, PLT, PCT and MPV values at any time during the experiment (Tables 4, 5, 6, and 7).

Discussion

For calves, most of the papers studied the immunomodulatory effects of simultaneous administration of levamisole and various vaccines. However, the information about the effects of levamisole in normal neonatal dairy calves is limited and controversial (Mohri et al. 2005). Anderson (1984) reported that the use of levamisole (2–3 mg/kg) with an

Table 5 MONO %, GR %, RBC, HGB, HCT values in control and treatment groups from birth to 22 day of age, *P<0.05, n.s. non-significant

	Days														
	0			1			8			15			22		
	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P
MONO % Groups															
Control	4	1,1	ns	4	0,7	ns	2	0,8	ns	2	0,7	ns	2	0,8	*
Levamisole	3	0,8	ns	3	0,3	ns	1	0,1	ns	4	0,9	ns	5	1,0	ns
Vitamin E	4	0,8	ns	4	0,8	ns	2	0,5	ns	4	0,9	ns	6	0,6	*
GRA % Groups															
Control	47	6,2	*	54	2,9	ns	33	4,0	ns	29	5,8	ns	25	2,5	ns
Levamisole	58	3,2	ns	52	2,7	ns	31	3,8	ns	29	3,0	ns	22	3,1	ns
Vitamin E	62	3,7	*	55	3,2	ns	35	4,3	ns	31	5,5	ns	20	2,9	ns
RBC (10ˆ6/µl) Groups															
Control	8	0,3	ns	7	0,3	ns	7	0,3	n.s.	7	0,4	ns	7	0,2	ns
Levamisole	8	0,3	ns	7	0,4	ns	7	0,4	n.s.	7	0,3	ns	7	0,4	ns
Vitamin E	8	0,3	ns	6	0,3	ns	7	0,3	n.s.	7	0,3	ns	8	0,3	ns
HGB (g/dl) Groups															
Control	9	0,5	ns	8	0,4	ns	7	0,4	ns	8	0,5	ns	7	0,3	ns
Levamisole	9	0,5	ns	7	0,5	ns	7	0,6	ns	7	0,5	ns	7	0,5	ns
Vitamin E	8	0,5	ns	7	0,4	ns	7	0,4	ns	7	0,4	ns	7	0,3	ns
HCT (%) Groups															
Control	30	1,7	ns	25	1,4	ns	24	1,4	ns	24	1,6	ns	21	1,1	ns
Levamisole	29	1,2	ns	22	1,5	ns	23	1,5	ns	22	1,5	ns	22	1,6	ns
Vitamin E	27	1,5	ns	22	1,2	ns	23	1,3	ns	23	1,2	ns	23	1,2	ns

intermittent treatment alters immune responsiveness more effectively than the continuous treatment. In the present study, i.m. injection of 3 mg/kg of levamisole starting at birth, weekly until the age of two weeks resulted with high Ig concentrations.

Nagahata et al. (1991) reported that antibody producing activity of lymphocytes are lower in calves within 3 weeks after birth, indicating that neonatal calf lymphocytes has a low antibody producing activity at least up to 1 month following birth. Alternatively, administration of higher amounts of vitamin E, not only enhances humoral immune response (Tengerdy 1980). Vitamin E may help in reducing morbidity due to pathogens, during the period from the loss of maternal antibodies to the production of antibodies by the calf (Hidiroglou et al. 1992). Hidiroglou et al. (1992) reported that, 2700 IU of vitamin E injected every 3 wk, from birth up to 12 wk, created higher concentration of IgM than control calves, and there were no significant differences among treatments in IgG_1 and IgG_2 concentrations. Our results are consistent with those of Hidiroglou et al. (1992), for the augmentation of IgM levels, but are in contrast regarding the serum IgG levels of the treatments. Levamisole and vitamin E administered groups resulted with high concentrations of IgM than control calves on day 22 and again high concentrations of IgG than that of control calves on day 1, 8, 15 and 22 of the trial.

There were no statistically important differences in the serum total cholesterol, HDL and triglyceride values among the control and levamisole groups during the trial, but there was

Table 6 MCV, MCH, MCHC, RDWc, PLT values in control and treatment groups from birth to 22 day of age, *P<0.05, n.s. non-significant

	Days														
	0			1			8			15			22		
	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P
MCV(fl) Groups															
Control	37	0,8	*	36	0,7	*	34	0,7	ns	32	0,5	ns	30	0,8	ns
Levamisole	34	0,9	ns	32	0,6	*	32	0,9	ns	31	0,9	ns	31	1,0	ns
Vitamin E	34	0,7	*	33	0,5	ns	32	0,5	ns	31	0,6	ns	30	0,4	ns
MCH (g/dl) Groups															
Control	11	0,2	ns	11	0,2	*	10	0,1	ns	10	0,2	ns	10	0,2	ns
Levamisole	11	0,3	ns	10	0,2	ns	10	0,3	ns	10	0,3	ns	12	2,0	ns
Vitamin E	10	0,2	ns	10	0,2	*	10	0,2	ns	9	0,2	ns	9	0,1	ns
MCHC (g/dl) Groups															
Control	29	1,3	*	31	0,3	ns	30	0,4	ns	32	0,3	ns	32	0,3	ns
Levamisole	32	0,5	*	32	0,4	ns	32	0,5	ns	32	0,4	ns	31	0,3	ns
Vitamin E	31	0,4	ns	30	0,3	ns	30	0,5	ns	31	0,3	ns	30	0,3	ns
RDWc (%) Groups															
Control	24	0,2	ns	24	0,2	ns	25	0,2	ns	29	0,9	ns	29	0,9	ns
Levamisole	25	0,8	ns	25	0,8	ns	27	0,8	ns	28	0,8	ns	28	0,5	ns
Vitamin E	25	0,6	ns	25	0,7	ns	26	0,6	ns	28	0,6	ns	28	0,5	ns
PLT (10^3/µl) Groups															
Control	684	123,8	ns	390	24,5	ns	883	94,7	ns	854	56,3	ns	703	51,9	ns
Levamisole	562	44,0	ns	310	21,6	ns	878	65,0	ns	876	59,0	ns	877	62,2	ns
Vitamin E	561	71,7	ns	297	43,6	ns	793	60,3	ns	999	122,5	ns	791	62,6	ns

a decrease in LDL values in the levamisole group only on day 22 compared to the control group.

There were no statistical differences in serum LDL, HDL, triglyceride and cortisol values among the control and vitamin E groups during the trial. Carter et al. (2005) studied the effects of duration (days) of vitamin E feeding during a 42-day receiving period, on animal performance, health, and serum cholesterol and vitamin E concentrations in calves. They did not record interactions between serum cholesterol and days of vitamin E supplementation. Furthermore, they suggested that vitamin E may provide some protection against some of the detrimental effects of stress and disease and that these effects might be time dependent. In our study, there was only a difference in total cholesterol levels on the first day, between the control and vitamin E groups.

Stogause and King (1995) suggested that, in the rat, levamisole administration caused lower levels of corticosterone and probably this reduction increased immune function. However, the only statistically important difference in cortisol value was observed on day 22 in the levamisole group in the present study, but this difference was within the reference ranges (Kurz and Willett 1991).

There was only a statistical difference in cortisol value on the delivery in the vitamin E group compared to the control group.

Table 7 PCT, MPV, PDWc values in control and treatment groups from birth to 22 day of age, *P<0.05, n.s. non-significant

	Days														
	0			1			8			15			22		
	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P
PCT (%) Groups															
Control	0,3	0,0	ns	0,2	0,0	ns	0,4	0,0	ns	0,4	0,0	ns	0,3	0,0	ns
Levamisole	0,3	0,0	ns	0,1	0,0	ns	0,4	0,0	ns	0,4	0,0	ns	0,4	0,0	ns
Vitamin E	0,3	0,0	ns	0,1	0,0	ns	0,3	0,0	ns	0,4	0,0	ns	0,3	0,0	ns
MPV (fl) Groups															
Control	6	0,1	ns	5	0,1	ns	5	0,0	ns	5	0,0	ns	5	0,0	ns
Levamisole	6	0,1	ns	5	0,0	ns	5	0,1	ns	5	0,0	ns	5	0,1	ns
Vitamin E	5	0,1	ns	5	0,0	ns	5	0,0	ns	5	0,0	ns	5	0,0	ns
PDWc (%) Groups															
Control	34	0,7	ns	33	0,6	*	32	0,4	ns	30	0,2	ns	31	0,3	ns
Levamisole	34	0,7	ns	32	0,5	ns	32	0,6	ns	30	0,4	ns	32	0,6	*
Vitamin E	32	0,5	ns	31	0,2	*	31	0,4	ns	30	0,3	ns	30	0,2	*

Asif et al. (1995) reported no significant changes in the leukocyte, neutrophil, eosinophil and basophil counts in Sahiwal heifers after oral administration of levamisole. In another study on buffalo heifers, Zia-ul-Rahman et al. (2003) reported an increase of WBC counts on the first day after levamisole administration, which returned to pre-dose levels at 7 and 14 days after administration. Neutrophil percentage decreased and lymphocyte percentage increased on days 7 and 14 after drug administration, but an increase in monocyte count was observed on days 7 and 14 of their experiment. Goranov and Bonovska (1987) reported no changes in the total count of leukocytes in sheep after levamisole injection, although an elevation in the phagocytic activity of neutrophiles was determined. Mohri et al. (2005) observed that there were significant differences between neutrophil and monocyte levels at the second and third weeks of the trial between groups, but there were no significant changes in the PCV and WBC values.

In our study there were no differences between levamisole group and other treatment and control groups for the WBC and LY values on day 1, 8, 15 and 22 following treatments. These results were similar to those of Asif et al. (1995), and Goranov and Bonovska (1987), while the results were not consistent with the findings of Zia-ul-Rahman et al. (2003). Similar results were observed in the GR, LY%, MONO%, GR% values in the treatment groups on the same days. There was only an increase in MONO values in the levamisole group rather than the control group on day 15, but this increase was within reference ranges and consisted with the findings of Mohri et al. (2005). The other RBC, HGB, HCT, MCH, MCHC, RDWc, PLT, PCT and MPV values of the levamisole group were not statistically different than the vitamin E and control groups. There was only a statistically important decrease for the MCV values on the first day in the levamisole group. But this decrease was in the reference ranges (Kurz and Willett 1991).

Reddy et al. (1987) studied the haematological responses in thirty-two Holstein heifer calves which received conventional rations and were supplemented with 0 (control), 125,

250, or 500 IU of vitamin E/calf per day. At 4 and 8 wk of age, MCH in calves given 500 IU was lower than others and MCHC in calves given 500 IU was lower than in calves given 125 or 250 IU. They did not observed significant differences in WBC and RBC counts, HGB, PCV, and MCV values among treatments. In the present study there were no differences observed in WBC, LYM, MONO, GRA, LY%, GR%, RBC, HGB, HCT, MCV, MCHC, RDWc, PLT, PCT, MPW values among the vitamin E and control groups on day 1, 8, 15 and 22 during the treatment. But there was an increase in MONO% value on day 22 in the vitamin E group than the control one, but again this increase was in the reference ranges (Kurz and Willett 1991). Furthermore, MCH and PDWc values in the vitamin E group on day one were statistically lower than control group, but on day 22 those were statistically lower than the levamisole group. And our results were similar to those of Reddy et al. (1987), for the WBC and RBC counts, haemoglobin, PCV, MCV and MCH values, controversial for the MCHC value.

Conclusions

With the present study not only the Ig enhancing effects of levamisole and vitamin E were investigated but also some biochemical and whole blood parameters of the neonatal Jersey calf's from the delivery to 22 day of age were observed. Our results further confirm the beneficial effects of vitamin E on immunomodulation and support its safety use with high amounts in neonatal Jersey calves. Furthermore, vitamin E and levamisole administrations within the first hour after birth before the colostrum intake and on days 7 and 14 may also indicate greater capacity for a primary immune response in calves and also it may constitute a practical way of increasing the calf's Ig levels rather than other costly applications. In conclusion, the observed stimulant effects of levamisole and vitamin E applications to neonatal Jersey calves could be used as an alternative way for their beneficial effects such as improving the humoral immune responses of calves against the neonatal period infections which encountered frequently resulting with major economical losses and their safety use in the field.

Acknowledgements This study was undertaken in The Directorship of Samsun Karaköy Agricultural Enterprises with the grate permission of the Chief Dr. Ahmet Cıra. The authors appreciate the valuable help of Filiz Akdag for statistical analysis of the data and Ertan Emek Onuk for the ELISA analysis of the samples and our Department chief Yucel Meral for his general support. The authors also wish to thank to Gokmen Zafer Pekmezci and Guvenc Gokalp for their technical assistance and Adem Usta for his general support. We are also grateful to the staff of The Directorship of Samsun Karaköy Agricultural Enterprises for the care and handling of calves.

Conflicts of interest The authors declare that there are no conflicts of interest.

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