

Plasma cortisol concentrations in gilts treated with immunomodulator during early gravidity

Dalibor Potočnjak^{1*}, Željko Pavičić², Ljiljana Bedrica¹, Boris Krsnik², Anamaria Ekert Kabalin³, Marin Torti¹, and Damir Žubčić¹

¹*Clinic of Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, Croatia*

²*Department of Animal Environment and Ethology, Faculty of Veterinary Medicine, University of Zagreb, Croatia*

³*Department for Animal Husbandry, Faculty of Veterinary Medicine, University of Zagreb, Croatia*

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ABSTRACT

The goal of this research was to ascertain if multiple immunomodulation can decrease stress consequences of gilt relocation from the service station to the prefarrowing unit during the early gravidity phase. Three groups of pregnant Swedish Landrace gilts, 8 to 9 months of age, were encompassed by the research. Each group consisted of 30 pregnant gilts, and each researched group was treated differently. The animals in group A were given the immunomodulator intramuscularly in doses of 2 ml (Baypamun®, Bayer Pharma, Leverkusen, Germany) on the 6th, 4th and 2nd days before the relocation from the service station to the prefarrowing unit. It was also administered to the animals in group B intramuscularly in doses of 2 ml on the 5th, 3rd and 1st days before the relocation from the service station to the prefarrowing unit. Group C was not treated. Blood was sampled from the aforementioned gilts on the 2nd, 4th and 6th days, during their stay at the prefarrowing unit. During the research period statistically significant differences in plasma cortisol concentration were established ($P < 0.01$) between the treated (groups A and B) and not-treated groups (group C). The mean plasma cortisol concentration was the lowest in group A, whereat values determined on the 4th and 6th days of stay in the prefarrowing unit were significantly lower in comparison to values in group B ($P < 0.01$). The mean plasma cortisol concentration in group C was higher than the reference values for pigs. In all three gilt groups an increase in plasma cortisol concentration was noted during their stay in the prefarrowing unit, so that mean values on day 6 were significantly lower ($P < 0.01$) in comparison to values determined on the 2nd day of stay in the prefarrowing unit. The aforementioned results demonstrate that immunomodulation alleviates hormonal changes that occur as a response to a stressful situation and refer to the positive effects of immunomodulator after the reduction of stress susceptibility.

Key words: plasma cortisol, gilts, gravidity, immunomodulation, stress

*Contact address:

Assist. Prof. Dr. Dalibor Potočnjak, Clinic of Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10 000 Zagreb, Croatia, Phone: +385 1 2390 347; Fax: + 385 1 2441 390; E-mail: dalibor.potocnjak@vef.hr

Introduction

Gilts go through different and unfamiliar stages under the influence of farm technology: changes in keeping, feeding, formation of new groups, first insemination, gravidity, labour, and raising offspring. Organism exertion is increased so those factors can act stressfully upon gilts. In pig farming the presence of stress has numerous and various negative consequences: loss of body mass and slow growth, disorders in reproduction, excitement and disturbance of animals. Today a great role is attributed to stress as one of the factors connected with weakening of the immune system and its potential role in the aetiology of many diseases (KELLEY and DANTZER, 1990; SANTORO, 1996). In regard to stress sensitivity of various domestic animals, pigs are the most sensitive and they adapt with difficulty to ambient changes. Stress does not always result in disease development, but more commonly causes a decrease in the animal's productivity and resistance to various diseases (COLES, 1986). Under the influence of stress animals' organisms (especially in pigs) are more susceptible to conditional infectious diseases, parasitic invasions, reproductive disorders, certain gastrointestinal disorders (stress causes decrease in secretory and motoric functions of the gastrointestinal tract), cardiovascular disorders and various metabolic disorders (FORENBACHER, 1983; RADOSTITS et al., 2000).

Stressful situations in pregnant animals can lead to changes in homeostasis which affect reproductive results, that is of breeding. Continuous recurrence of stressful situations can lead to chronic stress and can also result in the end of the reproductive cycle of very sensitive animals. Those animals often express fear as a reflex to traumatic experiences, even at the most benign, which occur throughout their life (SANTORO, 1996). A substantial role in the process of suppression of stressors is attributed to the harmful effects of metabolism regulation corticosteroids. They facilitate emergence of new energy sources and combat inflammatory and allergic processes in the organism. The main glucocorticoid in pigs is cortisol. Cortisol has a very high level of activity which makes plasma cortisol concentration one of the crucial biochemical indicators in pigs (CRONIN et al., 1991; LAWRENCE et al., 1994; PERREMANS et al., 1996).

Modifiers of immune response increase the non-specific resistance of the organism to tumours, bacterial, viral and fungal infections. Non-specific immunomodulation can potentially be applied during the neonatal period and periods of immunosuppression caused by stress, viruses or bacteria (BLECHA and CHARLEY, 1990). Application of certain immunomodulators is effective in immunostimulation and improvement of reproductive results in pigs (VALPOTIĆ et al., 1993; KYRIAKIS et al., 1998; KRŠNIK et al., 1999; SARATSIS et al., 1999; PAVIČIĆ et al., 2003; PAVIČIĆ et al., 2004; POTOČNJAK et al., 2006).

In intensive industrial pig farming ambient changes during the technological process are inevitable. One of the most sensitive phases in such a process is the relocation of gilts in early gravidity from the service station to the prefarrowing unit. Such relocation can

be associated with moderate to severe risk of acute stress which has a negative effect on a gilt's immune system and reproductive results.

The goal of this research, in regard to various immunomodulation treatments of various groups of gilts, was to ascertain if stress consequences associated with relocation to a new ambient can be ameliorated with the use of immunomodulators. Results of this research should make it possible to introduce new possibilities of stress reduction and prevention and to increase economic results in intensive pig farming and animal welfare, because only healthy animals which feel good have good reproductive results.

Materials and methods

This research encompassed three groups of pregnant Swedish Landrace gilts, 8 to 9 months of age. The researched animals were completely included in the productive and technologic processes on farm with all the other animals. Between 110 and 190 days of age (according to the technologic process on the farm) all gilts were intramuscularly vaccinated against swine erysipelas, leptospirosis, atrophic rhinitis, Aujeszky's disease, parvovirus, piglet influenza, and piglet respiratory and reproductive syndrome (PRRS). The animals were also vaccinated against neonatal colibacillosis and clostridial enterotoxaemia on day 90 of gestation.

Selection criteria of gilts for rearing, besides age, were minimal body mass of 85 kilos, back bacon thickness of 20 mm, phenotype characteristics related to the breed, number of mammary glands and their quality. Gilts that fulfilled the aforementioned criteria, were relocated to the service station where they were artificially inseminated by semen of hogs of the appropriate breed. The gilts stayed in the service station for 35 to 40 days. During their stay gravidity was diagnosed by ultrasound examinations. Those gilts that had not conceived were inseminated one more time, and if they did not conceive again they were excluded. After gravidity had been diagnosed, gilts were relocated in groups of 6 to 7 animals to boxes (16 m²) in the prefarrowing unit, where they stayed for 70 days. 3 to 5 days before labour pregnant gilts were transferred to the farrowing unit.

Each group consisted of 30 pregnant gilts, and each group was treated differently. The animals in group A were given the immunodulator in doses of 2 ml (Baypamun®, Bayer Pharma, Leverkusen, Germany) on the 6th, 4th and 2nd days before their relocation from the service station to the prefarrowing unit. The animals in group B were given the immunomodulator in same doses on the 5th, 3rd and 1st days before their relocation from the service station to the prefarrowing unit. Baypamun® (containing 10^{6.75} TCID₅₀ of inactivated strain D 1701 of Parapoxovirus virus) was administered intramuscularly. Animals in group C were not treated with immunomodulator before the relocation from the service station to the prefarrowing unit.

Blood was sampled from all gilts on the 2nd, 4th and 6th days of their stay in the prefarrowing unit, between 9 and 9.30 a.m. Before taking blood, each gilt was fettered with a wire through the snout. Blood was sampled from the cranial vena cava. After collection blood samples were centrifuged on 3500 rpm during 10 minutes. After centrifugation, plasma was pipetted to sterile vials and frozen at -20 °C.

Plasma cortisol concentration was determined by using the radioimmunoassay method (RIA) with reagents from the Department of Immunology and in the laboratory of the Department of Endocrinology and Metabolic Diseases, Clinic for Internal Diseases of the Rebro Hospital in Zagreb, Croatia. Impreciseness in the determination was 10.1%, at a concentration of 256 ± 26 nmol/L. The impreciseness between determinations were 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.

Statistical analysis was performed with the help of Statistica 7.1 program (StatSoft Inc., 2005). The spread was ascertained with the Kolmogorov-Smirnov test. The significance of differences in plasma cortisol concentration was determined with one-way variance analysis. Variance analysis with repeated measurements was used to determine the significance between plasma cortisol concentrations within the groups (ANOVA Repeated Measures, Tukey HSD test).

Results

Mean plasma cortisol concentrations in selected gilt groups determined in the course of research are shown in Table 1.

It is visible from Table 1 that during the research period statistically significant differences ($P < 0.01$) in plasma cortisol concentration were observed between the treated (groups A and B) and non-treated (group C) groups of gilts. Mean plasma cortisol concentrations were the lowest in group A, whereas values on the 4th and 6th days of the stay in the prefarrowing unit were significantly lower than the same values in group B ($P < 0.01$). In group C, mean cortisol concentration was above the reference range for pigs (49.7-218 nmol/L), whilst mean cortisol concentrations in groups A and B were within the reference range.

In all three gilt groups a decrease of plasma cortisol concentration was noted during their stay in the prefarrowing unit. The mean plasma cortisol concentrations determined on the 6th day of their stay in the prefarrowing unit were significantly lower ($P < 0.01$) than those determined on the 2nd day of their stay in the prefarrowing unit (Fig. 1). Despite the decrease of plasma cortisol concentration in relation to elapsed time from the stressful event, the plasma cortisol concentration in group C was above the reference range during their stay in the prefarrowing unit.

Table 1. Plasma cortisol concentration in gilts after relocation from service station to prefarrowing unit (n = 30)

Group	Cortisol concentration (nmol/L)		
	Day 2	Day 4	Day 6
	Mean ± SD	Mean ± SD	Mean ± SD
A	190.57 ^x ± 30.59	184.57 ^x ± 21.80	176.77 ^x ± 21.39
B	207.23 ^y ± 17.29	206.37 ^x ± 12.21	194.73 ^x ± 10.35
C	251.50 ^{x,y} ± 36.86	242.10 ^x ± 36.17	219.00 ^x ± 29.16

The same letters (x and y) within columns indicate statistically significant differences (P<0.01).

^{a,b} within selected groups labeled with same letter statistically significant differences were determined (P<0.01)

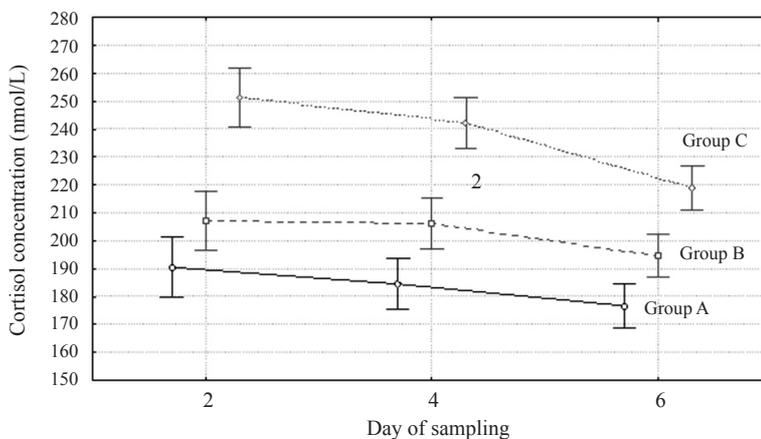


Fig. 1. Plasma cortisol concentration variations on days 2, 4 and 6 during the stay of gilts in the prefarrowing unit

Discussion

As late as 1983 it was officially established that immune response modifiers reduce corticosteroid secretion in rats under stress (BIEDERMANN, 1983). The efficacy of immune response modifier Baypamun in immune response stimulation and stress reduction in pigs was researched and described in several scientific papers (VALPOTIĆ et al., 1993; KYRIAKIS et al., 1998; KRSNIK et al., 1999; SARATSIŠ et al., 1999; PAVIČIĆ et al., 2001; PAVIČIĆ et al., 2003; PAVIČIĆ et al., 2004; POTOČNJAK et al., 2006). Baypamun can increase passive immunity in new-born piglets after immunization of pregnant sows and can reduce losses caused by gastrointestinal syndrome. Significantly high concentrations of total proteins and immunoglobulins in the sera and colostrums of pregnant gilts and their piglets treated with the immunomodulator Baypamun was also established (VALPOTIĆ et al., 1993). In gilts treated with Baypamun a significant difference in the number of liveborn and stillborn piglets was found (KRSNIK et al., 1999; POTOČNJAK et al., 2006). Also, beneficial effects of immunomodulator on reproductive results in transported pigs were noted (SARATSIŠ et al., 1999).

On the basis of the results obtained in our research it can be concluded that because of stress, which occurs as a result of pregnant gilt relocation from the service station to the prefarrowing unit, there is an increase in plasma cortisol concentration, which has been noted in other research as well (CRONIN, 1991; PAVIČIĆ et al., 2001; PAVIČIĆ et al., 2003; PAVIČIĆ et al., 2004). Using the procedures for gilt accommodation change, especially during the partal period and lactation, some effects on animal welfare can be obtained in regard to the fact that ambient change causes an increased cortisol response in pigs (BARNETT et al., 1984). In the non-treated group C, despite the decrease in plasma cortisol concentration during the research period, the mean plasma cortisol concentration (2nd day 251.50 nmol/L, 4th day 242.10 nmol/L and 6th day 219 nmol/L) was above the reference range for pigs, 49.7 to 218 nmol/L (HANNON et al., 1990). In both groups treated with immunomodulator, plasma cortisol concentration was within the reference range during the whole research period. PAVIČIĆ et al. (2001) noted an increase in plasma cortisol concentration in the group of gilts treated with immunomodulator during their stay in the farrowing unit, which is explained by severe stress caused by labour. In other research as well an increase in plasma cortisol concentration 24 hours before and after labour was noted (SEBRANEK et al., 1973; FRIEND et al., 1979; BARNETT et al., 1984; LAWRENCE et al., 1994). BARNETT et al. (1987) concluded that an increase of plasma cortisol concentration in piglets based on displayed consequences on protein metabolism, immune system and reproduction represents a proof of danger for the welfare of same piglets.

In our research, gilts from group A treated with immunomodulator tolerated stress most efficiently on the 6th, 4th and 2nd days before relocation to the prefarrowing unit, in regard to the fact that the lowest mean plasma cortisol concentration during the

researched period was determined in this group (2nd day 190.57 nmol/L, 4th day 184.57 nmol/L, 6th day 176.77 nmol/L). Gilts in group B were treated with immunomodulator on the 5th, 3rd and 1st days before relocation to the prefarrowing unit, and had a higher mean plasma cortisol concentration in regard to the concentrations established for group A (2nd day 207.23 nmol/L, 4th day 206.37 nmol/L, 6th day 194.73 nmol/L), and these values were within the reference range for plasma cortisol in pigs. Statistical significant differences in the concentration of cortisol, on the 4th and 6th day in the prefarrowing units between the gilt groups (A and B) treated with the immunomodulator, we can explain the time difference in the application of the immunomodulator in these two groups prior to their relocation to the prefarrowing units. Our results suggest that the gilts from group A showed the most significant effect of the immunomodulator in comparison to the gilts from group B. CRONIN et al. (1991) state that relocation of gilts not treated with an immunomodulator from one building to another results in a significant increase in plasma cortisol concentration, which we confirmed by the cortisol concentration established in group C gilts in our research.

The aforementioned results of our research indicate that immunomodulator application alleviates hormonal changes which occur as a result of a stressful situation and has positive effects on reduction of stress susceptibility. Further research and comparison of currently available results should determine the optimal time of immunomodulator application in intensive pig farming, with the final goal of significant stress reduction and improvement in reproductive results.

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SAŽETAK

Cilj ovoga istraživanja bio je utvrditi može li višekratna imunomodulacija smanjiti posljedice stresa zbog preseljenja nazimica iz pripuštališta u čekalište tijekom rane faze gravidnosti. Istraživanje je obuhvatilo tri skupine gravidnih nazimica pasmine švedski landras, u dobi od 8 do 9 mjeseci. Svaka skupina životinja sadržavala je 30 gravidnih nazimica. Tri istraživane skupine nazimica bile su različito obrađivane. Skupini A primijenjen je imunomodulator (Baypamun®) 6, 4 i 2 dana, a skupini B 5, 3 i 1 dan prije preseljenja iz pripuštališta u čekalište. Navedeni imunomodulator dan je intramuskularno u dozi od 2 ml u obje skupine nazimica. Skupina C nije bila obrađivana imunomodulatorom prije preseljenja iz pripuštališta u čekalište. Nazimicama svih navedenih skupina izvađena je krv 2., 4. i 6. dana boravka u čekalištu. U istraživanom razdoblju utvrđene su statistički značajne razlike ($P < 0,01$) u koncentraciji kortizola između imunomodulatorom tretiranih skupina A i B i netretirane skupine C. Prosječne koncentracije kortizola bile su najmanje u skupini A, pri čemu su vrijednosti utvrđene četvrtoga i šestoga dana boravka u čekalištu bile statistički značajno manje u odnosu na skupinu B ($P < 0,01$). Prosječne koncentracije kortizola utvrđene u netretiranoj skupini C bile su veće od referentnog raspona za svinje. U sve tri skupine nazimica zabilježen je pad koncentracije kortizola u krvnoj plazmi tijekom boravka u čekalištu, tako da su prosječne vrijednosti utvrđene šestoga dana boravka u čekalištu bile statistički značajno manje ($P < 0,01$) u odnosu na koncentracije kortizola utvrđene drugoga dana boravka u čekalištu. Navedeni rezultati pokazuju da imunomodulacija ublažava hormonalne promjene nastale kao odgovor na stresna stanja i upućuje na pozitivno djelovanje imunomodulatora u smanjenju stresne osjetljivosti.

Cljučne riječi: kortizol, nazimice, gravidnost, imunomodulacija, stres
