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ACUTE PHASE PROTEINS DURING THE REPRODUCTIVE CYCLE OF SOWS

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The objective of this study was to evaluate the concentrations of selected acute phase proteins – haptoglobin (Hp) and C-reactive protein (CRP) in blood serum of sows during different stages of reproductive period. The evaluation was performed in 24 sows of crossbreeds of large white and landrace after 1–5 farrowings. The animals were divided into 4 groups by 6 animals in each group according to certain phases of the reproductive cycle: 1 month before farrowing, 1 week before farrowing, 1 week after farrowing and 1 week after weaning. Blood samples from sows were collected in each group four times at intervals of two weeks. Hp and CRP were determined by the method of enzyme linked immunosorbent assay. The significantly highest average concentrations of Hp, as well as CRP ($P<0.05$ and $P<0.01$), we recorded in sows 1 week after farrowing. Later after farrowing we found a significant decrease in concentrations of the aforementioned indices ($P<0.05$ and $P<0.01$). Our results suggest important changes in the concentrations of acute phase proteins during the reproduction period of sows, which may be a consequence of numerous physiological reactions occuring in sows close to the time of parturition.

Key words: C-reactive protein, farrowing, haptoglobin, sows

INTRODUCTION

The acute phase proteins (APPs) are a group of blood proteins that change in concentration in animals subjected to external or internal challenges, such as infection, inflammation, trauma or stress (Pyörälä, 2000). Their serum concentrations may increase or decrease in response to the stimulus. The most frequently studied serum APPs in swine are haptoglobin (Hp), alpha 1-acid glycoprotein (AGP), and C-reactive protein (CRP) (Grellner *et al.*, 2002). Evaluating of APPs might provide information about the health status in a pig (Itoh *et al*., 1993). Measurements of some acute phase reactants can identify herds with poor hygiene, lack of surveillance or poor vaccination response is leading to immunological stress resulting in a reduction of feed efficiency (Pineiro *et al*., 2007).

In general, the time around parturition is a very challenging period, when the resistance of the organism to external and internal stimuli is impaired. The stress due to prefarrowing dislocation and farrowing might activate subclinically present chronic urogenital infections, resulting in postparturient diseases of sows (Glock and Bilkei, 2005; Kovac *et al.*, 2007). Urogenital diseases of sows and mastitis-metritis-agalactia syndrome (MMA) cause increased values of APPs and may contribute to a lower number of piglets in litter, inadequate lactation and even sow mortality (Mirko and Bilkei, 2004).

The objective of this study was to evaluate the concentrations of selected acute phase proteins – haptoglobin and C-reactive protein in blood serum of sows during some stages of the reproductive cycle, including the time before and after farrowing, as well as after weaning.

MATERIAL AND METHODS

Selected acute phase proteins – Hp and CRP were evaluated in 24 sows. The sows were crossbreeds of Large White and Landrace after 1–5 farrowings. The animals were fed complete feedstuff according to corresponding stages of the reproductive period twice a day, and they had free access to drinking water. The sows were divided into 4 groups by 6 animals according to the phases of the reproductive cycle at the beginning of the evaluation:

– Group 1. 4 weeks before farrowing
– Group 2. 1 week before farrowing
– Group 3. 1 week after farrowing
– Group 4. 1 week after weaning.

The afore mentioned acute phase proteins were evaluated in blood serum. Blood samples were collected by direct puncture of *v. cava cranialis* in each group four times at intervals of two weeks (Table 1).

Table 1. Blood sample collections in evaluated groups of sows

Groups of sows	Sample collections			
	1.	2.	3.	4.
1	4 weeks *a.p.*	2 weeks *a.p.*	1–2 days *p.p.*	2 weeks *p.p.*
2	1 week *a.p.*	1 week *p.p.*	3 weeks *p.p.*	1 week *p.*wean.
3	1 week *p.p.*	3 weeks *p.p.*	1 week *p.*wean.	7 weeks *p.p.*
4	1 week *p.*wean.	3 weeks *p.*wean.	5 weeks *p.*wean.	7 weeks *p.*wean.

a.p. – *ante partum*, *p.p.* – *post partum*, *p.*wean. – *post* weaning

Before each sample collection, the animals were clinically examined by standard clinical examination procedures (Jackson and Cockcroft, 2002).

In the first part of the study we evaluated the dynamics of changes in Hp and CRP concentrations during the reproduction cycle in the mentioned groups of sows. In the second part of the study we summarised the individual values of both

variables from these groups of sows according to 4 selected periods of the reproductive cycle – 4 and 1 week *ante partum*, 1 week *post partum* and 1 week *post* weaning.

The determinations of Hp and CRP were performed by the method of enzyme linked immunosorbent assay (ELISA) using ELISA kits (Tridelta Development, Ltd., Wicklow, Ireland) in microplates. The readings of absorbance and the consecutive calculations of final concentrations were performed on automatic microplate reader Opsys MR (Dynex Technologies).

Statistical analyses of the obtained results were performed by assessment of means (x) and standard deviations (sd) in each monitored group of sows according to sample collection. The significance of differences in average values of concentrations of the mentioned variables in relation to the corresponding monitored periods of reproduction was evaluated by one way analysis of variance (ANOVA). The significance of differences in means between sample collections in the groups was evaluated by Student's paired test (the 1st part of the study). The significance of differences in average values between selected periods of reproduction period was evaluated by Student's unpaired test (the 2nd part of the study). Statistical analyses were done with programme GraphPad Instat V2.04.

RESULTS

The results of assessments characterised by average values, standard deviations, and statistical evaluation are summarised in Table 2. and Table 3.

Table 2. Concentrations of Hp (mg/mL) in blood serum of sows (x ± sd)

Group of the sows	Sample collection				ANOVA P
	1.	2.	3.	4.	
I.	1.27±0.45	2.30±1.83	1.63±0.40	1.57±0.44	n.s.
II.	1.76±0.21^{a}	1.79±0.44	1.63±0.45	1.16±0.16^{a}	n.s
III.	2.96±1.06^{a,b,B}	1.91±0.65^{a,A}	1.27±0.40^{b}	1.20±0.59^{A,B}	<0.01
IV.	2.10±0.68^{a,b}	1.44±0.94	1.00±0.71^{b}	0.99±0.86^{a}	n.s.

n.s. – not significant
The same indexes in lines mean statistical significance of differences in average values between the groups: a, A – P<0.05; b, B – P<0.01

In Group 1, 2 weeks *ante partum* (2nd sample collection), we recorded a markedly higher Hp concentration compared with the 1st sampling, this difference was statistically not significant. Other sample collections (*post partum*) showed a continuous decrease in its concentrations. In CRP concentrations, we found at 3rd and 4th sampling nonsignificantly higher values compared with the 1st and 2nd samplings. The highest average concentration of CRP was recorded in sows after farrowing (3rd sampling). The changes in average concentrations of Hp and CRP during the monitored period in this group of sows were not

significant. All animals from this group throughout the whole monitored period were clinically healthy.

Table 3. Concentrations of CRP (ng/mL) in blood serum of sows (x ± sd)

Group of the sows	Sample collection				ANOVA P
	1.	2.	3.	4.	
I.	16.27±9.38	15.97±12.35	27.19±21.96	20.85±10.16	n.s.
II.	23.97±11.84	31.92±37.20	49.98±39.04	9.83±7.36	n.s.
III.	64.92±60.10^{a}	14.47±14.82	7.78±8.76^{a}	4.10±3.39	<0.05
IV.	23.59±17.67^{a}	52.44±48.27^{A}	8.25±7.68^{a}	6.93±12.91^{A}	<0.05

n.s. – not significant
The same indexes in lines mean statistical significance of differences in average values between the groups: a, A – $P<0.05$

In Group 2, 1 week before farrowing until 3 weeks after farrowing, we recorded nonsignificant changes in Hp concentrations. Significantly lower means compared with the 1st sampling were found 5 weeks after farrowing (4th sampling, $P<0.05$). In concentrations of CRP we found a nonsignificant increase of values by 2nd and 3rd sampling with consecutive more marked, however statistically not significant decrease, of its average concentrations below the level of the initial values at 4th sampling. The changes in means of Hp and CRP in this group of sows throughout the monitored period were not significant. All animals from this group during the monitored period were clinically healthy, and were without changes in the reproductive system after farrowing.

In Group 3, we recorded a trend of decrease in average Hp concentrations from the 1st (1 week *p.p.*) to the 4th sample collection (7 weeks *p.p.*). The means of Hp at the 1st sampling were significantly higher compared with the 3rd ($P<0.05$) and 4th samplings ($P<0.01$). The average Hp concentrations in sows 3 weeks after farrowing (2nd sampling) were also significantly higher compared with animals at the 4th sampling. Similar decreasing trends was found also in means of CRP. The concentration of CRP by the 1st sample collection was significantly higher compared with values found by the 3rd sampling ($P<0.05$). The changes in means of Hp and CRP in this group of sows during the monitored period were significant ($P<0.01$ and $P<0.05$). Almost in all the animals from this group, clinical signs of MMA syndrome were present at the 1st sampling (1 week *p.p.*), characterised by decreased food intake, vaginal discharge, erythema, and firmness of the mammary gland. By following samplings in these animals we did not record any clinically evident changes on the reproductive system.

In Group 4, we recorded different dynamics in the changes of concentrations of both monitored variables. Hp concentrations had a decreasing trend of average values. The concentrations of Hp by the 1st sampling time (1 week *post* weaning) were significantly higher compared with results by the 3rd and 4th sampling (5 and 7 weeks *post* weaning, $P<0.05$). In CRP values, after a nonsignificant increase of average values by the 2nd sampling, we recorded a

repeated decrease of their values by the 3rd and 4th sampling. While the changes in average values of Hp in this group of sows during the monitored time were not significant, the changes in average concentrations of CRP throughout the monitored period were significant ($P<0.05$). By the 1st sample collection we found in one animal from this group inflammatory changes of the carpal joint. Clinical examinations of monitored animals by the next sampling time did not reveal any disorders of the general health state.

The obtained results of individual values from the evaluated groups of sows are summarised also according to selected periods of reproduction cycle (Table 4). The highest average concentration of Hp (2.17 mg/mL) we found in sows 1 week after farrowing. This value was significantly higher compared with mean values recorded in sows 4 weeks before parturition and 1 week after weaning ($P<0.01$ and $P<0.05$, respectively). By evaluation of average concentrations of CRP a similar trend of significantly increasing values was faund. While the changes in average values of haptoglobin during the monitored time were not significant, the changes in average concentrations of C-reactive protein throughout the monitored period were significant ($P<0.05$).

Table 4. The average concentrations of Hp and CRP in blood serum of sows in selected periods of reproduction cycle ($x \pm sd$)

Variable	Sample collections				ANOVA P
	4 weeks *a.p.*	1 week *a.p.*	1 week *p.p.*	1 week *p.*wean.	
Hp	1.27 ± 0.45^{b}	2.09 ± 1.40	$2.17 \pm 0.93^{a,b}$	1.55 ± 0.64^{a}	n.s.
CRP	16.27 ± 9.38^{a}	19.17 ± 12.19	$42.52 \pm 44.34^{a,A}$	14.22 ± 14.05^{A}	<0.05

a.p. – *ante partum*, p.p. – *post partum*, p.wean. – *post* weaning
The same indexes in lines mean statistical significance of differences in average values between the groups: a, A – $P<0.05$; b – $P<0.01$

DISCUSSION

Reproductive state and parity are important factors influencing most of the biochemical parameters of sows, including APPs (Verheyen *et al*., 2007). At present, physiological reference values of Hp and CRP in pigs are not definitely established. However, it appears that the time of sampling (before or after farrowing) and parity have an important influence on most of the acute phase proteins.

Many researchers reported that APP values undergo significant changes around parturition (Alsemgeest *et al*., 1993; Ametaj, 2005). In horses and cows the highest concentrations were observed 1 day *post partum*. On the other side, data in sows at parturition are lacking so far (Gymnich *et al*., 2003). Our results suggest that in sows there are also important changes in concentrations of APPs connected to the reproductive cycle. In sows 1 week after farrowing we found a significantly higher average concentration of Hp, as well as CRP, than before and

later on after parturition. Kostro *et al*. (2003) reported similar findings, as they evaluated concentrations of Hp and CRP in sows, and recorded higher values after farrowing than previous to it. According to Verheyen *et al.* (2007), Hp concentrations show a significant increase one week after farrowing at over 2.09 g/L, at which, similar to our results, the mean concentrations of this parameter on the 94th and 108th day of gestation are roughly uniform (1.36 and 1.54 g/L). The increased Hp concentration *post partum* could be explained by physiological events during puerperium. The periparturient acute phase response recorded also in our study could be related to tissue damage occuring due to the increase in myometrial activity, subsequent relaxation and dilatation of the cervix, and the caudal part of the birth canal during expulsion. According to Regassa and Noakes (1999), higher values of APPs may be consequence of the involution of the uterus, as well as degeneration and regeneration of the endometrium. Higher values of Hp and CRP, recorded after calving, may be also a consequence of increased cortisol production in the adrenal cortex, as part of the response of the organism to stress (Uchida *et al*., 1993). According to Busch *et al.* (2003), Hp concentrations in young sows increase 2–8 weeks before farrowing, and the increase is higher than in older sows. Verheyen *et al*. (2007) reported also that primiparous sows had higher Hp concentrations. This finding is in contrast whit the results of Petersen *et al*. (2002) who described a Hp increase with age.

The reproductive tract of sows is susceptible to infection after farrowing because of the periparturient increase in the number of both nonpathogenic microflora and facultative pathogens in the caudal vagina and urinary bladder (Bilkei *et al*., 1994). Elevated concentrations of Hp and CRP in sows may reflect the inflammation in the reproductive tract and mammary gland. Mirko and Bilkei (2004) evaluated sows without postparturient complications of the normal process of puerperium and sows suffering from MMA syndrome after parturition. In both groups they recorded an increase of concentrations of Hp and AGP in the postparturient period. The concentrations of the aforementioned acute phase proteins on the 1st and 5th day after parturition were higher in sows suffering from MMA syndrome compared with sows without clinical signs of reproductive disorders. Friendship and Bilkei (2005) stated that in sows with MMA syndrome and other urogenital diseases the Hp concentration was higher on the 1st and 5th day of lactation. Monitoring the concentrations of these indicators during the first days after parturition may be used to diagnose early stages of MMA and to start suitable therapy (Gymnich *et al*., 2003).

In general, the acute phase response is considered to be useful in the prevention of microbial growth, and may help in the recovery of the organism. On the other side, an active cellular immune response in the sow indirectly influenced growth rate negatively in the preweaning piglets, presumably due to decreased milk production (Bilkei, 1995).

Aforementioned results suggest that the reproductive state influences the production of acute phase proteins and indicates that around farrowing also in sows there are important changes in concentrations of some acute phase proteins. In the monitored period we recorded significant changes in values of Hp

and CRP. However, the interpretation should always be performed together with a thorough anamnesis and clinical evaluation of individual animals.

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PROTEINI AKUTNE FAZE U TOKU REPRODUKTIVNOG CIKLUSA KRMAČA

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SADRŽAJ

Cilj ovih istraživanja je bio da se utvrde koncentracije odabranih proteina akutne faze (haptoglobina – Hp i C – reaktivnog proteina – CRP) u krvnom serumu krmača u različitim fazama njihovog reproduktivnog ciklusa. Ispitivanja su izvedena na ukupno 24 krmače, meleza bele mesnate svinje i landrasa. Prasnost krmača se kretala od 1 do 5. Krmače su bile podeljene u 4 jednake grupe u zavisnosti od faze reproduktivnog ciklusa: mesec dana pred prašenje, nedelju dana pred prašenje, nedelju dana posle prašenja i nedelju dana posle zalučenja. Uzorci seruma su prikupljani 4 puta od svake jedinke u intervalima od dve nedelje. Koncentracije Hp i CRP su određivane ELISA metodom. Najveće srednje koncentracije Hp i CRP su utvrđene nedelju dana posle prašenja dok je kasnije dolazilo do njihovog značajnog pada.