

## THE USE OF ACUTE PHASE PROTEINS IN PIG PRODUCTION

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### Objectives

Acute phase proteins (APP) are proteins present in blood which respond to disease or other stressors by increasing or decreasing in concentration. The objective of Work Package 3 in the EU project was to study whether APP determination during production will be a valuable tool to indicate periods of reduced performance and clinical or subclinical disease prevalence with the aim that specific diagnostic methods and therapeutic approach would follow only if necessary. Furthermore to prove if APPs could be established as screening parameters for the health status of pigs during the production chain.

### Materials and Methods

As variations exist in the processing of pig production in Europe studies in different European countries were necessary in order to find out an appropriate production process oriented strategy for the implementation of APP in the pig production chain in Europe. Different farms and production systems were included in the study in order to find out an appropriate production process oriented strategy for the implementation of APP in the pig production chain in Europe.

In Germany three studies were performed. In the first study five variants of customer-supplier contacts were tested consisting of 20 piglet rearing farms involving the collection of about 1100 blood samples. In addition seven piglet breeding farms and one fattener farm were available for samples. Out of these farms five piglet breeding farms, 12 piglet rearing farms and one fattener farm were selected with a total of 680 blood samples. The selection was based on the amount of serum which was obtained from the farmers or the veterinarians respectively. In aim the study 2 to evaluate if the serum concentration of haptoglobin respectively pig MAP is correlated to the concentration of these APPs in meat juice which is routinely collected at slaughterhouse for salmonella testing. Slaughter blood and muscle samples for extraction of meat juices from 299 slaughter pigs were collected. Study 3 was a short study where the acute phase protein haptoglobin was used as a screening parameter in five fattening farms. The aim of this work was to examine if haptoglobin could be used for single point investigations on the farms instead of time-course analyses.

The four Danish studies are presented, too. In study 1, the design was a case-control study including 340 finishing pigs in 15 commercial Danish pig herds was carried out in order to study haptoglobin concentration in serum as an objective marker of different clinical signs. Rectal temperature and haptoglobin concentration in serum was compared as markers of clinical disease. Finishing pigs aged 10 to 25 weeks with different clinical signs were matched to control pigs without clinical signs with respect to herd, pen, estimated weight and gender. Each pig was subjected to a standard clinical examination and a serum sample was obtained. In 86 of the case-control pairs, the rectal temperature was also recorded. In study 2, a cross-sectional study was conducted with 617 finishing pigs aged 10 to 25 weeks in 11 commercial herds of different health status as defined by the Danish monitoring program for specific pathogen free (SPF) herds. A standard clinical examination was performed and a blood sample was obtained from each pig for determination of haptoglobin concentration in serum. In study 3, a cross-sectional study was performed in 98 commercial finisher pig herds. Clinical signs were recorded for up to 1000 pigs in each herd and haptoglobin concentration in serum was determined in 30 pigs per herd. In study 4, paired blood samples were obtained at the farm before transport and at the abattoir to investigate the effect of routine transport and lairage and chronic lesions ante mortem in APP serum concentration. Four studies were conducted in Spain, two of them to assess the effect of stressors commonly present in pig production, such as mixing or an inadequate feeding pattern. In study 1 the effect of mixing animals, from different pens in the nursery, at entry to the fattening barn, on the levels of APP (PigMAP and haptoglobin) was studied. The possible interactions between different number of animals per pen (8 or 12 pigs per pen) and mixing were also evaluated. In study 2, the effect of a changeable pattern of food administration in the growth performance and APP levels (PigMAP, haptoglobin, Apo-AI, SAA, CRP and TT) of growing pigs was evaluated in the growing phase (74-116 d of age). Two hundred and forty pigs (26.3 ± 0.4 kg BW) were distributed into two treatments: fed ad libitum (AL) or disorderly (DIS), consisting in the administration of feed following a disorderly pattern, alternating periods of AL administration with periods of no feeding. Total feed intake (FI) was kept constant in both groups. In

study 3, the APP response (PigMAP, haptoglobin, ApoAI, SAA, CRP and TT) after a long transport (12 h) under commercial conditions was evaluated. Twenty pigs were bled in the farm the day before transport, at the arrival to the slaughterhouse and at the slaughter-line (about 24 h after the beginning of the transport). The fourth study included the determination of the serum concentration of the APP pig-MAP, haptoglobin, CRP and ApoA-I in pigs from twelve commercial farms located in Segovia, Spain. For this, two blood samplings were performed to establish a range of concentration of these proteins in normal state, as well as to analyse the potential of these markers to evaluate the general health status of farms.

## Results

### APP in the rearing and fattening period (Germany, Study 1)

A significant influence of the origin could be observed for Haptoglobin (Hp), Pig Major Protein (PigMAP), C - reactive protein (CRP) and Alpha Lipoprotein (ApoA1). There was a strong significant relationship between the hygiene status of the breeding farms and the four APPs. Furthermore pigs causing costs of medical treatment above € 1.15 in the rearing period had significant higher Hp, PigMap and CRP serum concentration respectively and lower ApoA1 concentrations. Concerning the daily weight gain lower haptoglobin and PigMap serum concentration at final inspection in the rearing phase could be observed in the group with the higher daily weight gain. Nevertheless the correlation was not significant. In contrast to these results are the results for CRP and ApoA1. These two parameters behaved vice versa.

### APP in meat juice (Germany, Study 2)

A significant correlation between slaughter blood and meat juice of the pars costalis diaphragmatis was found for haptoglobin ( $p < 0.001$ ,  $r = 0.7$ ) respectively PigMAP ( $p < 0.001$ ,  $r = 0.86$ ).

### Haptoglobin in the fattening period (Germany, Study 3)

Due to the average increases of daily weight gain as well as the average losses of all farms the pigs were divided into two classes. It was obvious that pigs living in farms where higher performance (daily weight gain > 715 g) and lower losses (< 3,14%) could be realized showed significantly lower haptoglobin concentrations. Samples 2 and 3, which were taken in the middle and at the end of the fattening, should give information on the health status of the animals in the farm. Again it turned out that pigs with a daily gain above 715g had significantly lower haptoglobin concentrations, compared with animals with lower performance.

This method of blood sampling of pig groups of different ages on one day showed that the parameter haptoglobin is suitable not only for a time-course analysis, but also for a single point investigation in the farms.

### APP and clinical signs (Denmark, Study 1)

A substantial and significantly elevated mean haptoglobin concentration in serum was found in pigs with lameness ( $p < 0.0001$ ), respiratory disease ( $p = 0.0002$ ), tail or ear bite ( $p < 0.0001$ ) and diarrhoea ( $p = 0.02$ ). Similarly, a higher mean rectal temperature was found in pigs with lameness ( $p < 0.0001$ ), respiratory disease ( $p = 0.002$ ) and tail or ear bite ( $p = 0.0003$ ) when compared to the controls. A significant but low correlation between rectal temperature and haptoglobin concentration in serum was observed ( $p = 0.003$ ,  $r = 0.20$ ). Maximum simultaneously sensitivity (0.61 - 0.71) and specificity (0.61 - 0.77) of serum haptoglobin for the different clinical signs was found at a cut-off value of 1.1 mg/mL. When using a cut-off value of 1.8 mg/mL, the sensitivity decreased (0.31 - 0.60) and the specificity increased (0.82 - 0.86). The area under the ROC-curve was found to be 0.67 - 0.78 for the different clinical signs. Defining a cut-off value which classified individual pigs according to clinical signs was not possible.

### Health status & slaughter (Denmark, Study 2)

A significant difference in haptoglobin concentration between herds was observed. This difference was not only related to the health status declarations. Likewise, SPF-status combined with age was found to influence the haptoglobin concentration. Pigs aged 10 to 14, 15 to 19 and 20 to 25 weeks in conventional herds had a significantly higher haptoglobin concentration compared to SPF-x pigs of the same age ( $p = 0.01$ ,  $< 0.001$  and  $< 0.001$ , respectively). No difference between SPF-x pigs of different age was observed. Conventional pigs aged 15 to 19 and 20 to 25 weeks were found to have a higher haptoglobin concentration than conventional pigs aged 10 to 14 weeks ( $p = 0.005$  and  $0.01$ , respectively). Lamé pigs and pigs with tail or ear bite were found to have an elevated haptoglobin concentration in serum ( $P < 0.001$ ). No significant effect of respiratory symptoms or umbilical hernia was found.

### Risk factors in finishing pigs (Denmark, Study 3)

Pigs from herds with high prevalences of clinical signs of respiratory disease and diarrhoea and lame pigs had high serum haptoglobin concentration. Increasing levels of antibodies against *A. pleuropneumoniae* serotype 2 and *M. hyopneumoniae* were associated with increasing serum haptoglobin concentration.

Finishing pigs between 25 and 50 kg had high haptoglobin concentrations. Regional differences in serum haptoglobin concentration possibly influenced by different observers were found. Herds with up to 12 hours quarantine for visitors and with high stocking density had high haptoglobin concentrations. Herds with continuous production had higher haptoglobin concentrations than herds with batch production without all-in/all-out. Serum haptoglobin concentration was found to be a promising indicator of clinical and subclinical disease in finishing pigs.

#### **Handling/ transport & slaughter (Denmark, Study 4)**

An increase in haptoglobin and CRP serum concentrations were observed following a relatively short transport (1.3 h) and lairage of pigs. Whether this was due to sub-clinical tissue damage or haptoglobin and CRP production due to transport stress or other causes could not be concluded. Chronic lesions, which are commonly observed at slaughter without consequences for meat inspection, did not increase haptoglobin serum concentration significantly in the present investigation. This may be due to the small sample size. The study showed that blood samples for ante-mortem control in pig herds should preferentially be obtained before handling and transport to abattoir.

#### **Effect of bad management. Mixing. (Spain, Study 1)**

The day following placement at the fattening barn, mixed pigs had higher PigMAP values (2.07 vs 1.36 mg/mL;  $P=0.0006$ ) than non-mixed pigs and were still higher 5 d after placement (1.18 vs 0.90 mg/mL;  $P=0.04$ ), and the difference disappeared thereafter. No consistent pattern was observed for haptoglobin, but the concentration was always higher for mixed pigs being significant at d 14 (1.83 vs 1.31 mg/mL;  $P=0.05$ ). Males were more sensitive to both stressors than females and showed higher PigMAP concentration ( $P<0.05$ ) and lower growth ( $P<0.05$ ). As conclusion, mixing of pigs at the start of the fattening period induces stress independently of pen size and serum concentration of APP's can be used to detect the intensity of stress.

#### **Effect of bad management. Feeding. (Spain, Study 2)**

Average daily gain (ADG) was higher in AL than in DIS group (592 vs 548 g/d;  $P<0.01$ ). Differences in ADG between AL and DIS animals in the 74-88 d subperiod were due to the males (523 vs 398 g/d), having the females of both groups similar ADG. Differences in the behaviour of the APP were found between DIS males and females. In females, no differences were found between feeding patterns. However, in DIS males, PigMAP and haptoglobin remained elevated respect the AL ones at 88 and 102 d of age ( $P<0.05$  and  $P<0.01$  for PigMAP and  $P=0.11$  and  $P<0.01$  for haptoglobin). SAA was also numerically higher in DIS males at 88 d of age and CRP was higher ( $P=0.07$ ) at 102 d. The concentration of negative APP ApoAI, increased slightly with time in AL males, whereas in DIS males the concentration remained lower than in the AL. No differences were observed in TT. The acute phase index also showed differences at 88 d of age and DIS males had a higher index than AL ones (58.7 vs 5.7;  $P<0.05$ ), with no differences in females. These results confirm a good correlation between daily gain and APP serum levels. Hence, APP can be proposed as an interesting parameter to be used in the detection of situations that cause stress in the pig.

#### **Long distance transport (Spain, Study 3 )**

All the positive APP determined showed a significant increase of concentration after transport ( $P=0.0001$  for PigMAP and CRP,  $P=0.001$  for SAA and  $P=0.02$  for haptoglobin), being values obtained in slaughter line the highest. The magnitude of the change of concentration observed after transport varied from one protein to another. The concentration of ApoAI decreased about 30% in the day after transport ( $P=0.0001$ ), whereas transthyretin did not show significant changes respect the values obtained in the farm the day before transport. These results suggest that APP may be interesting parameters to evaluate welfare during transport, and useful tools in the evaluation of transport conditions. It is interesting to remark that the use of the acute phase index (PigMAP x CRP/ApoAI) improved the sensitivity in the detection of the APP response that follows transport.

#### **APP in commercial farms (Spain, Study 4)**

In the first study, there were no differences in the concentration of Pig-MAP or haptoglobin depending on the number of parturitions of the sows. In the case of the negative APP ApoA-I, higher levels ( $p<0.0001$ ) were found in sows having none parturition, being the rest of the values similar. In fattening pigs, PigMAP concentration tended to decrease slightly with age, whereas haptoglobin showed a slight slope at 12 weeks of life. From this study, a cut off value for normal state based on the mean plus 1.96 sd was established for reproductive sows and boars and for fattening pigs.

In the second study, the acute phase index (PigMAP x CRP/ApoAI) was compared in two farms of different health status. The APP concentration pattern obtained in the high-health status farm showed constant APP levels in all the growing period, whereas the presence of disease or stress due to bad managing conditions would alter this APP pattern in the low-health status farm, that could be used in the

detection of problems in the farm. As conclusion, APP may become an useful tool when evaluating the general health status of farms, and the APP index increases the sensitivity.

### **Conclusions**

The project, divided in three work packages (WP) has come to a successful conclusion achieving its milestones and producing the deliverables expected. From the WP1, the assessment APP as markers of immunological stress, nutrition and the acute phase was positively validated. From the WP2 the identification of the best combinations that can be employed to identify animals with infectious/inflammatory processes was obtained. The establishment of the acute phase index (CRP x PigMAP) / (ApoA1) increase the robustness of analysis, whereas the combination of Hp, ApoA1 and PigMAP was also validated.

The studies performed in WP3 at differing stages of the pig production process and in different countries with differing production systems were able to demonstrate the usefulness of APP analysis in the production chain. There are numerous examples from the studies undertaken that demonstrate the value of APP analysis. However, the full potential of their measurement will need much further investigation as this project is undoubtedly only a starting point for our full understanding of how APP assay will be used to enhance the identification of immunological stress in pigs during production. The full range of APP was not measured in every study due to the interactions of time and resource availability. Nevertheless there were a number of fascinating discoveries made in the course of the project some of which were predictable from prior knowledge of the acute phase response in other species while others could suggest an even wider role for their analysis. In the former category of result would be the investigations confirming the APP are raised in animals with clinical conditions such as respiratory lesions, lameness or diarrhoea and that mean levels can vary between (especially rearing) farms with differing levels of hygiene. Economic implications of the results are also evident from the association between high positive APP (low negative APP) and the future cost of medical treatment. Important issues of welfare are relevant to the findings that stress of moving to new accommodation can cause an increase in APP and also the interesting observation that there is a sex difference in the response to disorderly feeding with males giving an APP response absent in females.

In conclusion the project has delivered a range of methods to monitor positive and negative acute phase protein and has utilised them to establish a core group of proteins to be assessed for their value in quantification of the acute phase reaction and immunological stress in pigs. These tests are already showing their ability to monitor health and disease in pigs through the production chain but there is a need for further extensive national and international studies to fully understand and exploit their diagnostic potential.

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