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MAJOR ACUTE PHASE PROTEINS IN PIG SERUM FROM BIRTH TO SLAUGHTER

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Abstract

Age-related changes in serum concentrations of C-reactive protein (CRP), haptoglobin (Hp), serum amyloid A (SAA), and pig major acute phase protein (pig-MAP) were investigated in healthy pigs from birth to slaughter under field conditions. Repeated blood samples were obtained from 60 pigs at ages of 1-19 weeks. Concentrations of acute phase proteins (APP) were measured with the use of commercial ELISA kits. Concentrations of all APP increased with age ($P<0.05$) and positive correlations were evidenced between their concentrations and the age of pigs. Great variations in CRP, Hp, and SAA concentrations were found, as can been seen from standard deviation values. The minimal individual variability was found in regard to pig-MAP. A significant increase in all APP was observed in pigs' serum after weaning, constituting an important characteristic of this period. The elevation of APP after weaning may be associated with stress induced by mixing animals after weaning or changes in the pattern of feed administration. The peak in APP may be also caused by the initiation of synthesis of these proteins by piglets. Because a significant association between age and APP concentrations exists, further studies are needed to decide whether the age may influence the diagnostic value of APP as a marker of infection. Additionally, studies are needed to estimate whether the APP response in infection is age-dependent to any clinical importance degree.

Key words: pigs, CRP, haptoglobin, SAA, pig-MAP, postnatal development.

The acute phase response (APR) is an early response of the organism caused by various factors (3, 4, 22, 23). This reaction is mediated by pro-inflammatory cytokines and involves local and systemic effects, including changes in the concentrations of acute phase proteins (APP). The APP consists of negative and positive proteins (22). The serum concentrations of positive APP *e.g.* haptoglobin (Hp), C-reactive protein (CRP), serum amyloid A (SAA), and pig major acute phase protein (pig-MAP), increase during the APR, whereas the serum concentrations of negative APP *e.g.* albumin (Alb), decrease (3, 7, 8). The exact biological effects of APP remain largely unknown, but they are thought to participate in the innate immune defence mechanisms and in controlling inflammatory responses to infection by binding to foreign substances, that have opsonising activities and modulating phagocytic cell functions (14).

During the last few years, a significant increase in interest in porcine APP has been observed, especially with respect to their potential usage as markers of health status and animal welfare, and indicators of infection or inflammation (1-4, 10). The APP assay has also been shown to be an interesting tool for the evaluation of treatment efficacy (11, 12). However, up to now, the knowledge about age related changes in the serum concentrations of APP in healthy pigs from birth to slaughter is restricted.

Therefore, the aim of the present study was to determine the serum concentration of major pigs' APP (CRP, Hp, SAA, and pig-MAP) in healthy pigs from commercial farm over a lifetime (from birth to slaughter).

Material and Methods

Animals. Sixty clinically healthy pigs (30 gilts and 30 barrows) from a commercial breeding farm with the high health status were used. The herd was seronegative to porcine reproductive and respiratory syndrome virus and pseudorabies virus. No evidence of pleuropneumonia, streptococcosis, and atrophic rhinitis was recorded at any age group of the pigs, based on clinical, serological, and pathological examinations from over a 5-year period. Complete management and health data were kept for the sows and their offspring. Production was an "all in-all out" procedure with a thorough cleaning between batches. The piglets were weaned at approximately 28 d of life, and slaughtered around the 21st week of life. Three pigs got sick during the period of study and all data from those animals were omitted from the statistical analysis. Additional two pigs

were excluded after postmortem examination carried out at the slaughterhouse (the macroscopic changes in their lungs were found).

Sample collection. Whole blood was obtained by venepuncture of the jugular vein at 1, 2, 3, 4, 6, 8, 10, 13, 16, and 19 weeks of their age. Serum was collected after centrifugation and stored at -70ºC for further analyses.

Measurement of acute phase proteins. Serum levels of APP CRP, Hp, SAA, and pig-MAP were quantified by the spectrophotometric methods using commercial ELISA kits: pig C-reactive protein (CRP) ELISA and pig haptoglobin ELISA (Life Diagnostics, Inc., USA), phase serum amyloid A assay (Tridelta Development Ltd, Ireland), and pig-MAP KIT ELISA (PigCHAMP Pro Europa S.A, Spain). For all analyses serum samples were tested in duplicate, according to the manufacturer's recommendations. Prior to analyses serum samples were diluted as follows: 1:100 for CRP, 1:35,000 for Hp, 1:10 for SAA, and 1:1,000 for pig-MAP

Statistical analysis. The age-dependent changes were tested with nonparametric Friedman test. Comparisons between gilts and barrows were assessed using the Mann-Whitney U test. For analysis of correlation between measured parameters, the Spearman rank correlation was used. All calculations were performed with the Statistica 8.0 (Statsoft, Poland) computer programme.

Results

The obtained results indicate that concentrations of all investigated APP generally increased with age and positive correlations existed between concentration of all APP and the animals' age. There were no significant differences between the levels of investigated proteins in barrows and gilts ($P \geq 0.05$).

C-reactive protein. The concentration of CRP ranged from 18.83 ±12.36 µg/mL in 7-day-old piglets to over 28 µg/mL in 13-week-old animals, and remained at that level to the end of the study. The lowest concentration of CRP (14.23 ±10.38 µg/mL) was observed on the 28th day of life. After weaning the level of CRP tended to increase. From weaning to the end of the study the concentration of CRP increased 2-fold. A significant difference was found between the level of CRP in 4 and 13-week-old pigs ($P<0.05$). Similarly as for other APP, the positive correlation was evident between the mean concentration of CRP and pig age (R-Spearman=0.30, $P<0.05$). Additionally, slight correlation was found between concentration of CRP and Hp (R-Spearman = 0.30, $P<0.05$). The mean (±SD) concentration of all APP during whole fattening period is shown in Fig. 1.

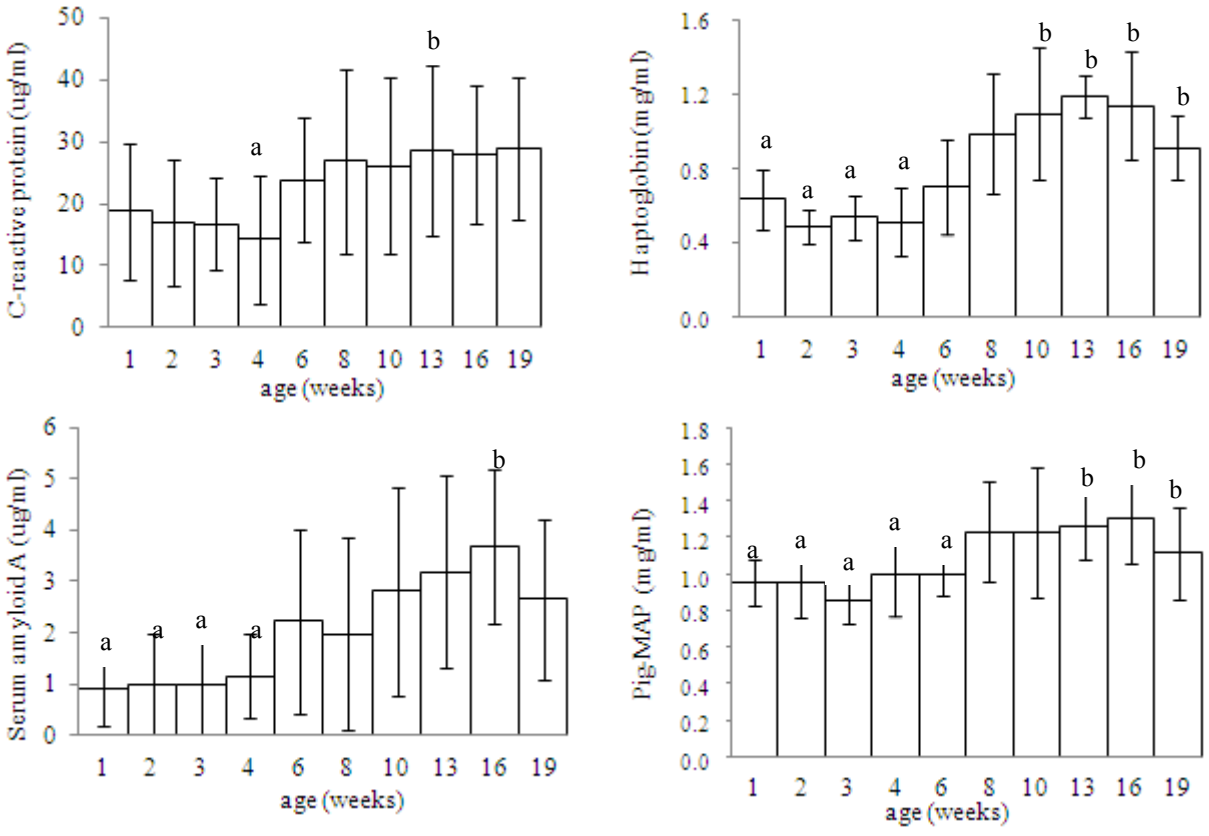


Fig. 1. Serum concentrations (mean ± SD) of investigated APP in pigs from 1 to 19 week of life. Different letters (a–b) indicate statistically significant differences ($P<0.05$) as determined by the nonparametric Friedman test.

Haptoglobin. The concentration of Hp also generally increased with age. Strong positive correlation was found, between concentration of Hp and the pigs' age (R-Spearman = 0.63, P<0.001). The increase in Hp serum concentration was from 0.63 ±0.16 mg/mL on the first week of life to over 1 mg/mL in older pigs (from 10 weeks of age). The short-term decrease observed before weaning (from 1 to 2 weeks of life) was not significant. Concentrations of Hp were the highest in pigs between 13 and 16 weeks of age. The levels of Hp observed from 10 weeks of life were significantly higher than in pigs before weaning (P<0.05). From the 10^{th} to 16^{th} week of life, the level of Hp in the serum was almost 2-fold higher than at the first weeks of life. In 19-week-old pigs, a slight decrease in Hp concentration was found. A positive correlation between concentration of Hp and pig-MAP (R-Spearman = 0.45, P<0.05) was also evidenced.

Serum amyloid A. The concentration of SAA also increased significantly with age. During the first 4 weeks of life, the mean levels of SAA were relatively stable and ranged from 0.92 to 1.16 μg/mL (P≥0.05). From 6 to 8 weeks of life, the concentration of SAA was 2-fold higher than before weaning. Significant differences were found between serum SAA concentration up to 4 weeks of life and those observed in 16-week-old animals (P<0.01). At this time, the level of SAA was over 3-fold higher than at the beginning. In 19-week-old pigs the concentration of SAA decreased to 2.02 μg/mL. A positive correlation was observed between concentration of SAA and the pigs' age (R-Spearman = 0.45, P<0.05).

Pig major acute phase protein. The mean concentration of pig-MAP ranged from 0.84 ±0.1 mg/mL to 1.30 ±0.24 mg/mL during the period of the study. The lowest value was observed in 3 weeks old piglets and the highest in 16 weeks old fatteners. No significant differences were observed between serum levels of pig-MAP in animals younger than 8 weeks (P≥0.05). Starting from 13 weeks of life, the mean concentrations of pig-MAP were significantly higher (P<0.05) as compared to the levels observed in animals younger than 8 weeks. The mean concentration of pig-MAP increased over 1.3-fold from 1 to 16 week of life. At the end of the study, the mean level of pig-MAP decreased slightly but not significantly. A positive correlation was found between serum concentration of pig-MAP and the pigs' age (R-Spearman = 0.45, P<0.001).

Discussion

Studies on APP of domestic animals have expanded greatly since the 2000s (2, 5, 11, 13, 23-25). This has largely been caused by the realisation that monitoring the concentrations of the APP can provide the means to assess the immune system response to disease, inflammation, or trauma (6, 7, 11) and on this basis to monitor animals' health status. It is clear, that before APP concentrations as the indicators of animals' health status will be accepted, and studies are needed to establish the range of concentration of APP in healthy pigs at different ages.

In the present study, the physiological concentration of four APP in the sera of pigs (from 1 to 19 week of age) was quantified using commercial ELISA kits. The results showed important changes in the protein concentrations during this period. The results obtained in this study represent the first reported data about changes in SAA and CRP concentrations from birth to slaughter in normal, apparently healthy pigs from commercial farm. However, it should be underlined that it is difficult to characterise a pig as an animal free of disease under field conditions, even when based on clinical and laboratory examinations. Therefore, we decided to examine pigs also in the slaughterhouse (post-mortem examination).

In accordance with the results of previous experiments (17, 26), a positive correlation between concentration of APP and age of pigs was found. In our study, most investigated APP showed maximum mean concentration between 13 and 16 weeks of age. The levels of most investigated APP decreased at 19 weeks of life, with exception of CRP. In accordance with Lipperheide *et al*. (11), and in contrast to Piñeiro *et al.* (20), no significant effect of gender on APP concentration in serum was found. However, in our study all males were castrated during the first 3 d of life.

Haptoglobin is one of the most studied APP in pigs and some data about its concentration at various stages of life have been published already (9, 16, 20). High levels of this protein were found in pigs affected with bacterial infections (5, 23, 24) or inflammation (9). The expression of this protein during foetal development in pigs is limited, but during the first days of life there is a significant increase in the serum concentration of Hp (13). Martin *et al.* (13), who investigated the concentration of selected proteins in sera of normal pigs during postnatal development, found that during the first days of life, an important increase in the levels of this protein, reaching values higher than those found in the sera of 6-month-old pigs, was observed.

In accordance to our findings, in the study of Martin *et al.* (13), a decrease in Hp concentration from 6 to 14 d of life was observed. However, the Hp concentration remained relatively constant until the time of slaughter, while in our study the subsequent increase of Hp concentration was found till 16 week of life, followed by a slight decrease in 19-week-old pigs. A possible explanation of these differences could be a different blood sampling schedule. In our study, the blood was taken every 2-3 weeks while in the study by Martin *et al.* (13), the blood was taken at 6, 14, 21, and 180 d of life. It is possible that between 21 and 180 d of life the concentrations of Hp may change significantly.

The mean Hp concentrations determined in 19-week-old pigs were slightly lower than those reported by Chen *et al.* (2) (1.47 mg/mL)) and Clapperton *et al.* (3) (1.2 mg/mL), and higher than the level observed by Martin *et al.* (13) (0.65 mg/mL) in pigs at similar age (5-6 month-old).

The changes in pig-MAP levels observed by Martin *et al.* (13) were very similar to those of Hp. At

birth, the concentration of Pig-MAP was very low and then increased rapidly to approximately 1.6 mg/mL at postnatal day 5, followed by a subsequent decrease from 3 to 26 weeks of life. In our experiment, a slight decrease in pig-Map concentration from 16 weeks of life was also evidenced; however, more sampling points revealed different kinetics of pig-MAP serum concentration during pigs' life. Similarly as in the study of Piñeiro *et al.* (20), a linear correlation between pig-MAP and Hp concentration was observed. However, in our study the concentration of both of these APP in pigs at respective age were slightly higher. In the study of Piñeiro *et al.* (20) in 4 to 12-week-old pigs, mean concentrations of pig-MAP were around 1 mg/mL, and about 0.8 mg/mL in finishing period. Haptoglobin concentrations increased in the course of time, from around 0.6 mg/mL at 4 weeks of age to 1.4 mg/mL at 12 weeks. In accordance to our study, mean values of around 0.9 mg/mL were observed in the finishing period.

As can been seen from SD values, great variations in CRP, Hp, and SAA concentrations were found. Other studies on pigs also reported a wide distribution of Hp concentrations in healthy pigs (20). The minimal individual variability at the same age was found in regard to pig-MAP.

To our best knowledge, this is the first study reporting serum levels of CRP and SAA in healthy conventional pigs from birth to slaughter. Moreover, up to now, there have been no data about concentrations of CRP and SAA in other healthy animals during their life. Only one report was published on this subject (12) but in this study changes in the CRP and SAA serum concentration of healthy piglets were investigated only during first 7 d of their life. As it was found, the concentration of CRP did not differ significantly during this period, while there was a significant effect of age on plasma SAA level. The level was elevated on days 1, 3, and 5. Subsequently, there was a significant reduction on day 7 (12). The authors concluded that it is possible that elevated levels of SAA result from the high cortisol concentrations found in piglets' serum just after birth. In the study of Chen *et al*. (2), who investigated the dependence of APP levels in the serum and swine health status, the concentration of CRP in healthy 6 to 7-month-old pigs was much higher than those observed in our experiment and reached 84 μg/mL. The possible reason of such high level of CRP observed in the previous report could be an early-stage acute condition, prior clinical or gross manifestation, which might be involved. The lower concentration (about 50 μg/mL), but still higher than in our experiment, was found by Pallares *et al.* (15) on 190-day-old healthy pigs. However, in this experiment, contrary to our investigation, the blood was collected after road transport of pigs to the abattoir. Thus, the influence of stress on the concentration of CRP cannot be excluded.

In our experiment, the concentration of CRP increased over 1.5 fold during the period of study. Similar increase was found by Hutchinson *et al*. (8) in human. In this study, the mean CRP level approximately was doubled with age, from ~1 mg/L in the youngest people (25-34 years of age) to ~2 mg/L in older ones (65-74 years of age), and tended to be higher in females. As it has been shown in our study, the CRP exhibited a similar kinetics as pig-MAP, although with greater variation within the same age group. The SAA concentrations profile was similar to those observed for Hp.

A significant increase in all APP was observed in pigs serum after weaning, constituting an important characteristic of this period. Weaning is a critical period in a pig's life. It is connected with the huge changes that take place in the pig's surroundings, and the exposure to many „new" antigens. During stress, cortisol provokes proteolysis in the muscles and amino acids, released due to protein degradation during the acute phase response, are utilised for APP synthesis by the liver (12). Therefore, the elevation of APP after weaning may be associated with stress induced by mixing animals after weaning or changes in the pattern of feed administration. The reason for the peak may be also the initiation of synthesis of APP by piglets. These data are in agreement with the results obtained by Piñeiro *et al.* (19), who investigated the effects of mixing animals on serum concentration of APP in growing pigs. The response of APP after weaning might be also a result of increasing expression of pro-inflammatory cytokines, which takes place in the gut post weaning (18).

On the basis of our study, we conclude that concentration of all investigated APP tended to increase with age. Positive significant correlations were evidenced with respect to serum concentration of CRP, Hp, SAA, as well as pig-MAP and the age of pigs. Because a statistically significant association between the age and APP concentrations exists, further studies are needed to decide whether the age may influence the diagnostic value of APP as a marker of infection. Additionally, studies are needed to estimate whether the APP response in infection is age-dependent to any clinical importance degree.

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References

1. Carpintero R. Alonso C., Piñeiro M., Iturralde M., Andres M., La Potier MF., Madec F., Alava M.A., Piñeiro A., Lampreave F.: Pig major acute phase protein and apolipoprotein A-I responses correlate with clinical course of experimentally induced African swine fever and Aujeszky's disease. Vet Rec 2007, **38**, 741-753.
2. Chen H.H., Lin J.H., Fung H.P., Ho L.L., Yang P.C., Lee W.C., Lee Y.P., Chu R.M.: Serum acute phase proteins and swine health status. Can J Vet Res 2003, **67**, 283-290.
3. Clapperton M., Bishop S.C., Cameron N.D., Glass E.J.: Association of acute phase protein levels with growth performance and with selection for growth performance in Large White pigs. Anim Sci 2005, **81**, 213-220.
4. Grau-Roma L., Heegaard P.M.H., Hjulsager C.K., Sibila M., Kristensen C.S., Allepuz A., Piñeiro M., Larsen L.E., Segalés J., Fraile L.: Pig-major acute phase protein and

haptoglobin serum concentrations correlate with PCV2 viremia and the clinical course of post-weaning multisystemic wasting syndrome. Vet Microbiol 2006, **138**, 53-61.

5. Heegaard P.M.H., Klausen J., Nielsen J.P., Gonzalez-Ramon N., Piñeiro M., Lampreave F., Alava M.A. The porcine acute phase response to infection with *Actinobacillus pleuropneumoniae*. haptoglobin, C-reactive protein, major acute phase protein and serum amyloid A protein are sensitive indicators of infection. Comp Biochem Physiol 1998, **119B**, 365-373.
6. Heegaard P.M.H., Stockmarr A., Piñeiro M., Carpintero R., Lampreave F., Campbell F.M., Eckersall P.D., Toussaint M.J.M., Gruys E., Sorensen N.S.: Optimal combinations of acute phase proteins for detecting infectious disease in pigs. Vet Res 2011, **42**, 50-62.
7. Hulten C., Johansson E., Fossum C., Wallgren P.: Interleukin 6, serum amyloid A and haptoglobin as markers of treatment efficacy in pigs experimentally infected with *Actinobacillus pleuropneumoniae*. Vet Microbiol 2003, **95**, 75-89.
8. Hutchinson W.L., Koenig W., Fröhlich M., Sund M., Lowe G.D., Pepys M.B.: Immunoradiometric assay of circulating C-reactive protein: age-related values in the adult general population. Clin Chem 2000, **46**, 934–938.
9. Lampreave F., González-Ramón N., Martínez-Ayensa S., Hernández M.A., Lorenzo H.K., García-Gil A., Piñeiro A.: Characterisation of the acute phase serum protein response in pigs. Electrophoresis 1994. **15**, 672–676.
10. Lauritzen B., Lykkesfeldt J., Skaanild MT., Angen Ø., Nielsen J.P., Friis C.: Putative biomarkers for evaluating antibiotic treatment: an experimental model of porcine *Actinobacillus pleuropneumoniae* infection. Res Vet Sci 2003, **74**, 261-270.
11. Lipperheide C., Diepers N., Lampreave F., Alava M., Petersen B.: Nephelometric determination of haptoglobin plasma concentrations in fattening pigs. J Vet Med A 1998, **45**, 543–550.
12. Llamas Moya S., Boyle L.A., Lynch P.B., Arkins S.: Age-related changes in pro-inflammatory cytokines, acute phase proteins and cortisol concentrations in neonatal piglets. Neonatology 2007, **91**, 44-48.
13. Martin M., Tesouro M.A., Gonzalez-Ramon N., Piñeiro A., Lampreave F.: Major plasma protein in pig serum during postnatal development. Reprod Fert Develop 2005, **17**, 439-445.
14. Orro T., Nieminen M., Tamminen T., Sukura A., Sankari S., Soveri T.: Temporal changes in concentrations of serum amyloid-A and haptoglobin and their associations with weight gain in neonatal reindeer calves. Comp Immunol Microb Infect Dis 2006, **29**, 79-88.
15. Pallares F.J., Martinez-Subiela S., Seva J., Ramis G., Fuentes P., Bernabe A., Muñoz A., Ceron J.J.: Relationship between serum acute phase protein concentrations and lesions in finishing pigs. Vet J 2008, **177**, 369-373.
16. Petersen H.H., Dideriksen D., Christiansen B.M., Nielsen J.P. Serum haptoglobin concentration as a marker of clinical signs in finishing pigs. Vet Rec 2002, **151**, 85-89.
17. Petersen H.H., Ersbøll A.K., Jensen C.S., Nielsen J.P.: Serum-haptoglobin concentration in Danish slaughter pigs of different health status. Prevent Vet Med 2002, **54**, 325–335.
18. Pie S., Lalles J.P., Blazy F., Laffitte J., Seve B., Oswald I.P.: Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. J Nutr 2004, **134**, 641-647.
19. Piñeiro C., Morales J., Piñeiro M., Lampreave F., Mateos G.G.: Effects of induced mixing and pen size on performance and serum concentration of acute phase proteins in growing pigs. J Anim Sci 2004, **82**, 505.
20. Piñeiro C., Piñeiro M., Morales J., Andrés M., Lorenzo E., del Pozo M., Alava M.A., Lampreave F.: Pig-MAP and haptoglobin concentration reference values in swine from commercial farms. Vet J 2009, **179**, 78-84.
21. Pomorska-Mól M. Acute phase proteins in pigs– the current state of knowledge. Medycyna Wet 2010, **66**, 732.
22. Pomorska-Mól.: Acute phase proteins in veterinary medicine: utility in diagnostics and monitoring of health status. Medycyna Wet 2010, **66**, 822-826.
23. Pomorska-Mól M., Markowska-Daniel I., Kwit K., Stępniewska K., Pejsak Z.: Kinetics of the response of four positive acute phase proteins in pigs experimentally infected with toxigenic *Pasteurella multocida*. Vet Microbiol 2011, **152**, 429-435.
24. Pomorska-Mól M., Urbaniak K., Markowska-Daniel I.: Porcine acute phase protein response to experimental infection with *Bordetella bronchiseptica*. Bull Vet Inst Pulawy 2011, **55**, 371-375.
25. Pomorska-Mól M., Markowska-Daniel I., Pejsak Z.: Acute phase protein response during subclinical infection of pigs with H1N1 swine influenza virus. Vet Microbiol 2012, DOI: 10.1016/j.vetmic.2012.04.030 – in press
26. Richter H.: Haptoglobin bei haussäugetieren. III. Mitteilung: der haptoglobingehalt im blutplasma und-serum von widerkaüern und sweinen unter verschiedenen physiologischen bedingungen. Arch Exp Vet Med 1974, **28**, 505–519.