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IMMUNOGLOBULIN CHANGES IN BOARS EXPOSED TO ADMINISTRATION OF LEVAMISOLE AND EXOGENOUS ADRENOCORTICOTROPIC HORMONE

BILANDŽIĆ NINA*, TERZIĆ SVJETLANA* and ŠIMIĆ B**

*Croatian Veterinary Institute, Zagreb, Croatia
**University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia

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The objective of this paper was to determine the effect of levamisole (LEV) on immunoglobulin concentration in the serum of boars exposed to 3-day stress induced by exogenous ACTH. Boars were assigned to 4 groups (n=7). The first group received LEV for 3 days (2.5 mg/kg BW), the second group received ACTH (10 μg/kg BW) for 3 days and the third group received LEV for 3 consecutive days and ACTH for the following 3 days (2.5 mg/kg BW; ACTH 10 μg/kg BW). The control group received saline solution during the 6 days. Concentrations of cortisol, total protein, globulin, albumin and immunoglobulin (IgG, IgA and IgM) were determined during treatments and on the 16th day post-administration.

Cortisol concentration was increased in both ACTH treated groups during all three days of administration and the day after the last ACTH treatment ($p<0.05$). ACTH increased total protein levels during the stress period and over the next 16 days ($p<0.05$). However, in the LEV+ACTH group total protein levels were elevated only on day 1 and 2 of ACTH injection ($p<0.05$) and after the end of treatment on day 11 and 22 ($p<0.05$). LEV stimulated the increase of protein concentrations compared to control values after LEV treatment, on days 5, 14, 18 and 22 ($p<0.05$). Serum albumins were not affected by LEV or ACTH treatment.

Globulin concentrations were increased throughout and on the 16th day after administration of ACTH in the ACTH and LEV+ACTH groups ($p<0.05$). Globulin concentrations did not differ between LEV and control groups of boars. ACTH treatment elevated serum IgG concentration during the stress period ($p<0.05$) and over the next 16 days ($p<0.05$). However, in the LEV+ACTH group of boars, IgG levels were elevated on days 1 and 3 after ACTH injection ($p<0.05$) and days 1 and 5 in the post-treatment period ($p<0.05$). LEV had no impact on IgG levels compared to the control boars. However, increased IgA concentrations in boars treated with LEV were determined on day 2 ($p<0.05$) and day 11 ($p<0.05$) after administration of LEV compared to animals in the LEV+ACTH group. These results show that LEV

application may protect boars from the negative influence of stress and provoke improved non-specific immunity.

Key words: ACTH, albumins, boars, globulins, immunoglobulins, levamisole, total proteins

INTRODUCTION

Pig breeding processes on farms commonly involve several stressful situations, including temperature changes, transport, mixing of animals from different litters, and competition within groups. The animal response to stressful stimuli includes the release of corticotropin releasing factor from the hypothalamus, which in turn leads to the release of adrenocorticotropic hormone (ACTH) and other peptides from the anterior lobe of the pituitary gland. Elevated ACTH stimulates the release of glucocorticoids from the adrenal cortex into the circulation of stressed animals (Danzer and Mormede, 1983). Furthermore, neuroendocrine signals caused by stress stimulate changes in the ratio of specific types of leukocytes, affecting their distribution from the blood into peripheral organs or vice versa (Westermann and Pabs, 1990). Stressful situations stimulate a decreased lymphocyte count (Brown-Borg *et al*., 1993, Bilandžić *et al*., 2006), as well as eosinophils and monocytes (McGlone *et al*., 1993), and increase neutrophil counts in the blood of pigs (Tuchscherer *et al*., 1998; Stull *et al*., 1999). The type of stress and its duration can cause changes in total proteins, globulins and albumins in the blood of pigs (Jensen *et al*., 1996; Hicks *et al*., 1998; Bilandžić *et al*., 2005).

Levamisole (LEV), a potent broad-spectrum anthelmintic drug has shown positive effects as an immunomodulator in the treatment of certain diseases, postoperative infections, chronic infections, inflammatory processes and autoimmune diseases (Symoens and Rosenthal, 1977; Mutch and Hutson, 1991; Van Wauwe and Janssen, 1991; Szeto *et al*., 2000). The action of LEV seems to enhance or restore depressed immune responses, acting primarily on the cell-mediated immune response such as macrophages and T cells (Szeto *et al*., 2000). The mechanism by which LEV stimulates the immune system depends on several factors, such as the administered dose, timing, and immune status of the animal or human patients (Symoens and Rosenthal, 1977; Kimball *et al*., 1991; Cuesta *et al*., 2002). Efforts are made to determine the reasons for its administration for the stimulation of the immune response in various states of reduced activity of animal immune cells (Blecha, 1988; Nagy and Fekete, 1999; Karakowski *et al*., 1999; Božić and Mrljak, 2001).

The objective of this study was to determine the potential impact of LEV administered on 3 consecutive days prior to 3 days activation of the HPA axis by ACTH on immunoglobulin concentrations and blood proteins. Therefore, during LEV and ACTH treatment and in the 16-day post-treatment period, concentrations of total protein, albumin, globulin and also immunoglobulins (IgG, IgA and IgM) were measured.

MATERIALS AND METHODS

Animals and Treatments

Swedish Landrace boars aged 6 months (n=28) and weighing between 110 and 145 kg were included in the study. Boars were housed in individual pens on farms. Stable temperatures varied between 9°C and 15°C during the experiment. The animals were given 3 kg of standard corn-soy ration *per* day. Water was available *ad libitum*.

Boars were randomly assigned to four experimental groups. During the 6 experimental days, LEV, LEV and ACTH, ACTH and saline were administered at 10:00 a.m. All animals were handled using restraint with a snare during the procedures of LEV, ACTH and saline administration, and blood collection. The control group (CONTROL, n=7) received 1 mL of placebo (sterile 0.9% saline) intramuscularly during the 6 experimental days. The first experimental group (LEV, n=7) intramuscularly received LEV (Nilverm, Veterina d.o.o., Zagreb, Croatia; preparation contains 75 mg LEV hydrochloride/mL) at the dose of 2.5 mg/kg BW on 3 consecutive days and then received 1 mL of placebo intramuscularly on days 4, 5 and 6. The second group (ACTH, n=7) was injected intramuscularly with 1 mL of placebo on days 1, 2 and 3, and then with ACTH (ACTH, from porcine pituitary, 80 IU/mg, Sigma-Aldrich Co., St. Louis) in a dose of 10 μg/kg BW on days 4, 5 and 6 into the ear vein. The third experimental group of animals (LEV+ACTH, n=7) was given LEV (preparation of 75 mg LEV hydrocloride/mL; Nilverm®, Veterina d.o.o., Zagreb, Croatia) intramuscularly on days 1, 2 and 3 (2.5 mg/kg BW), and animals were then injected with ACTH (ACTH, from porcine pituitary, 80 IU/mg, Sigma-Aldrich Co., St. Louis) in a dose of 10 μg/kg BW on days 4, 5 and 6 into the ear vein.

Blood sampling

All boars were frequently handled and used to the blood collection procedure to be performed *via* the jugular vein 90 min after administration of LEV, ACTH or saline on treatment days 1, 3, 4, 5 and 6 and throughout the 16 days post-treatment (day 7, 11, 14, 18 and 22). Blood samples were collected using a sterile syringe (Becton Dickinson SA, Fraga, Spain) and immediately transferred to ice-cold glass tubes for serum separation (SST, DB Vacutainer®, Preanalytical Solutions Belliver Industrial Estate, Plymouth, UK). Subsequently, blood samples were centrifuged for 10 min at 750 x g, the serum was separated and stored at –20°C until analysis.

Cortisol analysis

Serum cortisol concentration was determined by radioimmunoassay using commercially available RIA Coat-A-Count Kit (Diagnostics Products Corp., Los Angeles, USA) according to the manufacturer's instructions. Samples were quantified in two assays, with average intra- and interassay coefficients of variation of 7.5% and 12.0%, respectively. The assay sensitivity was 0.1 nmol/L.

Serum proteins analysis

Technicon RA-1000 System Spectrophotometer (Technicon® Instruments Corporation, Tarrytown, New York) was used for total protein and albumin analyses.

Total protein concentration was determined by the Biuret method using commercially available kits (Randox Laboratories Ltd., Crumlin, Co. Antrim, United Kingdom). Albumin content was determined by bromocresol green method using Randox Laboratories Ltd. kits. Intra- and inter-assay variation coefficients were 5.5% and 7.6%, respectively, for total protein and 4.3% and 5.1%, respectively, for albumin.

Globulin levels in serum samples were calculated as the difference between total protein and albumin concentrations.

Immunoglobulins analysis

Radial immunodiffusion (RID) was used for the determination of pig immunoglobulins (Mancini *et al.*, 1965). Test plates for the determination of IgG were prepared by diluting 2 g of agarose in 100 mL of barbiturate buffered saline (0.1 M) with the addition of anti-pig IgG antiserum (1:10) and 0.1% sodium azide. Five microlitres of reference standard solutions of IgG and diluted serum samples (1:20) were pipetted to a separately identified well of test plates. The plate was securely covered and incubated for 48 to 72 hours at room temperature. Following incubation, the plates were removed and placed over a source of illumination to clearly see the precipitation rings. The external diameters of the rings were measured to the nearest 0.1 mm by using an ocular scale. A reference curve was plotted using the diameters measured from standard solutions. From the reference curve, the IgG concentration of each diluted test sample was calculated by multiplying the concentration read from the curve by the dilution factor to obtain the actual concentration. Intra- and inter-assay variation coefficients were 3.5% and 5.4%, respectively.

The concentrations of immunoglobulins A and M were determined using commercially available Pig IgA and IgM VET-RID kits (Bethyl Laboratories, Inc., Montgomery, Texas). Intra- and inter-assay coefficients of variation were 1.3% and 2.5%, respectively, for IgA and 0.5% and 1.2%, respectively, for IgM.

Statistical analysis

Data were analyzed using the Statistica software package 6.0 (StatSoft® Inc., Tulsa, USA). Results were expressed as mean ± SE. Differences in studied cortisol levels and immunoglobulin parameters in the control and treated groups of boars with LEV or ACTH and the combined treatment by LEV and ACTH were examined using analysis of variance (ANOVA). To evaluate the differences in means between the control and treated groups of boars on specific days of measurement, the *t*-test for independent samples was used. Probability values of 0.05 or less were considered to be statistically significant.

RESULTS

Cortisol levels

The mean serum cortisol response in experimental groups of boars is shown in Table 1. The administration of 10 μg/kg body weight of ACTH in the experimental groups (ACTH and LEV+ACTH) induced an increase in serum cortisol concentration after 90 minutes on all three days of ACTH administration. The administration of saline and LEV had no effect on cortisol concentrations.

In both ACTH treated groups of boars the increase in cortisol concentrations was highly significant as compared with cortisol concentration after saline infusion on each treatment day ($p<0.05$). Also, the concentrations of cortisol were significantly higher in both ACTH treated groups 24 hours after the last ACTH dosage in comparison with control and levamisole treated boars. Thereafter, the concentration of cortisol returned to normal levels on days 11, 14, 18 and 22 after the last drug dosage. There was no significant difference in cortisol concentration between the two groups treated with ACTH.

Serum proteins

The effects of LEV, ACTH and the combined treatment LEV and repeated ACTH administration in the experimental group of boars (ACTH and ACTH+LEV) on serum concentrations of total protein are shown in Table 2. Total protein levels were elevated during the stress period ($p<0.05$) and over the next 16 days ($p<0.05$) in the ACTH group of boars compared to control animals. However, in comparison to the control group total protein levels were elevated in LEV+ACTH group on day 1 and 2 of ACTH injection ($p<0.05$) and day 5 (day 11) and on the last day of follow-up (day 22) ($p<0.05$). There were no differences in total protein levels between LEV+ACTH and LEV group of boars. However, administration of LEV during 3 days induced an increase in protein concentration compared to control values on day 2 (day 5) and on days 11, 15 and 24 (day 14, 18 and 22) after LEV treatment ($p<0.05$).

According to the determined total protein concentrations, globulin concentrations (Table 3) were increased throughout and after the ACTH treatment (ACTH) in relation to the control group of boars ($p<0.05$). Globulin concentrations were elevated in the LEV+ACTH group in comparison to the control group during all 3 days of ACTH treatment and in the next 16 days after the cessation of treatment ($p<0.05$). Higher globulin levels were measured in the ACTH group than in LEV treated animals on day 1 and 2 during treatment (day 4 and 5) and after treatment on day 7 ($p<0.05$). There were no differences in globulin levels between LEV+ACTH and LEV group of boars and also between LEV and control groups of boars ($p>0.09$).

There were no differences in serum levels of albumin (Table 4) among all four groups of boars throughout the study period ($p>0.07$).

Immunoglobulins

Mean serum concentrations of immunoglobulin IgG, IgA and IgM levels during and after LEV and ACTH treatments are shown in Tables 5, 6 and 7. IgG

Table 1. Serum cortisol concentration (mean ± SE; nmol/L) in boars treated with saline (CONTROL, n=7), levamisole (LEV, n=7), ACTH (ACTH, n=7) and levamisole and ACTH (LEV+ACTH, n=7)

	Levamisoletreatment		ACTH treatment			Post-treatment					
	Days										
Groups	1	3	4	5	6	7	11	14	18	22
Control	39.5±2.86	35.9±5.25	43.5±5.12^{ab}	36.2±4.31^{ab}	41.9±4.61^{ab}	45.1±5.99^{ab}	44.9±5.00	33.5±2.20	37.5±4.18	34.6±2.24
ACTH	41.7±4.00	38.3±3.47	426±34.5^{ad}	376±27.4^{ad}	379±39.9^{ad}	63.9±5.34^{ad}	48.3±8.20	43.6±5.28	31.8±3.17	45.1±8.38
LEV	46.6±6.30	40.0±4.62	30.5±5.23^{cd}	37.5±5.65^{cd}	36.4±4.10^{cd}	37.6±4.49^{cd}	36.7±3.66	45.6±6.35	33.7±3.66	39.5±4.56
LEV+ACTH	34.2±3.28	31.6±4.0	393±37.1^{bc}	327±25.5^{bc}	298±27.8^{bc}	52.4±5.43^{bc}	49.2±9.70	37.5±2.88	33.4±5.65	32.9±2.97

Different letters indicate significant differences ($p<0.05$) between treatment groups: ^{a}ACTH and CONTROL, ^{b}LEV+ACTH and CONTROL, ^{c}LEV+ACTH and LEV, ^{d}LEV and ACTH.

Table 2. Total protein concentrations (mean ± SE; g/L) in boars treated with saline (CONTROL, n=7), levamisole (LEV, n=7), ACTH (ACTH, n=7) and levamisole and ACTH (LEV+ACTH, n=7)

	Levamisole treatment		ACTH treatment			Post-treatment				
	Days									
Groups	1	3	4	5	6	7	11	14	18	22
Control	68.2±0.89	69.7±1.38	71.9±1.35^{ab}	69.3±0.72^{abc}	68.5±1.41^{a}	70.2±0.64^{a}	67.4±0.98^{ab}	70.2±1.67^{ac}	70.5±0.86^{ac}	68.4±0.76^{abc}
ACTH	69.3±1.33	71.4±1.52	77.8±1.12^{a}	76.6±1.37^{a}	78.2±1.35^{ad}	76.2±0.56^{a}	78.6±1.04^{ad}	78.7±2.04^{a}	79.8±0.90^{a}	76.0±0.76^{a}
LEV	71.5±1.26	71.7±1.33	72.5±2.26	73.4±0.83^{c}	73.7±1.57^{d}	72.3±1.63	73.2±0.75^{d}	76.3±2.02^{c}	77.3±1.51^{c}	76.6±2.16^{c}
LEV+ ACTH	70.8±1.86	70.4±1.14	77.2±1.29^{b}	74.5±1.63^{b}	73.5±1.53	73.5±1.98	72.4±2.40^{b}	72.9±2.75	74.6±2.88	78.1±2.29^{b}

Different letters indicate significant differences ($p<0.05$) between treatment groups: ^{a}ACTH and CONTROL, ^{b}LEV+ACTH and CONTROL, ^{c}LEV and CONTROL, ^{d}LEV and ACTH

Table 3. Globulin concentrations (mean ± SE; g/L) in boars treated with saline (CONTROL, n=7), levamisole (LEV, n=7), ACTH (ACTH, n=7) and levamisole and ACTH (LEV+ACTH, n=7)

	Levamisole treatment	ACTH treatment	Post-treatment							
	Days									
Groups	1	3	4	5	6	7	11	14	18	22
Control	27.6±1.22	30.4±2.00	28.9±1.80^{ab}	25.7±1.02^{ab}	25.6±0.98^{ab}	27.9±2.44^{ab}	25.8±1.03^{ab}	26.6±1.10^{ab}	26.6±0.84^{ab}	26.9±0.95^{ab}
ACTH	27.0±1.09	31.6±0.59	35.2±0.85^{ac}	35.2±0.94^{ac}	36.4±1.07^{a}	34.6±0.39^{ac}	34.1±0.86^{a}	33.5±0.94^{a}	34.6±0.68^{a}	32.9±0.88^{a}
LEV	28.5±1.09	29.6±1.22	30.3±1.67^{c}	28.1±1.15^{c}	30.1±1.39	28.3±1.22^{c}	30.1±1.54	31.2±2.28	31.1±2.02	31.0±2.49
LEV+ ACTH	29.6±2.06	29.8±1.88	35.8±1.89^{b}	34.1±2.14^{b}	32.5±1.60^{b}	34.7±2.20^{b}	31.7±1.78^{b}	35.3±1.55^{b}	31.3±1.91^{b}	35.7±0.90^{b}

Different letters indicate significant differences ($p<0.05$) between treatment groups: ^{a}ACTH and CONTROL, ^{b}LEV+ACTH and CONTROL, ^{c}LEV and ACTH

Table 4. Serum albumin concentrations (mean ± SE; g/L) in boars treated with saline (CONTROL, n=7), levamisole (LEV, n=7), ACTH (ACTH, n=7) and levamisole and ACTH (LEV+ACTH, n=7)

	Levamisole treatment	ACTH treatment	Post-treatment							
	Days									
Groups	1	3	4	5	6	7	11	14	18	22
Control	40.6±1.18	39.2±1.43	43.1±0.95	43.6±0.62	42.9±0.71	42.4±1.75	41.6±0.49	43.6±0.96	43.8±0.33	41.5±0.85
ACTH	42.3±1.31	39.8±1.35	42.5±1.02	41.3±0.97	41.8±1.44	41.6±0.48	44.5±1.59	45.2±1.52	45.2±0.77	43.1±0.52
LEV	42.9±1.19	42.0±1.18	42.3±1.58	43.8±1.17	42.9±1.24	43.4±1.09	42.8±1.06	43.7±1.58	44.2±0.98	42.8±1.23
LEV+ ACTH	41.2±0.69	40.8±1.16	41.1±0.94	41.6±0.89	41.1±2.12	40.6±1.90	42.6±1.20	41.2±1.25	42.0±1.21	42.4±2.16

Table 5. Imunoblobulin G concentrations (mean ± SE; g/L) in boars treated with saline (CONTROL, n=7), levamisole (LEV, n=7), ACTH (ACTH, n=7) and levamisole and ACTH (LEV+ACTH, n=7)

	Levamisole treatment		ACTH treatment			Post-treatment				
	Days									
Groups	1	3	4	5	6	7	11	14	18	22
Control	20.67±1.02	21.4±0.98	23.06±0.76^{ab}	23.4±1.05^{a}	23.4±0.84^{ab}	24.4±0.90^{ab}	25.7±1.68^{ab}	24.5±2.02^{a}	21.5±0.55^{a}	23.3±1.88^{a}
ACTH	21.1±0.99	21.0±0.89	21.9±1.66^{a}	22.6±1.18^{a}	22.6±1.04^{a}	20.5±1.10^{a}	21.9±0.41^{a}	22.4±1.06^{a}	21.2±1.34^{a}	21.7±1.25^{a}
LEV	19.7±1.56	21.4±1.32	24.8±0.87	25.8±0.74	24.5±1.31	26.7±1.03	25.8±0.98	22.9±0.97	23.5±0.74	24.4±0.69
LEV+ ACTH	19.2±0.55	19.9±0.54	19.4±1.14^{b}	18.8±1.18	18.1±1.18^{b}	19.2±0.85^{b}	18.1±0.73^{b}	17.8±1.13	18.7±1.05	19.1±1.28

Different letters indicate significant differences ($p<0.05$) between treatment groups: ^{a}ACTH and CONTROL and ^{b}LEV+ACTH and CONTROL

Table 6. Imunoblobulin A concentrations (mean ± SE; g/L) in boars treated with saline (CONTROL, n=7), levamisole (LEV, n=7), ACTH (ACTH, n=7) and levamisole and ACTH (LEV+ACTH, n=7)

	Levamisole treatment		ACTH treatment			Post-treatment				
	Days									
Groups	1	3	4	5	6	7	11	14	18	22
Control	0.99±0.072	1.00±0.107	0.96±0.098	0.97±0.052	0.89±0.089	0.92±0.085	1.04±0.099	0.95±0.072	0.97±0.147	1.08±0.119
ACTH	0.93±0.074	1.11±0.128	1.02±0.124	1.09±0.122	1.16±0.139	1.15±0.205	1.38±0.331	1.16±0.131	1.24±0.107	1.36±0.227
LEV	0.97±0.102	0.96±0.088	1.04±0.084	1.24±0.146^{a}	0.94±0.056	1.31±0.164	1.15±0.174	1.37±0.146^{a}	1.14±0.059	1.12±0.075
LEV + ACTH	1.00±0.076	0.91±0.074	0.86±0.072	0.80±0.039^{a}	0.81±0.057	0.93±0.082	1.06±0.178	0.95±0.108^{a}	1.05±0.112	1.00±0.101

^{a} Significant differences ($p<0.05$) between treatment groups LEV+ACTH and LEV

Table 7. Imunoblobulin M concentrations (mean ± SE; g/L) in boars treated with saline (CONTROL, n=7), levamisole (LEV, n=7), ACTH (ACTH, n=7) and levamisole and ACTH (LEV+ACTH, n=7)

	Levamisole treatment		ACTH treatment			Post-treatment					
	Days										
Groups	1	3	4	5	6	7	11	14	18	22
Control	4.56±0.26	5.01±0.25	5.41±0.39	5.45±0.29	5.26±0.45	5.43±0.43	5.18±0.42	4.99±0.47	5.03±0.45	5.56±0.28
ACTH	4.79±0.61	5.24±0.44	4.99±0.41	5.16±0.49	4.99±0.42	5.33±0.19	4.66±0.34	5.23±0.52	5.33±0.43	5.11±0.35
LEV	4.51±0.60	4.78±0.56	4.37±0.65	5.68±0.41	4.81±0.53	4.92±0.54	4.64±0.47	4.96±0.33	4.59±0.18	5.08±0.23
LEV+ACTH	4.61±0.22	5.13±0.29	5.10±0.49	4.68±0.53	5.13±0.53	5.12±0.27	6.21±0.26	5.78±0.34	5.52±0.33	5.77±0.35

concentrations were elevated in the ACTH group during the stress period ($p<0.05$) and over the next 16 days ($p<0.05$). However, IgG levels were not changed in the ACTH group compared to the LEV group of boars ($p>0.09$). In the LEV+ACTH group, IgG levels were elevated on day 1 and 3 of ACTH injection ($p<0.05$) and day 1 (day 7) and day 5 (day 11) after treatment ($p<0.05$). There were no differences in IgG levels between LEV+ACTH and LEV group ($p>0.08$). LEV had no impact on IgG levels compared to the control group ($p>0.08$).

There were no differences in serum IgA and IgM concentrations between treated groups and the control group throughout the study period ($p>0.1$). Also, there were no differences in IgA and IgM levels between ACTH and LEV group of animals ($p>0.09$). Higher IgA concentrations were determined in boars treated with LEV on day 2 ($p<0.05$) and day 14 ($p<0.05$) after administration of LEV compared to animals of LEV+ACTH group.

DISCUSSION

Different stress situations in animals can suppress the non-specific defence mechanism and cause increased susceptibility to disease and infection (Blecha, 1988; Griffin, 1989). In an intensive husbandry system, stressful events for pigs such as weaning, bacterial infections or a new social environment can change the behaviour or physiology, and also decrease the cellular and humoral immune response (Morrow-Tesch *et al*., 1994; Nagy and Fekete, 1999). Also, prenatal stress affects humoral and cellular immune responses of neonates and is able to alter the postnatal response of the immune system to stress (Tuchscherer *et al*., 1998). Protection from stress, or at least reduction of its effects, is significant for specific stages of breeding and reproduction of pigs. LEV has been shown to increase serum antibody titers after immunization, the number of leucocytes, phagocyte activity, expression of cytokines by monocytes/macrophages, lymphocyte proliferation and antitumor responses (Kimball *et al*., 1992; Szeto *et al*., 2000; Holcombe *et al*., 2001; Janjatović *et al*., 2008).

In this study the measured baseline cortisol levels were comparable to previously obtained concentrations in pigs (Becker *et al*., 1985; Hessing *et al*., 1994; Wallgren *et al*., 1994). Adrenocortical response to ACTH administration in this study induced a significant increase in serum cortisol concentration 90 min after application on all 3 days of ACTH treatment as shown previously (Bilandžić *et al*., 2006). Used dose of ACTH has been reported to induce a high cortisol-response in treated animals (Janssens *et al*., 1994; Haussmann *et al*., 2000; Mwanza *et al*., 2000). In the present study, the significant increase in cortisol concentration persisted for 24 hours after the last ACTH dose as compared with the control group and levamisole treated animals.

Results obtained in this study have shown increased protein and globulin concentrations in both treated groups of boars during ACTH administration and during the two week period following completion of treatments. Previous studies had shown that the biosynthesis of immunoglobulins in lymphocytes depends on the duration of the stress situation and on the individual perception of stress in pigs (Hessing *et al*., 1995; Tuchscherer *et al*., 1998). Acute stress does not cause

changes in the concentrations of total protein, globulin, albumin and IgG in boars (Hicks *et al*., 1998). However, in chronic stress situations, increased total protein concentrations were determined in sheep (Antunović *et al*., 2002) and pigs (Tuchscherer *et al*., 1998; De Groot *et al*., 2001). Chronic lice irritation in pigs was followed by an increase in total protein serum, globulin and albumin (Davis and Williams, 1986). Stress caused by transfer stimulates a lower proliferation of lymphocytes and synthesis of IgM, cytokine IL-10 and interferon γ in boars than in sows (De Groot *et al*., 2001). Also, mixing of animals from various litters caused an increase in total protein and IgG concentrations in the dominant pigs in the group (Tuchscherer *et al*., 1998; De Groot *et al*., 2001). In pigs exposed to prenatal stress, immunoglobulin concentrations were reduced in the postnatal period (Otten *et al*., 2000). It was shown that increased cortisol excretion causes changes in the effects of macrophages, neutrophils, lymphocytes, and other immune system components. Changes in the lymphocyte subpopulations were observed, including changes in the biosynthesis of cytokines of helper T-cells (a type of T-lymphocyte) T_h1 or T_h2 (Yang and Glaser, 2002). Increases in the number of T_h2 cells were observed. These induce the activity of B-lymphocytes and production of immunoglobulin through biosynthesis of cytokines (IL-4, IL-5, IL-6) (DeKruyff *et al*., 1998; Yang and Glaser, 2002).

In this study, LEV was applied to investigate the effects of immunomodulating substances on the immune system and physiological processes in pigs exposed to 3 days of stress. Previous studies have shown varying effects of LEV on immune cells (Symoens and Rosenthal, 1977; Van Wauwe and Janssen, 1991). When applied in doses for prevention and treatment of parasites in pigs, it has an immunosuppressive effect by reducing cell immune response, i.e. migration of leukocytes (Symoens and Rosenthal, 1977; Blecha, 1988; Jenkins and Hurdle, 1989). However, a stimulation of the immune response, increase of leukocyte maturation and lymphocyte differentiation is achieved with a dose of 2 to 3 mg/kg BW (Renoux and Renoux, 1974). LEV affects the immune response more efficiently when applied at regular intervals, and its administration is recommended during 3 consecutive days or as one weekly dose (Brunner and Muscoplat, 1980). Administration of LEV prior to stress induction in the previous study resulted in a balanced ratio of neutrophyls and lymphocytes in the blood of boars (Bilandžić *et al*., 2005).

In the present study, administration of LEV in a dose of 2.5 mg/kg BW in boars stimulated an increase in total protein concentrations in the serum of boars by 9.6% and IgG concentrations by 13.8 to 24.6% compared to control animals, while IgA and IgM concentrations were unchanged. Administration of 6 mg/kg BW LEV reduced the level of antibodies in herpes virus infection in cows (Blecha, 1988). However, where cows were exposed to stress during transport, LEV increased the concentration of antibodies. At a dose of 2.5 mg/kg BW, LEV increased IgM concentrations in rats aged up to 10 days (Hunter *et al*., 1981). Administration of the same dose in mares in the final stage of gravidity increased the concentrations of total protein, IgG and IgM in the colostrum (Flesh *et al*., 1982; Karakowski *et al*., 1999). In the serum of foals of treated females, a

significant IgG concentration increase was observed compared to foals of mothers that had not received LEV (Karakowski *et al*., 1999).

In this study, administration of LEV in boars for 3 days prior to ACTH induction reduced total protein levels and values were similar to those in boars receiving only LEV. The applied treatment with LEV and ACTH had no impact on albumin concentrations and was in compliance with control concentrations. However, globulin concentrations were increased, which may be explained by increased globulin concentrations caused by ACTH effects. Accordingly, IgG concentrations were significantly increased during and two weeks after the treatment with ACTH in both groups of boars. LEV used before ACTH administration stimulated the reduction in IgG concentrations particularly in the week after stress, which confirmed its impact on balancing the stress-induced immune response. During stress, LEV had no impact on albumin, IgA and IgM concentrations.

LEV acts primarily on T-cells (Symoens and Rosenthal, 1977). It may act directly or indirectly on B-cells by affecting T-lymphocytes and macrophages, which in turn activate B-cells and immunoglobulin synthesis through specific cytokine synthesis (Kühlmann-Rabens *et al*., 1987; Jenkins and Hurdle, 1989). One of the possible mechanisms of the impact of LEV on the immune system is an increase in the synthesis of cytokine IL-1 in macrophages as determined in *in vitro* research on mice macrophages (Kimball *et al*., 1991; Szeto *et al*., 2000). IL-1 is the main agent in T-lymphocyte activities and in the synthesis of cytokine IL-2 and IL-6 which stimulate the activity of B-cells. LEV reduces gene expression for cytokine IL-4 (T_h2 cells) in rats and induces the activity of T_h1 cells (Szeto *et al*., 2000). It acts selectively on the increase of synthesis of cytokine IL-18 in macrophages by inducing biosynthesis of INF-γ in T_h1 cells, which then stimulate the activity of B-lymphocytes. Recent investigations have suggested that LEV may act as an adjuvant for preventive vaccines and stimulate both humoural and cell-mediated immune responses (Božić *et al*., 2002; Jin *et al*., 2004; Božić *et al*., 2006).

In conclusion, the presented results indicate that administration of LEV may have a practical application regarding its impact on immune response. LEV stimulates the production of immunoglobulins in boars exposed to stress. Application of LEV in investigated concentrations may protect boars from the negative influence of stress and provoke improved non-specific immunity. Protection of stress could also reduce economical losses in pig production.

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Adress for correspondence:
Dr. sc. Nina Bilandžić
Laboratory for Residue Control
Department for Veterinary Public Health
Croatian Veterinary Institute
Savska 143, Zagreb
Croatia
E-mail: bilandzic@veinst.hr

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PROMJENE KONCENTRACIJE IMUNOGLOBULINA U NERASTOVA PRIMJENOM LEVAMISOLA I EGZOGENOG ADRENOKORTIKOTROPNOG HORMONA

BILANDŽIĆ NINA, TERZIĆ SVJETLANA i ŠIMIĆ B

SADRŽAJ

Cilj ovog istraživanja bio je utvrditi utjecaj levamisola (LEV) na koncentraciju imunoglobulina u serumu nerastova izloženih tri dana stresu administracijom ACTH. Nerastovi su podijeljeni u 4 skupine (n=7). Prva eksperimentalna grupa primala je LEV kroz 3 dana (2,5 mg/kg tj. mase), druga grupa tretirana je s ACTH (10 μg/kg tj. mase) tijekom 3 dana, a treća grupa kombinacijom LEV (2,5 mg/kg tj. mase) kroz 3 dana i zatim s ACTH (10 μg/kg tj. mase) 3 sljedeća dana (LEV+ ACTH). Kontrolna grupa primala je fiziološku otopinu tijekom 6 dana. Tijekom tretmana i 16 dana nakon tretmana određivane su koncentracije kortizola, ukupnih proteina, albumina i globulina, te imunoglobulina (IgG, IgA i IgM).

Koncentracija kortizola je bila povećana u obje grupe tretirane s ACTH tijekom sva tri dana tretmana ($p<0,05$) te dan nakon posljednje primjene ACTH ($p<0,05$). Primjena ACTH utjecala je na povećanje ukupnih proteina tijekom primjene ACTH te tijekom 16 dana nakon tretmana ($p<0,05$). Međutim, u grupi nerastova tretiranih s LEV+ACTH ukupni proteini povišeni su samo na dane 1 i 2 primjene ACTH ($p<0,05$) te nakon završetka tretmana na dane 11 i 22 ($p<0,05$). Primjena LEV stimulirala je povećanje koncentracije proteina u odnosu na kontrolne nerastove nakon tretmana na dane 5, 14, 18 i 22 ($p<0,05$, svi). Primjena LEV i ACTH nije utjecala na koncentraciju albumina.

Koncentracija globulina je povećana tijekom tretmana te 16 dana nakon primjene u nerastona tretiranih s ACTH i LEV+ACTH ($p<0,05$, svi). Koncentracija globulina se nije razlikovala između LEV i kontrolne grupe nerastova. Primjena ACTH utjecala je na povećanje razina IgG tijekom administracije ACTH te tijekom 16 dana nakon tretmana ($p<0,05$, svi). Međutim, u grupi životinja LEV+ACTH koncentracija IgG je bila povišena tijekom 1. i 3. dana tretmana s ACTH ($p<0,05$), te na dane 1 i 5 u periodu nakon tretmana ($p<0,05$). LEV nije imao utjecaj na koncentracije IgG u odnosu na kontrolu grupu nerastova. Povećane koncentracije IgA određene su na dane 2 i 11 ($p<0,05$) nakon primjene LEV u odnosu na životinje LEV+ACTH grupe. Rezultati ukazuju da primjena LEV može imati svrhu prevencije negativnog utjecaja stresa te inducuranja boljeg odgovora nespecifične imunosti u svinja.