

**ACUTE PHASE PROTEINS, SERUM CORTISOL AND PREWEANING LITTER PERFORMANCE  
IN SOWS SUFFERING FROM PERIPARTURIENT DISEASE**

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*The objective of this study was to evaluate serum concentrations of the acute phase proteins (APP), haptoglobin (HPT) and alpha 1-acid glycoprotein (AGP), as well as cortisol in sows with postparturient mastitis metritisagalactia (MMA) (Group one, n=21) and in healthy sows (Group two, n=20) and to determine whether changes in APPs are associated with litter performance. Serum samples were taken from each sow one day after parturition, and at days 5, 10, 15, and 20 of lactation.*

*The total number of pigs born (Group one:  $12.4 \pm 1.3$  vs. Group two:  $12.5 \pm 1.2$ ), number of liveborn pigs (Group one:  $11.9 \pm 1.1$  vs. Group two:  $11.8 \pm 1.3$ ), number of stillborn pigs (Group one:  $0.5 \pm 0.03$  vs. Group two:  $0.7 \pm 0.02$ ), and number of mummified pigs (Group one:  $0.4 \pm 0.03$  vs. Group two:  $0.2 \pm 0.01$ ) did not differ significantly between the groups. Prewaning mortality showed significant ( $P < 0.001$ ) differences between the sows suffering from MMA ( $22.1 \pm 5.2\%$ ) and the healthy sows ( $12.6 \pm 2.1\%$ ). Weaning litter weights at four weeks differed significantly ( $P < 0.01$ ) between the group suffering from MMA and the healthy animals ( $68.2 \pm 2.1$  kg vs.  $77.3 \pm 3.2$  kg).*

*Mean serum AGP concentrations were higher ( $P < 0.001$ ) at day ten and twenty ( $P = 0.02$ ) after parturition in sows suffering from MMA. Mean serum HPT was higher in sows suffering from MMA at days 1, 5 ( $P < 0.001$ ) and 10 ( $P = 0.04$ ) of lactation. Serum cortisol concentrations were significantly ( $P < 0.001$ ) higher in sows suffering from MMA compared to the healthy ones throughout the study. AGP was negatively correlated with litter weight, indicating that activation of the cellular immune response of the sow negatively affects growth rate of her litter. Correlations were found between the overall means for weight and acute phase proteins.*

*The authors conclude that AGP negatively correlates with litter weight, indicating that an activation of cellular immune response of the sow may negatively affect growth rate of the suckling piglets and that HTP may be a valuable indicator of stress and disease in postparturient sows.*

*Key words: sow, parturition, mastitis metritisagalactia, acute phase proteins, cortisol, preweaning litter performance.*

## INTRODUCTION

Acute phase proteins (APP) include a group of liver-synthesized proteins the serum concentrations of which vary in response to infections, stress and inflammatory processes (Burger *et al.*, 1992). The most frequently studied serum APPs in swine are haptoglobin (HPT), alpha 1-acid glycoprotein (AGP), and C-reactive protein (CRP) (Hall *et al.*, 1992; Eurell *et al.*, 1992). More than 30 APPs have been identified (Grellner *et al.*, 2002). Changes in APP can be used to monitor the systemic response to physiological insults (Hall *et al.*, 1992) and weight gain in pigs (Eurell *et al.*, 1992). Haptoglobin (HPT) and alpha 1-acid glycoproteins (AGP) have potential as indicators of subclinical diseases (Eckersall *et al.*, 1996). Evaluating a particular APP might provide information about the health status in a pig unit (Itoh *et al.*, 1993). Serum concentrations of APP may decrease or increase in response to the same stimulus (Horadagoda *et al.*, 1999). High AGP levels may negatively influence the pharmacological effects of antimicrobial drugs (Son *et al.*, 1996). No literature data are available on the possible association between APP, HPT, AGP, CRP, mastitis metritis agalactia (MMA), and litter performance.

## MATERIAL AND METHODS

The trial was performed during September and October 2003, in a Slovakian large pig production unit. At the beginning of September 2003 the unit suffered a sudden increase of the MMA symptom-complex in postparturient sows. Pre-trial bacteriological examination of postparturient vulval discharges and milk from the affected mammary glands revealed the presence of *Clostridium* spp, *Actinobaculum suis*, *Klebsiella* spp, *Proteus* spp, (and) Gram-positive streptococci, staphylococci, *Erysipelothrix rhusiopathiae* and *Escherichia coli*.

The sows were fed as follows:

- from mating to day 89 of gestation 2 kg and from day 90-114 of pregnancy 3 kg of a commercial gestating ration containing per kg 12.2 MJ digestible energy (DE), 125 g crude protein, 6.5 g lysine, 8 g calcium and 6 g phosphorus.
- from the first day of parturition the sows were fed *ad libitum* with a lactating ration containing per kg 13.0 MJ DE, 180 g crude protein, 10 g lysine, 8 g calcium and 6 phosphorus.
- From weaning to mating the sows received *ad libitum* the same lactating ration, supplemented with 500 g potato starch / sow / day ("flushing")

Sows were identified with ear tags of different colour. Weight loss and loss of back fat during lactation was not recorded. The sows were housed during lactation in identical large farrowing barns. The observers were not blind to the treatment. No cross-fostering was performed.

The sows had free access to a nipple waterer. During pregnancy the animals received erysipelas and *E coli* vaccination. Weaning age was  $28 \pm 1.1$  days.

Twenty one sows (group one) were diagnosed during the first week of September as suffering from MMA according to an early MMA diagnosis system

(Bilkei *et al.*, 1995). Twenty healthy sows of similar body condition and age were chosen as the control group (Group 2). Diagnosis of MMA was performed by the farm manager on the basis of elevated temperature, level of milk production and appetite (Table 5). Sows diagnosed with periparturient disease were treated with an Enrofloxacin (Baytril® Bayer, Germany, 3 mg/kg body weight) antibiotic by intravenous infusion (according to the sensitivity test), three days of parenteral antibiotics (according to the sensitivity test) and oxytocin (10 IU/day, Oxytocin® TAD, Germany), and on the day of positive diagnosis for MMA one prostaglandin F<sub>2α</sub> injection (175 µg cloprostenol, Estrumate® Essex, Germany).

In order to monitor changes in the concentrations of HTP, AGP and cortisol, serum samples were taken from each sow one day after parturition and at days 5, 10, 15, and 20 of lactation. Serum AGP and HPT were determined by ELISA according to ESAP (as described by Son *et al.*, 1996 and Grellner *et al.*, 2002). Cortisol concentrations were determined using a commercial kit (Diagnostic Co, LA, CA, USA).

For statistical analysis, the individual sow was the experimental unit. Data were analyzed using Statview software (1998). Between-group comparisons between MMA suffering and healthy sows, were made at specific time points according to the unpaired *t* test, while within-group comparisons between two data collection points were made using the paired *t* test. Correlation coefficients (*r*) were reported on the overall means for each variable for MMA suffering and healthy sows, using Fishers's *r* to *z* analysis to describe linear relationships between two variables within the same group. Variables used for correlation comparisons were serum concentrations of cortisol, AGP and HPT. Correlation comparisons were made between parameters of interest, and the positive or negative change in the linear relationship of the parameters compared over the same time period were reported. In all comparisons, *P* values <0.05 were considered significant.

Sow data were analyzed separately, using SAS (1998). Independent variables included breeding month, and treatments not related to reproduction. Parity category, interaction between parity category and previous lactation lengths (treated as continuous variables) were included in the model. Previous weaning-to-mating interval category and its interaction with parity category were also included in the model. The GLM procedure was used for analysis of litter data. Dependent variables were total number of pigs born, number of liveborn pigs, number of stillborn pigs, and number of mummified pigs. Independent variables included litter conception month and treatments not related to reproduction. Age at first mating (days), treated as a continuous variable, was included in the model for parity 1 sow data. Least-squares means were requested with "lsmeans" following the model statement. Standard errors for the means and probability comparison between the means were requested using options of "stderr" and "pdiff," respectively.

## RESULTS

Total number of pigs born (group one:  $12.4 \pm 1.3$  vs. group two:  $12.5 \pm 1.2$ ), number of liveborn pigs (group one:  $11.9 \pm 1.1$  vs. group two:  $11.8 \pm 1.3$ ), number of stillborn pigs (group one:  $0.5 \pm 0.03$  vs. group two:  $0.7 \pm 0.02$ ), and number of mummified pigs (group one:  $0.4 \pm 0.03$  vs. group two:  $0.2 \pm 0.01$ ) did not differ significantly between the groups. Preweaning mortality showed significant ( $P < 0.001$ ) differences between the MMA suffering and healthy sows (group one:  $22.1 \pm 5.2\%$  vs. group two:  $12.6 \pm 2.1\%$ ). Weaning litter weights at four weeks differed significantly ( $P < 0.01$ ) between the sows suffering from MMA and healthy sows ( $68.2 \pm 2.1$  kg vs.  $77.3 \pm 3.2$  kg).

Mean serum AGP concentrations were higher ( $P < 0.001$ ) at days 10 and 20 ( $P = 0.02$ ) after parturition in sows suffering from MMA (Table 1). Mean serum HPT was higher in sows suffering from MMA on days one, five ( $P < 0.001$ ) and ten ( $P = 0.04$ ) of lactation (Table 2). Serum cortisol concentrations were significantly ( $P < 0.001$ ) higher in sows suffering from MMA compared to the healthy ones throughout the study (Table 3). AGP was negatively correlated with litter weight, indicating that an activation of cellular immune response of the sow negatively affects growth rate of the suckling piglets. Correlations were found between the overall means for weight and acute phase proteins (Table 4).

Table 1. Mean ( $\pm$ SD) serum concentrations of alpha 1-acid glycoprotein (AGP  $\mu$ g/ml) in healthy sows and those with MMA

Interval after parturition (d)	Control g / ml ( $\pm$ SD)	MMA $\mu$ g / ml ( $\pm$ SD)	P-value
1	766.4 (101.1)	816.1 (112.4)	0.70
5	1132.2 (120.4)	1186.2 (104.2)	0.41
10	869.1 (110.1)	628.5 (102.1)	<0.001
15	529.5 (96.2)	517.6 (112.2)	0.08
20	429.3 (93.8)	519.4 (64.1)	0.02

Table 2. Mean ( $\pm$ SD) serum concentrations of haptoglobin (HPT mg/dl ( $\pm$ SD) in sows suffering from MMA and healthy sows

Interval after parturition (d)	Control	MMA mg / dl ( $\pm$ SD)	P-value
1	1.81 (0.2)	21.01 (3.0)	<0.001
5	3.7 (0.4)	21.36 (3.0)	<0.001
10	27.82 (3.7)	40.73 (5.1)	0.04
15	29.07 (7.2)	31.05 (5.0)	0.75
20	35.03 (4.8)	35.05 (7.2)	0.98

Table 3. Mean ( $\pm$ SD) serum concentrations of cortisol (ng / ml ( $\pm$ SD) in sows suffering from MMA and healthy sows

Interval after parturition (d)	Control (ng/ml ( $\pm$ SD))	MMA ng/ml ( $\pm$ SD)	P-value
1	22.1 (0.1)	48.1 (0.3)	<0.001
5	20.8 (0.4)	42.60 (3.1)	<0.001
10	2.76 (0.4)	22.98 (0.9)	<0.001
15	1.79 (5.1)	12.82 (7.3)	<0.001
20	1.99 (0.4)	2.84 (0.4)	<0.001

Table 4. Correlation coefficients between weaning litter weights, serum AGP, HPT, cortisol in sows suffering from MMA and healthy sows

Parameter compared	Healthy sows		MMA suffering sows	
	Correlation	P value	Correlation	P value
Weight (kg), AGP ( $\mu$ g / ml)	-0.479	0.0008	-0.542	<0.0001
Weight (kg), HPT (mg / dl)	0.281	0.602	0.706	<0.0001
Weight (kg), cortisol ( $\mu$ g / dl)	-0.015	0.9096	0.086	0.5656
AGP ( $\mu$ g / ml), HPT (mg / dl)	-0.226	0.1379	-0.343	0.084
AGP ( $\mu$ g / ml), cortisol ( $\mu$ g / dl)	0.271	0.435	0.106	0.4943
HPT (mg / dl), cortisol ( $\mu$ g / dl)	0.113	0.3870	0.324	0.294

Table 5. Diagnosis of periparturient diseases. Sows were examined for three days post-partum and diagnosis of disease made if a sow had a total score of at least + + + based on the severity of fever, on milk production and on appetite (Bilkei and Horn, 1991)

Fever °C	40.0 – 40.3 +	40.4 – 40.6 + +	Over 40.6 + + +
Milk production	Hypogalactia +	Agalactia without clinical signs of mastitis + +	Agalactia with clinical signs of mastitis + + +
Appetite	Diminished appetite +	Moderate anorexia + +	Total anorexia + + +

## Legend:

Hypogalactia: hungry, lethargic but otherwise healthy looking piglets that have regular suckling periods.

Agalactia without clinical signs of mastitis: weak, hungry dehydrated piglets that have no regular suckling periods or no suckling at all.

Agalactia with clinical signs of mastitis: swollen, painful, mammary glands with piglets not suckling at all.

Diminished appetite: more than 50 % but not all the daily feed provided (3 kg) eaten.

Moderate anorexia: less than 50 % (<1,5 kg) of the daily feed provided eaten.

Total anorexia: no feed eaten at all.

## DISCUSSION

The present paper reports on the acute phase response in sows with MMA, as indicated by some APP known to be important in some other mammals in serious inflammatory diseases (Horadagoda *et al.*, 1999).

The classification of MMA was based on three simple symptoms usually encountered in large East European pig production units (Bilkei, 1995). Although the diagnosis of MMA in the present paper may be criticized as a "non solid and non scientific" classification, lacking proper veterinary clinical examination such as heart rate, respiratory rate and bacteriological examination, the present diagnostic system is widely accepted in large East European pig breeding units (Bilkei and Horn, 1991)

While there are numerous publications on hematological and blood chemical data (Bilkei, 1995; Nachreiner *et al.*, 1972; Hermansson *et al.*, 1978), no data on serum AGP, HTP and cortisol and their correlation in lactating sows suffering from MMA could be found in the available literature. Similarly no data were available on the association of serum AGP, HTP and cortisol in sows with preweaning parameters of the piglets and their at litter weights weaning. Having no such data, discussion of the present results is more difficult. The differences found in preweaning mortality and litter weights at weaning might be explained by a complex effect of APP on sow and piglet production and health. Bilkei (1995) observed that APP decreased food intake, body growth rate, efficiency of feed utilisation and protein synthesis for muscle development. Growing pigs under low infectious pressure produce less APP than pigs in a highly infectious environment (Williams *et al.*, 1997; Wiseman, 1997). Thus, it is reasonable to suggest that sows suffering from a periparturient disease would show similar patterns in the development of APPs as the growing pigs examined by Williams *et al.* (1997) and Wiseman (1997). An active cellular immune response in the sow negatively influenced growth rate of the preweaning piglets (indirectly, presumably due to decreased milk production, Bilkei, 1995).

According to Itoh *et al.* (1993) the upper normal limit of serum AGP is around 500µg per ml for young growing swine. Itoh *et al.* (1993), found that serum AGP increases in response to infections and concluded that a sudden increase in serum AGP may be associated with acute, rather than chronic infections. MMA is an acute disease affecting the sow within hours post partum. Thus, an increase in serum AGP concentrations may be considered as an indicator of the intracellular immune communication, indicating an increase of cellular immune response (French, 1989) 5-10 days post partum.

In the present study, the negative correlation between AGP and weaning litter weights, suggests that an active cellular immune response negatively influences growth rate of the preweaning piglet. Itoh *et al.* (1993) suggested that in

pigs chronically exposed to pathogens, both disease and stressors may contribute to increases in AGP.

The increase in serum HTP concentrations in the present study are similar to the findings of Grellner *et al.* (2002) who evaluated growing pigs, raised under high *Mycoplasma hyopneumoniae* environmental pressure. The present findings indicate that the sows suffered long lasting stress due to MMA, which influenced their immune system. Moreover, weight gains were significantly correlated with HPT in the MMA sows.

Consistent with Grellner *et al.* (2002), who conducted a study on growing pigs, in the present trial, serum concentrations of AGP and HPT in the sows suffering from MMA did not correlate with changes in serum cortisol concentrations. Similarly, consistent with Bilkei (1995), Nachreiner *et al.* (1972) and Hermansson *et al.* (1978) high cortisol concentrations were found in sows with MMA in the present study. In a previous study, (Bilkei, 1995) serum cortisol values of sows suffering from MMA ranged between 40-50 ng per ml, while unaffected sows had 10-15 ng / ml during the first days post partum. Adrenal gland weights were higher in MMA affected sows. The present serum cortisol concentrations (42-48 ng / ml) confirm the findings of Bilkei *et al.* (1995) and Pejsak and Tarasiuk (1989) in sows suffering from coliform mastitis. Pejsak and Tarasiuk (1989) stated that *E coli* endotoxins (or "mediators") absorbed from the mammary gland or urogenital tract of the sow suffering from MMA, stimulate the release of interleukin -1 ("or other factors") that alter hypothalamic and adrenal function.

#### CONCLUSION

The present results indicate that serum AGP concentrations are negatively correlated with body weight, indicating that cellular immune stimulation negatively influences growth of the preweaning piglets. Further, serum HPT may be a valuable indicator of stress and disease in preweaning piglets and postparturient sows. In combination with higher cortisol concentrations, HPT seems to be a more reliable indicator of disease status than HPT or cortisol alone.

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PROTEINI AKUTNE FAZE, SERUMSKI KORTIZOL I OSOBINE LEGLA PRE ODBIJANJA  
KOD KRMAČA SA PERIPARTALNIM OBOLJENJIMA

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SADRŽAJ

Cilj ovog rada je bio određivanje koncentracije proteina akutne faze (APP), haptoglobina (HPT), alfa 1 – kiselih glikoproteina (AGP) i kortizola u serumu



krmača sa postpartalnom mastitis-metritis-agalakcijom (MMA) i utvrđivanje povezanosti promena u koncentraciji APP sa osobinama legla. Uzorci seruma su uzimani od svake krmače, dan nakon partusa, kao i 5., 10., 15., i 20. dana laktacije.

Broj ukupno rođene prasadi, broj živorođene prasadi, broj mrtvorodne prasadu kao i broj mumificirane prasadu nije se značajnije razlikovao između grupa obolelih i zdravih krmača. Međutim, mortalitet pre odbijanja ukazuje na značajne razlike između krmača, obolelih od MMA sindroma i zdravih krmača. Ukupna težina legla u momentu zalučivanja (4 nedelje po prašenju) se značajno razlikovala između krmača, obolelih od MMA sindroma i zdravih životinja.

Srednja vrednost koncentracije serumskih APP je bila veća 10. i 20. dana nakon partusa kod krmača koje imaju MMA sindrom. Srednja vrednost koncentracije serumskog HPT je bila veća kod krmača koje imaju MMA sindrom i to 1., 5. i 10. dana laktacije. Koncentracija serumskog kortizola je tokom istraživanja bila značajno veća kod krmača koje imaju MMA sindrom, u odnosu na zdrave krmače. AGP je bio u negativnoj korelaciji sa ukupnom težinom legla, što ukazuje da aktivacija celularnog imunskog odgovora krmača negativno utiče na razvoj legla. Na ovaj način je potvrđena korelacija između težine prasadu, koncentracije proteina akutne faze i koncentracije serumskog kortizola.

Autori zaključuju da koncentracija HPT može biti koristan indikator stresa i zdravstvenog stanja krmača u postpartalnom periodu.