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Plasma cortisol levels in gilts treated with Parapoxvirus ovis - based immunostimulator in the late phase of gravidity

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ABSTRACT

The goal of these investigations was to check if two-time treatment of gilts by Baypamun® before transferring from the pre-farrowing to farrowing unit can activate natural killer cells, stimulate leukocyte production and lymphocyte proliferation, and decrease the rise in plasma cortisol caused by stress. The investigation comprised 30 one-year-old Swedish Landrace gilts in the late phase of gravidity, which were divided into three groups. Group 1 formed a control group, from blood which was taken in the the pre-farrowing unit in order to enable comparison of the results of plasma cortisol with results obtained in the other two groups. In group 2, Baypamun in a dose of 2 ml i/m at days 7 and 5 was applied, and in group 3 at days 3 and 1 prior to transferral from the pre-farrowing to farrowing unit. After gilts in groups 2 and 3 had been transferred four days prior to farrowing, blood was taken from them at days 1, 3 and 5 of their stay in the farrowing unit. Plasma cortisol was analyzed by the radioimmunochemical (RIA) method. The obtained results by groups were compared with each other using one-way analysis of variance. According to the obtained results an average level of plasma cortisol of the gilts in groups 2 and 3 throughout the research was higher than in control group 1. A significant statistical difference ($P < 0.05$) appeared between control group 1 and group 3 at day 1 of the stay of gilts in the farrowing unit. Therefore, an anti-stress effect of Baypamun occurred between days 1 and 3 of the stay in the farrowing unit, i.e. by the day of farrowing, when the level of plasma cortisol again began to rise due to stress caused by partus.

Key words: gravid gilts, Baypamun, plasma cortisol, stress, farrowing unit

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Introduction

In intensive pig-breeding it is the practice for sows in the farrowing period and during lactation to be housed in farrowing crates. Such a system, which is widely accepted around the world, decreases mortality caused by piglets being crushed (CRONIN et al., 1991), but greatly reduces the living space of sows, causing stress, which is a reaction of the organism to harmful environmental conditions (DANTZER, 1988; TOPEL and CHRISTIAN, 1986). In veterinary medicine, stress is the final stage of an animal's physiological reaction to the detrimental effects of its environment and production processes (FRASER, 1975). In stock-breeding, the word stress is used to describe states which lead to losses in production (DANTZER and MORMÉDE, 1983). In other words, the occurrence of stress before and during farrowing contributes to problems in production, because stress hormones may influence the action of reproductive hormones (HANSEN and CURTIS, 1981), may prolong gravidity, as well as an increased percentage of still-born piglets (BAXTER and PETHERICK, 1980). Corticosteroids are just one group among the many hormones increasingly secreted as a result of the stress syndrome (ROTH, 1985). Of these hormones, cortisol has the highest degree of activity (RIJNBERK and MOL, 1989) and therefore its level in blood plasma is used as one of the biochemical indicators in pigs (CRONIN et al., 1991; LAWRENCE et al., 1994; BLACKSHAW et al., 1994; PERREMANS et al., 1986). To date, it has been proved that an increased level of corticosteroids in blood retards proliferation of lymphocytes (KELLEY, 1988), decreases the quantity of produced antibodies and the size of lymph nodes (MARTIN, 1987) and decreases the ability of the pig to resist infection (ENGLISH and EDWARDS, 1992). Therefore, the possibilities for reducing stress factors are presently being widely investigated, where immunomodulator Baypamun has been proved effective in immunostimulation as well as in improving reproduction and production performances of swine (CUTLER and PRIME, 1988; STEINMASL and WOLF, 1990; VALPOTIĆ et al., 1993; KYRIAKIS et al., 1996; KRSNIK et al., 1999). One of the most sensitive phases in the technology of industrial pig-breeding, i.e. the transfer of highly-gravid gilts from the pre-farrowing to farrowing unit, is followed by high risk of acute stress and other consequences which may affect the immunocompetence not only of the gilts themselves but also of their young. Normal farrowing and behaviour

of gilts during suckling may also be affected. The aim of this research was to discover whether Baypamun immunostimulator could alleviate the consequences of stress of gilts after transfer from the pre-farrowing to the farrowing unit and if, as an activator of natural killer cells, it could stimulate leukocyte production and lymphocyte proliferation, decrease the rise of plasma cortisol, as well as maintaining its level within normal values.

Materials and methods

These preliminary analyses comprised three groups of Swedish Landrace gravid gilts, 365±15 days old. The criteria for selection of gilts for mating, in addition to age, was a lowest body mass of 85 kg, phenotypic characteristics related to breed, and a thickness of back bacon of 20 mm. Protective vaccination against erysipelas, atrophic rhinitis, Aujeszky´s disease, parvovirosis, porcine reproductive and respiratory syndrome, was performed on all gilts which were 110-190 days old. Vaccination of gilts against neonatal colibacillosis and clostridial enterotoxaemia was performed on day 90 of gravidity.

Each selected group of animals comprised 10 gravid gilts. Group 1 was a control group. Blood was taken during the stay in pre-farrowing conditions in order to compare the obtained results of plasma cortisol with the results of the other groups. Two ml of Baypamun® ($10^{6.75}$ $TCID_{50}$ inactivated stock 1701 D of Parapoxvirus ovis; Bayer, Germany) was applied i/m in group 2 at days 7 and 5, as well as in group 3 at days 3 and 1, prior to transfer from the pre-farrowing to farrowing unit. In both groups of gilts Baypamun was applied in the early morning, immediately after the animals had been fed (8:30 to 9:00 h).

Gilts were fed meals containing per kg: 12.5 MJ of digestive energy, 16% raw proteins, 1% calcium and 0.6% total phosphor. Daily amount of food per gilt was 1.6 kg up to the day that gravidity was diagnosed, 1.8 kg up to day 60 of gravidity, 2.4 kg up to day 80 of gravidity, and 3.0 kg up to day 110 of gravidity. The daily amount of food was gradually decreased from day 111 of gravidity until farrowing.

On day 4 prior to expected farrowing, all groups of gravid gilts moved, on their own, through a 15 m-long hall connecting the pre-farrowing unit

with the farrowing unit and were transferred to single farrowing crates with clutches and slatted floors. Prior to that the animals had been kept for 70 days in common pre-farrowing conditions (16 m^2), which are suitable for housing 6 gilts. The length of a single farrowing crate was 2.15 m, and with a width of 1.65 m. In the farrowing crate, the width of clutches for the sow was 0.65 m, the left part for piglets was 0.40 m, and the right part for piglets was 0.60 m.

Blood was taken from group 1 gilts immediately before transfer from the pre-farrowing to farrowing unit, i.e. at 10 A.M. Blood was taken from gilts in groups 2 and 3 on days 1, 3 and 5 of their stay in the farrowing unit. On day 1, blood was taken at 10.30 A.M. after the gilts had been transferred from the pre-farrowing to farrowing unit. On days 3 and 5 of the stay in the farrowing unit, blood was taken at 8.30 A.M., after the gilts had been fed. Before taking blood, each gilt was fettered with a wire through the snout. Blood was centrifuged at 3500 rpm for 10 minutes. After centrifugation, plasma was pipetted in sterile bottles and frozen at –20 ^{0}C. Plasma cortisol was determined by the radioimmunochemical (RIA) method (HASLER et al., 1976) with reagents from the Department of Immunology, Zagreb, in the laboratory of the Department of Endocrinology and Metabolic Diseases, Clinic for Internal Diseases of the Rebro Hospital, Zagreb. Impreciseness in the determination was 10.1%, at a concentration of 256±26 nmol/L. Impreciseness between determinations was 11.6%, at a concentration of 180±21 nmol/L, and 10.9% at a concentration of 492±54 nmol/L. Limit of detection was 27 nmol/L.

The obtained data for the level of plasma cortisol in the groups were compared using one-way analysis of variance.

Results

Comparing the results of control group 1 and the other two groups for level of plasma cortisol at day 1 of stay in the farrowing unit (Table 1), it is found that a significant statistical difference ($P < 0.05$) occurred between control group 1 and group 3, which was treated by Baypamun preparation at days 1 and 3 prior to transfer from the pre-farrowing to farrowing unit. According to the average results, the highest concentration of plasma

Table 1. One-way analysis of variance of the level of plasma cortisol (mean ± sd nmol/L) of gilts* before (day 0) and after (days 1, 3 and 5) of transfer from the pre-farrowing to farrowing unit.

Treatment	Day following transfer of gilts from pre-farrowing to farrowing unit			
Day prior to transfer	0	1	3	5
Control group	137.1±69.2[a]	-**	-**	-**
Baypamun® (-7/-5)***	-**	185±72.1[a,b]	194±86[ab]	223.1±134
Baypamun® (-3/-1)***	-**	295.3±123.7[b]	212±73.6[a,b]	215±98.4

* Each group comprised 10 gilts;
** not tested;
*** days 7 and 5, i. e. 3 and 1 before transfer of gilts from the pre-farrowing to farrowing unit when Baypamun was applied
[a,b] arithmetic means in the same row or column which do not share the same letters in superscript have statistical difference $P < 0.05$

cortisol (295.3 nmol/L) was found in group 3, and the lowest (137.10 nmol/L) in control group 1. It is also the lowest average level of plasma cortisol found during the research (Tables 1 and 2).

Comparing the results of level of plasma cortisol of control group 1 with the other two groups during days 3 and 5 of stay in the farrowing unit, no significant statistical difference was found. During day 3 of stay in the farrowing unit the highest level of plasma cortisol (212 nmol/L) was found in group 3, and during day 5 (223.10 nmol/L) was found in group 2.

Table 2. One-way analysis of variance of groups 2 and 3 for level of plasma cortisol during days 1, 3 and 5 of gilts' stay in the farrowing unit

Groups				
	2		**3**	
day	M	sd	M	sd
1	185.0	72.1	295.3	123.7
3	194.0	86.0	212.0	73.6
5	223.1	134.0	215.0	98.4

Comparing the level of plasma cortisol of the two groups of gilts that were transferred from the pre-farrowing to farrowing unit (groups 2 and 3), it can be concluded that none of the comparisons has shown a significant statistical difference (Table 2). Bearing in mind the level of plasma cortisol

in groups 2 and 3, according to the days of research it appears that between days 1 and 3 Baypamun reduced the average level of plasma cortisol, whereupon it started to rise again due to the stress caused by partus in the groups treated by Baypamun (except group 3 at day 1).

Discussion

Baypamun preparation is an inducer of paraimmunity, comprising parapoxivirus isolated in sheep affected by pustulous dermatitis, inactivated in the described way (BÜTTNER et al., 1987; STRUBE et al., 1989). Its effectiveness in reduction of stress and stimulation of non-specific immunity in pigs has been the subject of several investigations (CUTLER and PRIME, 1988; STEINMASL and WOLF, 1990; VALPOTIĆ et al., 1993; KYRIAKIS et al., 1996). Namely, it was found that the preparation was effective in prevention or control of infections and alleviation of the effects of stress factors, through the mechanism of direct antigenic stimulation of nonspecific immunoreaction of an animal, which is established within several hours after application and which maintains for from 8 to 12 days (MAYR and BÜTTNER, 1984).

Presently, it is understood that application of Baypamun may increase passively obtained immunity in new-born piglets after non-specific immunization of gravid sows and may reduce losses caused by the gastrointestinal syndrome during the perinatal period (VALPOTIĆ et al., 1993). The same authors prove a significantly higher level of total proteins and immunoglobulin in the serum and colostrum of gilts and their piglets treated by Baypamun. Accordingly, they clarify the standpoint that Baypamun enhances the humoral immunity of gilts and increases their lacteal immunity, which was investigated earlier in gilts at farrowing (GREGORKO et al., 1989). Baypamun served in the investigation of the reproductive performance of the transported gilts, where it was concluded that three-time immunostimulation of gilts during transport can enhance their reproductive performance, in terms of live-born piglets and average mass of piglets at farrowing (KYRIAKIS et al., 1996). A similar investigation with Baypamun proved that two-time immunostimulation of gravid gilts before transfer from the pre-farrowing to farrowing unit may alleviate stress factors

and thus influence a decrease in still-born piglets in the litter (KRSNIK et al., 1999).

Bearing the foregoing in mind, i.e. that transfer from one unit to another causes stress in the pregnant sow, we attempted to discover whether two-time treatment of gilts with Baypamun in different time periods could induce non-specific immunity and thus indirectly influence the immunosuppressive effect of cortisol, and its level in plasma.

All gilts used in these investigations were completely included in the production technology of the farm, which means that they were protected against the severe specific disease-causers by vaccination. However, such a scheme did not protect the gilts from stress factors, which may negatively influence the production performance of the animals. Blood was taken from all gilts at about the same time during the forenoon (taking in account the limits set by production technology) because it is known that secretion of corticosteroids is not uniform during the day and night (RIJNBERK and MOL, 1989). Where no irregular stress situation exists, the strongest secretion of cortisol is in men, in most domestic animals, including pigs, it occurs at the end of the night and at the beginning of the day. The lowest secretion occurs in the late afternoon and during the night (BOTTOMS et al., 1972; FAVRE and MOATTI, 1977; RIJNBERK and MOL, 1989).

On the basis of the obtained results, it may be concluded that an average level of plasma cortisol in animals in groups 2 and 3 on all the three days of the investigation was higher than in control group 1, which accords with the investigations of CRONIN et al. (1991), where the level of plasma cortisol increased when the gilts were transferred from the pre-farrowing to farrowing unit. Having in mind investigations of the normal level of plasma cortisol in conscious pigs, which, determined by the RIA method, was 49.7 – 218 nmol/L (HANNON et al., 1990), we are able to state that the average level of plasma cortisol in the group of gilts treated by Baypamun preparation was within the mentioned limits, except in group 2 at day 5 (223 nmol/L) and group 3 (241.3 nmol/L) at day 1 of the stay in the farrowing unit. In the groups of gilts treated by Baypamun preparation during days 1 and 3 of stay in the farrowing unit, the average level of plasma cortisol was lower compared to day 5. Therefore, Baypamun may have indirectly affected the decrease in the average level of plasma cortisol

in the treated groups of gilts up to farrowing, through induced non-specific immunity. A small increase in the average level of plasma cortisol in the groups of gilts treated by Baypamun preparation during day 5, compared to day 3 of investigation is attributed to farrowing, which occurred on day 4 of the gilts' stay in the farrowing unit. Therefore, oscillations of the average level of plasma cortisol when comparing the groups treated by Baypamun preparation are somewhat different, which certainly depended on the individual ability of each the gilt in the group to resist stress caused by farrowing. Further investigations on the indirect influence of Baypamun preparation to plasma cortisol during repeated applications are to be performed on a greater number of gilts immediately before farrowing, because of the possible influence of decreasing plasma cortisol after farrowing.

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SAŽETAK

Cilj ovih istraživanja bio je provjeriti može li dvokratno tretiranje nazimica Baypamunom prije premještanja iz čekališta u prasilište aktivirati prirodne stanice ubojice, potaknuti stvaranje leukocita i proliferaciju limfocita te sniziti porast kortizola uzrokovan stresom. Istraživanje je provedeno na 30 jednogodišnjih nazimica pasmine švedski landras u kasnoj fazi gravidnosti podijeljenih u tri skupine. Prva skupina bila je kontrolna. Drugoj skupini nazimica dano je 2 ml Baypamuna im. 7. i 5. dana prije preseljenja iz čekališta u prasilište, a trećoj skupini 3. i 1. dana prije preseljenja. Nakon što su nazimice iz druge i treće skupine preseljene, njima je prvog, trećeg i petog dana boravka u prasilištu izvađena krv. Plazmatični kortizol je određivan radioimunokemijskom (RIA) metodom. Dobiveni podatci međusobno su uspoređeni jednosmjernom analizom varijance. Prosječna razina plazmatičnog kortizola nazimica u drugoj i trećoj skupini bila je veća nego u kontrolnoj skupini. Pri tome je značajne statistička razlika ($P < 0.05$) ustanovljena prvog dana boravka u prasilištu između kontrolne i treće skupine. Prema dobivenim rezultatima protustresni učinak Baypamuna očitovao se 1. i 3. dana boravka u prasilištu.

Ključne riječi: gravidne nazimice, Baypamun, plazmatski kortizol, stres, prasilište