BIO-MARKERS MEASURING HEALTH STATUS AND MANAGEMENT TOOLS TO IMPROVE PRODUCTIVE PERFORMANCE AND ANIMAL HEALTH ON SWINE COMMERCIAL FARMS

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BIO-MARKERS MEASURING HEALTH STATUS AND MANAGEMENT TOOLS TO IMPROVE PRODUCTIVE PERFORMANCE AND ANIMAL HEALTH ON SWINE COMMERCIAL FARMS

（養豚農場における健康状態を測定するバイオマーカーと生産成績

と健康状態を向上させる管理ツール）

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BIO-MARKERS MEASURING HEALTH STATUS AND MANAGEMENT TOOLS TO IMPROVE PRODUCTIVE PERFORMANCE AND ANIMAL HEALTH ON SWINE COMMERCIAL FARMS

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF MEIJI UNIVERSITY
BY

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ABSTRACT

Current swine production is led by competitiveness worldwide. This must be understood as achieving highest possible efficiency (combined pigs produced per sow per year with pig growth rate and feed efficiency) under high quality standards including compliance with welfare, control of pollutant emissions and food safety assurance including reduced or even zero use of antibiotics.

For this purpose, traditional methods of raising pigs cannot be enough and the need of new procedures and tools arise. Among the proposals in the last decade, the biomarkers and new procedures have been used to detect early problems or risks that can lead to later problems, lack of efficiency, loss of quality or animals delivered outside market specifications.

Chapter 1 reviews biomarkers in the swine industry. Biomarkers have been studied during the last decades as candidates to detect stress, losss of performance, subclinical disease of pigs or even for early warnings. Probably the most reliable group recently characterized have been acute phase proteins (APP), which are plasma proteins whose hepatic synthesis rate is significantly modified under stress situations. The plasma concentrations of these proteins may be decreased (in which case they are called APP-negative, such as prealbumin, transferrin, albumin and others), or increase considerably (APP-positive). Among the positive APP are C-reactive protein, serum amyloid A, pig major acute phase protein (Pig-MAP) and haptoglobin.

In Chapter 2, two of the most relevant biomarkers were studied (Pig-MAP and haptoglobin) to determine the respective reference levels on commercial farms, and then in a later experiment the impact of induced stressors on plasma levels and productive performance was investigated. Blood samples were collected from 270 sows and 450 pigs with 5 nursery to finishing stages on 10 commercial farms, and 40 boars in two boar stud centers. Pig-MAP serum

concentrations were lower in sows than in adult boars (mean values 0.81 vs. 1.23 mg/mL) while the opposite was observed for haptoglobin (1.47 vs. 0.94 mg/mL). No differences were found between parities, except for a minor decrease in haptoglobin concentrations in the 4th parity. A linear correlation was observed between Pig-MAP and haptoglobin concentrations. In pigs between 4–12 weeks old, the Pig-MAP mean concentration was around 1 mg/mL, being lower in the finishing period from 13 to 20 weeks of age (0.7–0.8 mg/mL). Haptoglobin concentrations increased with time, from around 0.6 mg/mL at 4 weeks of age to 1.4 mg/mL at 12 weeks. The mean value was around 0.9 mg/mL in the finishing period. A wider distribution of values was observed for haptoglobin than for Pig-MAP concentrations. Differences between herds were observed, with the highest values obtained in a herd with signs of respiratory disease.

In Chapter 3, an experiment was carried out to determine the impact of stressors on APP levels and productive performance. A total of 240 pigs, that were 74 d old and comprised half boars and half females, were included in a trial designed to assess the effect of the stress caused by changes in the pattern of food administration on the concentrations of APP and productive performance parameters. Half of the animals (pigs fed ad libitum, AL group) had free access to feed, while the other pigs were fed in a disorderly pattern (DIS group), in which animals had alternating periods of free access to feed and periods of no feeding, when food was removed from the feeder. The periods of free access to feed (two daily periods of 2-hour duration) were randomly assigned, and varied from day to day. Total feed supplied per day was identical in both groups, and exceeded the minimal amount required for animals of these ages. Pen feed intake, individual body weights and the main positive pig APP and Pig-MAP, haptoglobin, serum amyloid A, C-reactive protein concentrations, as well as negative APP apolipoprotein A-I (ApoA-I) and transtherytin concentrations were determined every 2 weeks between 76 - 116 d of age. Animals in the AL group had better average daily gain (ADG) than DIS animals throughout the whole experimental period ($P < 0.01$), but the differences in ADG only occurred in the two first experimental sub-periods (60 to 74 and 74 to 116 d of age),

suggesting that the stress diminished when the animals got used to the DIS feeding. Interestingly, differences in ADG between DIS and AL pigs only occurred with males, whereas no differences were observed between females. The same differences observed for ADG were found for APP in DIS males, which had higher Pig-MAP concentrations than AL males at 74 and 116 d of age, lower ApoA-I concentrations at 74 d of age and higher haptoglobin and C-reactive protein concentrations at 116 d of age ($P < 0.05$). The results obtained in this trial show an inverse relationship between weight gains and APP levels, and suggest that APP is a biomarker for the evaluation of distress and welfare in pigs.

In Chapter 4, a protocol based on parity segregation of the progeny was assessed, including productive performance, immunoglobulin (IgG) and APP serum concentrations from birth to slaughter. A total of 20 sows, 10 primiparous (PP) and 10 multiparous (MP; from 3rd to 5th parity), were used to study the effect of parity of the gestation sow and of the lactation sow on growth of piglets from birth to slaughter and their health status. There were 4 treatments in a 2 x 2 factorial arrangement design, with piglets from PP sows suckled by PP or MP sows, and piglets from MP sows suckled by PP or MP sows. Growth performance was controlled from weaning to 144 d of age and concentrations of IgG and Pig-MAP were measured as markers of health status throughout the study. Parity of the gestation and lactation sow affected growth performance of the pigs to different extents depending on the experimental period (28-76, 76-116, 116-144 d). Total ADG was higher in piglets born from MP sows than in those born from PP (669 vs. 605 g/d; $P = 0.001$), and was also higher in piglets suckled by MP sows than in piglets suckled by PP sows (655 vs. 620 g/d; $P = 0.037$). Total G:F tended to be higher for pigs suckled by MP sows than for those suckled by PP sows (0.43 vs. 0.41 g/d; $P = 0.076$). At weaning, IgG serum concentrations were higher ($P = 0.013$) in pigs suckled by MP sows than in piglets suckled by PP sows. However, IgG concentrations were also higher for pigs born from PP sows than for pigs born from MP sows at 116 d ($P < 0.001$) and 146 d of age ($P = 0.088$). The Pig-MAP tended to be lower in pigs suckled by MP sows than in pigs suckled by PP sows

at d 40 (P = 0.070) and 60 (P = 0.089) of age. The results suggest that both gestation and lactation sows are important for growth performance and health status of the offspring.

In Chapter 5, a new management protocol called Individual Pig Care (IPC) to control the impact of the acute phase reaction by means of the early detection of immunological stress due to infectious diseases, was assessed both in a small group of pigs and at large scale in more than one million pigs in several countries. Both in the nursery and in the growing periods, average daily gain (ADG) and average daily feed intake (ADFI) was higher and feed conversion ratio (FCR) was lower in IPC pigs than in the control group ($P < 0.05$). Body weight (BW) homogeneity tended to be higher in IPC than in the control group at d 40 and 67. Final BW was higher in the IPC group than in the control group. Moreover, application of IPC to more than one million pigs on 160 farms showed epidemic curves and mortality patterns for every disease, resulting in useful information for vets and manager that can be delivered real-time.

In conclusion, this thesis shows that acute phase proteins, and Pig-MAP in particular, are useful biomarkers indicating health and welfare status of pigs on commercial farms. Also, the thesis provides some references for Pig-MAP concentrations in nursery-growing pigs, finisher pigs, adult boars and sows on commercial farms. Furthermore, this study indicates that growth performance and health status of the offspring of PP sows can be improved by cross-fostering with MP sows. Finally, individual care and treatment should be emphasized in order to improve health status of pigs and reduce antimicrobial treatment on commercial farms.

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CHAPTER 1

LITERATURE REVIEW

ABSTRACT

During the last two decades, there has been a clear trend in animal production aiming to meet growing quality standards related to several aspects, such as animal welfare, environmental pollution or food safety. This trend is either based on national and international regulations (European Commission) and consumers' desire to know how the meat they eat is produced. On the other side, productive sector must achieve higher efficiency every day to maintain competitiveness, either in reproduction or productive performance (growth and feed efficiency). To cope with this situation, the sector is changing its approach, adopting new ways of production like group sows, best available techniques to control pollutant emissions or protocols to control and limit antibiotic usage, normally using prevention rather than later control or curing.

Most of the problems that pigs must face on farms are related to stress, being either the cause or its consequence. Stress can be defined as the biological response obtained when an individual perceives a threat to their homeostasis. Once the animal perceives a stressor as a threat, it reacts with different responses, which may affect its behavior, the autonomic nervous system, the neuroendocrine system or the immune system. It is the so-called acute phase response, which comprises a set of complex mechanisms that start immediately after the stimulus, producing endocrine, physiological and metabolic changes. All this has a biological cost, since the animal diverts energy that should have been devoted to growth or reproduction to attend to the stress.

Plasma proteins whose hepatic synthesis rate is significantly modified in these situations are called acute phase proteins (APP). The plasma concentration of these proteins may decrease (in which case they are called APP-negative, such as prealbumin, transferrin, albumin and others), or increase significantly (APP-positive). Among the positive APPs in humans are C-reactive protein, serum amyloid A, acid α1-glycoprotein (GPA), some protease inhibitors, haptoglobin and fibrinogen.

There are different types of stress, including social stress due to mixing, regrouping, transport or available space, environmental stress because of inadequate levels of temperature, humidity, light or gases or lack of certain materials, such as bedding, paper or straw, metabolic (feed or water restriction), or immunological stress, mainly related to infectious diseases, viral or bacterial, or transport. All of them generate different degrees of acute phase response and variation in the levels of acute phase proteins. These proteins can be determined either in serum or meat juice since there is a significant correlation between both levels and they have been strongly correlated with health (type and extension of lung lesions) and carcass meat quality, even having a predicting value since levels detected at 13 weeks of age predict later carcass quality.

Introduction

The livestock sector is moving into a new paradigm where new rules and requirements are defined. During the last few decades, the main objective was to produce meat in the cheapest and most efficient way, and little restrictions were set around that. This was affecting different topics around production that were steadily evolving including, food safety, animal welfare and pollutant emissions. In 2006 the European Union banned growth promoters, which was the final step in the phasing out of antibiotics for non-medicinal purposes. In 2004, the first draft of the BREF to define Best Available Techniques to control pollutant emissions from pigs and poultry farming was released, and it has been recently updated in 2017 (EU Commission 2017). In addition, and with the support and close co-operation of the Member States, the European Commission has been promoting animal welfare for over 40 years, gradually improving the lives of farm animals. An important step was the 1998 Council Directive 98/58/EC on the protection of animals kept for farming purposes, which gave general rules for the protection of animals of all species kept for the production of food, wool, skin or fur or for other farming purposes, including fish, reptiles or amphibians. These rules are based on the European

Convention for the Protection of Animals kept for Farming Purposes and they reflect the so-called 'Five Freedoms':

- Freedom from hunger and thirst
- Freedom from discomfort
- Freedom from pain, injury and disease
- Freedom to express normal behaviour
- Freedom from fear and distress

When the Lisbon Treaty came into force in 2009 it amended the 'Treaty on the Functioning of the European Union' (TFEU) and introduced the recognition that animals are sentient beings. Article 13 of Title II states that:

"In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions and customs of the Member States relating in particular to religious rites, cultural traditions and regional heritage."

It is clear that these topics are prominent in the political agenda in the EU and slowly being adopted on other continents. This trend is based on scientific facts, ethic concepts and consumers' opinions, where quality of production, food quality and safety are central issues in today's food economics. This is affecting even the willingness to pay an extra price for food safety, quality and environmental attributes (Grunert, 2006).

So, a big challenge for the industry is how to keep these quality standards and respect the regulations, while at the same time keep or improve the efficiency that producers need to

remain competitive in the market. The reaction has been around adapting its working schemes to improve efficiency and quality by means of different approaches (multisite production, medicated or segregated early weaning, more strict biosecurity protocols, feed additives of very different types including probiotics, prebiotics, essential oils and enzymes among others). It is interesting that certain countries have achieved the highest efficiency in terms of pigs produced per sow per year, under the highest ever quality standards, (Denmark or The Netherlands, Hoste, 2017) or adopted a national strategy to differentiate themselves from competitors, by implementing regulations even stricter than EU requirements (Denmark and The Netherlands, for welfare and environment).

Many of the factors that contribute to a loss of efficiency on farms are related to stress that can affect animal health. This was defined as the absence of disease, the normal functioning of an organism and as normal behavior by Baker and Greer (1980). In livestock, health can also be defined as the state allowing the highest productivity (Gunnarson, 2004). This definition often is enriched by concepts of a balance between the animal and its environment, and of the animal's welfare. Changes in modern veterinary medicine are linked to this broader definition. Veterinary medicine is focusing increasingly on prevention rather than cure, and this makes the animal's environment and welfare important factors (Ducrot et al., 2011).

Once the animal perceives a stressor as a threat, it has various responses, which may affect its behavior, the autonomic nervous system, the neuroendocrine system or the immune system (Baumann and Gauldie, 1994). It is the so-called acute phase response, which comprises a set of complex mechanisms that start immediately after the stimulus, producing endocrine, physiological and metabolic changes (Johnson, 1997, Heinrich et al., 1990). These changes involve increased protein catabolism, increased gluconeogenesis, altered lipid metabolism, proteolysis with negative nitrogen balance, and decreased lipogenic enzymes. All this has a biological cost, since the animal uses energy to attend to the stress that should have been devoted to growth or reproduction (Moberg, 1992). The body's response appears to be

quantitative, with correlation between stress intensity and metabolic change (Eurell et al., 1992).

To date, there are very few objective parameters that have been applied to the analysis of animal welfare and pig production yield. In the last decade, there have been different attempts to quantify the response to stress through different endocrine, behavioral, autonomic nervous system or immunological measures, although none of them has clearly demonstrated its efficacy. One of the main reasons may be the great individual variability in the response, which can be explained, among other factors, by genetics, age or previous experience (Moberg, 2000). Another factor that complicates such studies is the difficulty in obtaining samples, since sometimes the method itself can cause stress and produce measurable physiological effects (fixation, bleeding). These factors are most evident in studies developed under commercial conditions.

Plasma proteins whose hepatic synthesis rate is significantly modified in these situations are called acute phase proteins (APP). The plasma concentration of these proteins may decrease (in which case they are called APP-negative, such as prealbumin, transferrin, albumin and others), or increase significantly (APP-positive). Among the positive human APPs are C-reactive protein, serum amyloid A, acid α1-glycoprotein (GPA), some protease inhibitors, haptoglobin and fibrinogen (Steel and Whitehead, 1994). Cortisol has been largely used but can be influenced by factors such as circadian rhythm and genetics that could limit its use as a stress biomarker (Desautés, 1997). Cortisol concentrations follow a circadian rhythm, being morning levels up to 40 % higher than afternoon ones (Ruis, 1997), although a peak during the afternoon has been observed by several authors (de Leew, 2004). Moreover, the average concentration of cortisol in pigs decrease with age, reaching a stable profile around 20 weeks of age, when levels were about 37 % lower than at 12 weeks of age (Ruis, 1997). In addition, gender is another source of variation, with concentration in barrows being about 15 % higher than in gilts (Ruis, 1997).

The types of APPs vary between species (Gruys et al., 1994) and concentrations of

positive APPs can increase relative to healthy animals, by 2 to 4 times for APPs like GPA, fibrinogen and haptoglobin, and even by tens of times, such for C-reactive protein and serum amyloid measured in humans. During the 1990s, some authors proposed that the measurement of different APP in the blood of pigs may be of great practical interest for such an analysis (Saini and Webert 1991; Toussaint et al., 1995; Francisco et al., 1996). Thus, animals or production systems in which operating conditions, other than clinical or subclinical reasons, lead to an acute phase process, would be more easily identified. APP determinations would allow comparison of different production systems to identify operating factors or procedures that cause greater stress on pigs, which would serve to evaluate and improve production systems. APP can detect infectious diseases before of the appearance of clinical symptoms (Sorensen et al., 2006) and even discriminate the severity of the lesions on certain diseases like pneumonia (Saco et al., 2011). Furthermore, correlates with the clinical course and viremia of diseases like porcine circovirus type 2 (Grau-Roma et al., 2009). Furthermore, the optimal combinations of acute phase proteins for detecting infectious diseases in pigs have been defined by Heegaard and coworkers (2011). Several studies suggested the utility of haptoglobin to identify batches of animals in which poor hygiene and poor management have induced immune stress in animals, characterized by poor response to vaccinations and a reduction in efficiency in food conversion (Lipperheide et al., 1998, Petersen et al., 2002, Nielsen and Petersen, 2003).

Pigs face many different types of stress while they are being raised. One of the most accepted definitions of stress is “the biological response elicited when an individual perceives a threat to its homeostasis” (Moberg, 2000). Various classifications of stress have been described in the literature. From a practical point of view, it is of interest to classify the stress according to its duration and also, to its causes. Regarding the duration of the stress, it can be acute (short in duration; lasting minutes or various d) or chronic (lasting weeks, months or even years). In addition, depending on its cause, the stress can be classified as social, environmental, metabolic, immunological or transport.

Social stress

Pigs are regrouped at different times in their productive life, such as during gestation or after weaning, during the fattening period or before transport to slaughter. In these situations, it is usual that pigs fight to establish a new dominance hierarchy and this generates stress (Pitts et al., 2000). This can be acute, immediately following regrouping, or chronic, as a consequence of repeated social regrouping (Coutellier et al., 2007) or when the animals are socially submissive or isolated (Arnone and Dantzer, 1980).

Social stress can vary depending on the group size, since larger groups seem to have less social stress than smaller groups, reflecting a lower probability of monopolizing resources as the group size increases (Andersen et al., 2004). Available space affects stress because less available space per animals restricts movement and access to feed (Verdon et al., 2015). In both group-housed sows (Remience et al., 2008) and fatteners, the growth rate decreases linearly as the space allowance per pig decreases (Randolph et al., 1981). Finally, gender can affect the social response to stress, with higher stress in males than in females (de Groot et al., 2001, Piñeiro et al., 2007a).

The effect on APP has been demonstrated by Piñeiro and coworkers (2004) where induced mixing in piglets after weaning increased the serum concentration in the post-weaning period.

Environmental stress

Environmental factors are one the key stressors in intensive pig farming. Control of temperature, humidity, light, and gas concentrations (mainly ammonia and CO_2) is routine in the industry using sophisticated farm equipment, looking to keep the values within a non-stressful range (temperature, humidity, light) or under a threshold (gas) and defined for every production phase. However, sometimes optimal environmental conditions cannot be maintained on farms and this produces stress in animals. For example, in areas where there are extreme hot

or cold seasons, in farms located in areas of high noise or in the case of equipment failure. In addition, a lack of certain materials (bedding, paper or straw) that prevent natural behavior (e.g. nesting before farrowing) could be another source of stress (Oczak et al., 2015). Ammonia has been described as able to increase the levels of APP from a certain threshold (von Borell et al., 2007).

Quite often, stressors do not act alone, and pigs in commercial conditions can be subjected to 2 or more stressors at the same time. When this happens, the effect of individual stressors can be additive, as Hyun and coworkers demonstrated (Hyun et al., 1998), combining high stocking density, high temperature and regrouping. Stressor additivity was further corroborated by examining the effect of stressors imposed simultaneously, because when the number of stressors increased from 0 to 3, ADG, average daily feed intake (ADFI) and gain:feed (G:F) decreased linearly. Similar results were obtained by Ekkel and coworkers (Ekkel et al., 1996), who demonstrated that health, welfare, and production performance of pigs were improved when pigs were kept in a specific stress-free housing system where they were not mixed or transported.

This inhibition of growth is linked to pro-inflammatory cytokines, secreted from a variety of cells, including activated macrophages, and the secreted cytokines alter the host's metabolism. Three of these cytokines (tumor necrosis factor alpha [TNF-alpha], interleukin-1 [IL-1], and interleukin-6 [IL-6]) have profound behavioral, neuroendocrine, and metabolic effects. Moreover, in laboratory animal species, IL-1, IL-6, and TNF-alpha have been found to modulate intermediary metabolism of carbohydrate, fat, and protein substrates, regulate hypothalamic-pituitary outflow, and act in the brain to reduce food intake (Johnson, 1997).

Metabolic stress

Feed or water restriction can also generate stress (Toscano et al., 2007). Group-housing can result in submissive sows having less access to feed (Brouns and Edwards, 1994). The same

scenario of restricted feeding can generate chronic hunger in sows (Arellano et al., 1992). Recently, feed deprivation has been described in broiler chickens as able to generate increases in APP representing a chronic stressful situation (Najafi et al, 2016).

Immunological stress

Immunological stress is the status of an animal with an active immune system when they are challenged by bacteria or virus. It is associated with immunological, neurological, and endocrinological responses (Song et al., 2014). Stress can be considered as a possible cause, but also a consequence of infectious diseases. Such stress produces changes in numbers and proportions of blood leukocytes (Tuchscherer et al., 2009), natural killer cell cytotoxicity and circulating inflammatory factors (Wrona et al., 2001), which can result in increased susceptibility to any infectious disease (Wirtz et al., 2007).

Acute phase proteins have been studied in different infections, either bacterial, viral or mixed. Within the bacterial diseases, an induced controlled infection with *A. pleuropneumoniae* showed clear evidence that pig haptoglobin, C-reactive protein, pig major acute phase protein (Pig-MAP) and serum amyloid are prominent acute phase proteins (Heegaard et al., 1998). Similar results were found for another important pathogen in pig production, *S. suis*, which can generate meningitis, arthritis or be part of the so-called swine respiratory complex (SRC) by Sorensen and coworkers (2006).

The APP response to an infection caused by *H. parasuis*, the etiological agent of Glässer's disease in pigs, was characterized by Martin de la Fuente and coworkers, in 2010, by measuring serum concentrations of Pig-MAP, haptoglobin, C-reactive protein and apolipoprotein A-I in colostrum-deprived pigs. In this study, the APP response reflected ongoing Glässer-disease, showing a correlation between the severity and duration of clinical signs and lesions and the magnitude of changes in the APP levels.

Viral diseases have also been reported to generate an acute phase reaction, estimated through the levels of acute phase proteins. Segalés and coworkers (2004) determined the serum concentration levels of haptoglobin and Pig-MAP in postweaning multisystemic wasting syndrome (PMWS) affected pigs and porcine circovirus type 2 (PCV2) -subclinically infected pigs. The study showed that mean haptoglobin and Pig-MAP levels were significantly increased in clinically PMWS-affected pigs when compared to levels found in healthy pigs, but not in infected pigs who weren't showing clinical symptoms, suggesting that APP levels are significantly increased in pigs that develop PMWS, but not in animals with a PCV2 subclinical infection. Grau-Roma and coworkers, in 2009 demonstrated a significant correlation between PCV2 loads and both Pig-MAP and haptoglobin concentrations in serum of PMWS affected pigs, indicating that the acute phase response in PMWS affected pigs occurred concomitantly to PCV2 viremia.

Swine influenza caused by H1N1 is often complicated with secondary bacterial pneumonia, including *P. multocida*. Pomorska-Mól and coworkers (2013) studied the APP concentrations several d post infection (dpi) in pigs with clinical signs and viral shedding infected with the 2 pathogens. The concentration of C-reactive protein increased significantly at 1 dpi compared to control pigs, and remained significantly higher until 3 dpi. Also, the level of serum amyloid was significantly higher from 2 to 3 dpi, haptoglobin was significantly elevated from 3 dpi to the end of the study, and Pig-MAP elevated from 3 to 7 dpi. The concentrations of C-reactive protein, haptoglobin and serum amyloid significantly increased before specific antibodies were detected. Positive correlations were found between serum concentration of haptoglobin and serum amyloid and lung scores, and between clinical score and concentrations of Pig-MAP and serum amyloid. These results of current study confirmed that monitoring of APP may reveal ongoing infection, before antibodies can be detected, which would let farmers identify infected pigs before they are transported to an uninfected herd.

Enteric pathogens such as *E. coli* can induce an acute phase response (Morales et al., 2015), the intensity of which varies depending on the treatment used to control it, which will effect productivity, just like other non-infectious stressors.

Stress is also a factor increasing pathogen shedding in animals (Hussein et al., 2001). Jones and coworkers, (2001) reported that weaning, mixing and cold stress in piglets resulted in increased shedding of enterotoxigenic *E. coli* (in a disease challenge model) without affecting humoral immunity. Lack of food may also influence gastric function and shedding of pathogens. Following a 48-h fast, pigs (miniature) were found to have increased numbers of *C. jejuni* in their gastrointestinal tract. These differences were no longer present 5 d after the fast. No difference was seen in *C. jejuni* following a 4-H transport (Harvey et al., 2001). Fasting has also been observed to increase cecal concentration of *E. coli* (Nattress and Murray, 2000) and Salmonella in pigs.

Stress by transport

Transportation is inherently stressful for pigs, and many times complicated by unavoidable circumstances such as loading and unloading, vibration, new environment, overstocking density, mixing with other pigs, poor ventilation and deprivation of feed and water. All these factors can impair welfare and impact meat quality. Furthermore, they can increase consumer concerns about the way the meat they eat is produced.

It has been suggested that a combination of different measures, including behavioral and physiological, will provide the best estimation of animal discomfort (Grandin, 1997). Saco and coworkers (2003) described the effect of two pre-slaughter treatments: a) short-duration transport (1 h 15 min transport and 2 h lairage); or b) long-duration transport (6 h transport and 14 h lairage) in pigs susceptible or not to halothane sensitivity (NN or Nn). The study showed that the short-duration transport did not modify the levels of haptoglobin or of Pig-MAP, whereas cortisol was increased just after transport. In contrast, there was an increase in

haptoglobin and Pig-MAP in animal serum after long-duration transport, as observed in the post-mortem samples (20–21 h after the beginning of transport); cortisol levels were not increased in these conditions. In this experiment, homozygotes for the halothane gene tended to have higher values of haptoglobin after slaughter than did heterozygotes.

Piñeiro and coworkers showed that the quality of the transport is important (Piñeiroet al., 2007b). Results showed that when pigs were transported in higher quality in the truck that transported the pigs the increase of APP, Pig-MAP in particular, were lower than in those pigs transported in standard conditions, compared with the levels found one month after the arrival. These results showed the importance of the quality of the transport in the intensity of the acute phase reaction. Similar results were found by Salamano and coworkers (2008), indicating that not only transportation but the stress of adapting to a new accommodation can increase APP concentrations.

Abattoirs are a critical point to determine the levels of acute phase proteins, since the levels can be related to health and performance of the animals delivered from farms. One of the first authors to demonstrate an interest in measuring acute phase proteins at the abattoir was Yamane and coworkers in 2006. They found that Pig-MAP was 7-fold higher in wasting pigs than in normal abattoir pigs. Sometimes a high accuracy can be achieved in terms of investigating and discriminating lesions by means of the APP. Saco and coworkers (2011) tried to investigate the relationship between the existence of lung lesions in pigs at slaughter and the concentrations of the serum APP, haptoglobin, Pig-MAP and C-reactive protein, measuring cranio-ventral consolidation or pleuritis, with or without presence (positive or negative) in a large study involving 24 farms. All APP concentrations were significantly higher for pleuritis positive farms than for negative ones. However, only haptoglobin and Pig-MAP showed significantly higher concentrations for cranio-ventral consolidation positive farms than for the negative ones. Pig-MAP was the most sensitive biomarker since it was able to clearly discriminate between cranio-ventral negative and positive and pleuritis negative or positive

($p<0.001$ in both cases). It is interesting how a large study run under field conditions can discriminate so accurately the type and intensity of lung lesions in swine herds at slaughter just using APP's.

Probably the most relevant work is from Klauke and coworkers in 2013, showing the coherence of health, welfare and carcass quality in pork production chains. The authors sampled pigs between 30 - 105 kg BW and found significant correlations for growth (negative), feed efficiency (positive), intramuscular fat (positive) and water content in *L. dorsi* (negative). Also, they found a negative correlation with dressing yield, the weight of the loin and a positive correlation with the size of the belly. A total of 94 significant correlations with performance, carcass and meat quality where found (57 Pig-MAP, 37 haptoglobin). It is interesting to study their findings regarding the correlation with organ abnormalities. The threshold was defined as above 0.8 mg/ml for haptoglobin and/or 0.7 mg/ml for Pig-MAP for pigs with the respective increased risk of organ abnormalities being 16.00 and 10.58 times higher. These values are similar to those in our first study described in chapter three. All the effects described by Klauke and coworkers (decrease in performance, slaughter weight, dressing percentage, the weights of primal cuts and lean meat content), due to increasing APP concentrations might be caused by the presence of disease and the biological cost of the acute phase reaction. The changed metabolism results in negative energy balance (Gruys et al., 2005). Thus, the effects lead to catabolic metabolism in pigs with increased APP concentrations and so growth retardation is ameliorated (Knura-Deszczka, 2000). Eurell and coworkers, (1992) and Gymnich and coworkers (2004) proved the negative correlation between haptoglobin concentrations and weight gain in fattening pigs. Increased APP concentrations were correlated with an increase of subcutaneous and intramuscular fat. Kouba and Sellier (2011) stated that little is known about genetic and non-genetic control of intramuscular fat development and composition in pigs. Maybe the acute phase reaction leads to an increased storage of rapid mobilizable energy in adipose tissue.

To take this concept to a possible practical application, a study from Piñeiro in 2009 must be taken into account, since it demonstrated the relationship between APP concentration in plasma and meat juice, and therefore validated the use of meat juice as a valid sample. In this study, the concentrations of Pig-MAP and haptoglobin, were determined in approximately 300 paired samples of plasma and meat juice from the diaphragm (*pars costalis*), obtained after freezing and thawing the muscle. Acute phase protein concentrations in meat juice were closely correlated with those in plasma (r=0.695 for haptoglobin, r=0.858 for Pig-MAP, P<0.001). These results open new possibilities for the assessment of animal health in pig production, with implications for food safety and meat quality.

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CHAPTER 2

Pig Major Acute Phase Protein and haptoglobin concentration reference levels on commercial swine farms

ABSTRACT

Major Acute Phase Protein (Pig-MAP) and haptoglobin concentrations were determined in pigs from commercial farms, and reference intervals obtained for different productive stages. Pig-MAP serum concentrations were lower in sows than in adult boars (mean values 0.81 vs. 1.23 mg/mL), whereas the opposite was observed for haptoglobin (1.47 vs. 0.94 mg/mL). No differences were found between parities, except for a minor decrease in haptoglobin concentration in the 4th parity. A linear correlation between Pig-MAP and haptoglobin concentration was observed. Between 4–12 weeks of age, Pig-MAP mean concentrations were around 1 mg/mL, being lower in the finishing period from 13-20 weeks of life (0.7–0.8 mg/mL). Haptoglobin concentrations increased with time, from around 0.6 mg/mL at 4 weeks of age to 1.4 mg/mL at 12 weeks. Mean values of around 0.9 mg/mL were observed in the finishing period. A wider distribution of values was observed for haptoglobin than for Pig-MAP concentrations. Differences between herds were observed, with the highest values obtained in a herd with signs of respiratory disease.

Keywords: Pig-MAP; Haptoglobin; Pig; Commercial farms; Reference levels

1. Introduction

Acute phase proteins are plasma proteins that modify their concentration in response to inflammation caused by tissue injury, infections or stress (Murata et al., 2004). Acute phase proteins are mainly of hepatic origin and their synthesis is regulated by pro-inflammatory cytokines, which are hormone peptides that act as mediators between the damaged tissues and the liver (Baumann and Gauldie, 1994). Changes in the concentration of APP are only part of the physiological alterations that take place during the innate, non-specific, defence mechanism known as the acute phase response that also includes fever, increase in muscle catabolism, and hormonal changes or alterations in sleep and appetite patterns (Gabay and Kushner, 1999; Baumann and Gauldie, 1994). Acute phase proteins are used in human medicine as indicators

of infection or inflammatory problems and for monitoring the progression of disease (Kushner and Mackiewicz, 1987; van Leeuwen and van Rijswijk, 1994). Interest in animal Acute phase proteins and their value as disease markers has greatly increased during the last decade. It has been proposed that the measurement of APP in blood samples of pigs could be of practical use to evaluate animal health and welfare on farms, or at slaughterhouses for the detection of sick animals during the meat inspection process (Saini and Webert, 1991; Skinner, 2001; Petersen et al., 2004; Yamane et al., 2006). The APP assay has also been shown to be an interesting tool for the evaluation of antibiotic treatment efficacy (Lauritzen et al., 2003; Hulten et al., 2003).

The behaviour of Acute phase proteins (type and intensity of the concentration change) differs between species (Petersen et al., 2004). In swine, Pig-MAP (Major Acute phase Protein) and haptoglobin are two of the main Acute phase proteins, showing increases of more than 10 times in different experimental acute phase models, such as inflammation induced by turpentine oil injection, surgical trauma or acute bacterial infections (Lampreave et al., 1994; González-Ramón et al., 1995; Heegaard et al., 1998; Knura-Deszczka and coworkers, 2002; Carpintero et al., 2005). Significant increases in the concentration of these Acute phase proteins have also been observed in viral experimental infections, as well as in field studies on animals with different diseases (Alava et al., 1997; Asai et al., 1999; Petersen et al., 2002 a,b, Segalés et al., 2004). In addition, concentrations of Pig-MAP and haptoglobin were elevated compared to control animals when pigs were under the effect of stressors such as road transport or changes in the pattern of food administration (Piñeiro et al. 2007 a,b). Increased levels of these Acute phase proteins correlated with decreased weight gain (Clapperton et al. 2005; Piñeiro et al. 2007a). Studies suggest that the APP assay may have great potential in assessing animal health and welfare; however, it is necessary first to establish reference ranges for the normal state concentrations of these proteins, taking into account factors such as sex, age and herd. Therefore, as a first approach, we have measured the concentrations of Pig-MAP and haptoglobin in more than 800 pigs from commercial farms located in Spain, and used the data to determine reference

ranges for reproductive sows, boars, and growing pigs of different ages.

2. Materials and methods

2.1 Animals and serum samples

The study included 10 commercial farms located in the center of Spain. Nine farms (1–9) were closed-cycle farms, with 300–700 reproductive sows, while farm 10 was a three-phase farm with about 2000 reproductive sows. All farms sampled included sows from the same genetic origin (Large White x Landrace) and followed similar feeding, management, vaccination and medication programs. For each herd, all samples were obtained on the same day, and collection of all the samples at the different farms was completed within 2 months. Blood samples were collected from the caudal vein of gestating sows in the second month of gestation on farms 1–8 and 10. On each farm, samples were obtained from five sows per parity, including nulliparous and 1st to 6th parities. The sows were randomly selected, but any animal with evident respiratory or digestive disease signs and/or poor body condition was discarded. Blood samples were also collected from growing pigs by jugular venipuncture on farms 1–9. On each farm, 10 animals (half males and half females) aged 4, 8, 12, 16 and 20 weeks were randomly selected and sampled. Only animals without evident clinical signs of disease, including respiratory signs, diarrhea, and/or poor body condition or retarded growth, were included in the study of reference levels. At this time, the fattening animals on Farm number 7 showed signs of respiratory disease, so these animals were not included in the calculation of Acute phase protein concentration reference intervals. Samples from boars were collected from two artificial insemination centers also located in the central region of Spain, each housing about 100 boars (Large White, between 8 months and 4 years old). In each center, 20 animals were randomly selected to be included in the study, and sampled over a period of 1 year, at 3

monthly intervals. Samples from both centers were obtained by saphenous venipuncture. In all cases, after blood clotting, sera were separated by centrifugation, and stored at -20°C until Acute phase proteins analysis. The animal care and experimental procedure used in this study conformed to regulations and guidelines of the Real Decreto Español 223/88 BOE 67: 8509-8511, on the protection of animals used for scientific research.

2.2 Quantification of haptoglobin and Pig-MAP

The concentrations of haptoglobin and Pig-MAP were determined by radial immunodiffusion (Mancini et al., 1965) in 1% agarose gels containing specific antisera. Antisera against Pig-MAP and haptoglobin were raised by injection of the purified protein in rabbits as previously described (Lampreave et al., 1994; González-Ramón et al., 1995). As a result of the European Concerted Action QLK5-CT-1999-0153, a European Acute phase proteins Reference Serum has recently become available (Skinner, 2001). Data shown in this work were calibrated according to the European standard.

2.3 Statistical analysis

Pig-MAP and haptoglobin serum concentration data are presented as means ± standard deviation (SD) and % coefficients of variation (CV). Reference intervals are expressed as the 2.5th to 97.5th percentiles. Descriptive statistics were performed using the SAS program (SAS Institute). APP concentration data were subjected to ANOVA according to the general linear model procedure of SAS, to assess the effects of herd, parity (in sows) and age (in growing pigs). The farm of origin was included as block effect and the experimental unit was the animal. Analyses of correlation between Pig-MAP and haptoglobin concentrations were carried out by the REG procedure of SAS (linear regression analysis).

3. Results

3.1 Pig-MAP and haptoglobin concentration in sows

Table 1 shows mean values of Pig-MAP and haptoglobin concentrations in sows from the different farms studied. Statistically significant differences ($P < 0.05$) between herds were observed (Table 1). Mean concentration values and reference intervals obtained for each parity and for the total of animals are outlined in Table 2. There were no differences in the concentrations of Pig-MAP between parities (Table 2). Haptoglobin showed slightly lower concentrations at 4th parity ($P < 0.05$). Mean Pig-MAP and haptoglobin concentrations in sows were 0.81 mg/mL (range 0.41–1.94) and 1.47 mg/ mL (range 0.08–2.88), respectively. A linear correlation was found between both Acute phase proteins concentrations ($r2 = 0.31$; $P = 0.0001$).

3.2 Pig-MAP and haptoglobin levels in reproductive boars

There were no significant differences in the mean concentrations of haptoglobin or Pig-MAP between the two insemination centers (Table 3), and values remained constant during the 12 months of the study (Table 4). Mean Pig-MAP and haptoglobin concentrations in boars were 1.23 mg/mL (range 0.71–1.86) and 0.94 mg/mL (range 0.11–2.06), respectively.

3.3 Pig-MAP and haptoglobin in growing pigs

Mean values of Pig-MAP and haptoglobin concentrations on the different farms are shown in Table 5. Mean values of Pig-MAP and haptoglobin for all animals (excluding farm 7) were 0.90 mg/mL (SD = 0.46) and 0.92 mg/mL (SD = 0.68), respectively. Significantly higher values were

obtained on farm 7 that experienced respiratory disease (Table 5) in 12–16 week-old pigs. Respective mean Pig-MAP and haptoglobin concentrations in these pigs were 1.90 and 1.80 mg/mL at 12 weeks old, and 2.68 and 2.67 mg/mL fat16 weeks old (P = 0.0001). As in the case of sows, there was a linear correlation between both APP concentrations (r2 = 0.51; P = 0.0001). In Table 6 shows APP mean concentrations and reference ranges for the different ages studied. The highest concentrations (P < 0.05) of both Acute phase proteins occurred at 12 weeks of age (1.10 and 1.45 mg/mL for Pig-MAP and haptoglobin, respectively). The lowest values of Pig-MAP were during the finishing period (at 16 weeks of age), while for haptoglobin it was in the nursery period, increasing from 4–12 weeks of age.

4. Discussion

Interest in farm animal Acute phase proteins has steady increased in recent years, and studies have suggested that Acute phase proteins may in future be routinely assayed on farms to assess animal health and welfare, to optimize production rates or to monitor antibiotic therapy or vaccine efficacy (Eckersall, 2004). However, before APP concentrations can be used widely as a marker for herd or farm health status, extensive studies are needed to establish the range of concentrations of these proteins in healthy and sick animals

Data presented in this work represent the first reported reference values for Pig-MAP concentrations in different productive phases. Haptoglobin is the most studied APP in pigs, and although a number of workers have measured haptoglobin concentrations in growing pigs on commercial farms (e.g. Lipperheide and coworkers, 1998; Petersen and coworkers, 2000a,b; Chen and coworkers, 2003), data about reproductive pigs are limited. Both Pig-MAP and haptoglobin have been shown to be valid markers of disease in different experimental models, and their kinetics of induction after inflammation or bacterial infection have been found to be quite similar, with both proteins classified as intermediate fast and protracted responders

(Heegaard et al., 1998; Carpintero et al., 2005; Sorensen et al., 2006). In the present study, the correlation between Pig-MAP and haptoglobin concentrations at the animal level was significant, although lower in the case of reproductive sows (r2 = 0.31) than in growing pigs (r2 = 0.61) probably due to the fact that in growing pigs a higher percentage of animals had elevated APP concentrations. Greater variations were found in haptoglobin concentrations than in Pig-MAP concentrations, as can be seen from the CVs and reference intervals. The pronounced baseline variation observed for haptoglobin could reduce the sensitivity for detecting an acute phase condition (Heegaard et al., 2005). In the EU funded Shared Cost Project Acute Phase Proteins in Pigs (QLK5-2001-02219), the APP response to experimental bacterial, parasitic, and viral infection and inflammatory models has been studied to determine the optimal combination of Acute phase proteins for an effective APP index to boost information about the health status of a pig. It was concluded from the experimental models, that the most sensitive combination would be composed of Pig-MAP, the negative APP apolipoprotein A-I (ApoA-I) and, either C-Reactive Protein (C-reactive protein) or haptoglobin, (i.e. Pig-MAP/ApoA-1/haptoglobin or Pig-MAP/ ApoA-1/C-reactive protein; Petersen et al., 2005). It was also noted that, contrary to the other Acute phase proteins, haptoglobin pre-challenge concentration values differed widely between experiments, which could make it difficult to determine a cut off value for this protein (Heegaard et al., 2005). Other studies with pigs on commercial farms have also reported a wide distribution of haptoglobin concentration values in apparently healthy animals (Petersen et al., 2002). All commercial farms selected for our study were located in the same geographical area, and included animals of the same genetic origin. However, statistically significant differences were still observed between farms (Tables 1 and 4).

All reproductive sows had Pig-MAP levels below 1 mg/mL, which EU Shared Cost Project No. QLK520 001-02 219 proposed as the likely threshold for affecting health and performance. However, various different levels were detected; below 0.68 mg/mL, below 0.92 mg/mL and

below 0.97 mg/mL. Similar levels were also found for haptoglobin, below 1.30, 1.60, 1.66 and 1.73 mg/mL. These different levels may be related to some factors not considered in the study, mainly related to health status and reproductive performance. Some of those factors could be the farm's structure (farrow-to-finish or multisite), health status in terms of being stable or not to Pig Reproductive and Respiratory Syndrom virus (PRRS). All the farms where positive at the time of the study, but its stability status was not determined then since this concept was defined later (Holtkamp et al., 2011) in positive farms unstable, positive farms stable, positive farms stable undergoing elimination, provisional negative farms or negative farms based on their shedding and exposure status. . PRRS infection has been described as being able to generate a major alteration in APP's levels, in particular Pig-MAP (Janssen, 2014), and so a more accurate definition of the disease status at the time of the study would have helped to define a better correlation between APP levels and Pig-MAP in particular. On the other hand, PRRS is usually not the only pathogen that affects herds, and it usually affects herds in combination with other pathogens such as A. pleuropneumoniae (Pol et al., 1997), *M. hyopneumoniae* (Chae, 2016), Porcine Circovirus type 2 (Sinha et al., 2011), Influenza virus (Pol et al., 1997) and *S. cholerasuis* (Wills et al., 2000). These effects were not considered in the current study model, but would probably help to improve the accuracy and the quality of the results.

At the time the study data was collected, the current EU welfare regulations were not in force, and all the results were obtained from sows housed in stalls. However, more recent studieshave not shown any important effect of this housing factor (Sorrells et al., 2007, Chapinal et al., 2010), showing a similar effect on welfare and acute phase proteins of group sows vs stalls, and therefore it is very likely this did not affect the results.

Regarding boars, the study showed clear results on acute phase proteins levels without the presence of seasonal effects. All studied pigs were Large White, as was the tendency in the industry at that time, but the industry has evolved and now some other breeds are becoming

more common in many countries, including Pietrain and Duroc as global breeds and many other local breeds for niche markets (e.g. Iberico, Basque Country Pig, Chato Murciano, Hungarian Mongolitza, German Schwäbisch-Hällisches, English Tamworth or Berkshire). Some authors have described important differences in health and immune traits between breeds (Merlot et al., 2012), with differences seeming to be much more important between indigenous or local and global breeds (Tao et al., 2005).

Even though it would be almost impossible to include all of the boar breeds related to meat production, the study could have been imporved by including some other breeds important for the sector, such as Pietrain and Duroc boars, and also Iberico boars as well since the study was in Spain. Including these other breeds would have provided a wider and better picture of the acute phase proteins reference levels in terminal boars used in the industry.

In the study with growing pigs, APP levels obtained at farm 7, which had respiratory disease problems at the time of sampling, were clearly higher than those obtained in the rest of the farms ($P< 0.05$). Even on the other farms, although small, significant differences were observed, these could be attributed to subclinical disease or differences in managing conditions. A herd effect on haptoglobin concentration has been found in other works and attributed to differences in health status (Lipperheide et al., 1998; Petersen et al., 2002b).

Experimental studies have shown increases in APP levels in pigs infected with *A. pleuropneumoniae* (Heegaard et al., 1998; Hulten et al., 2003; Lauritzen et al., 2003), *S.suis* (Knura-Deszczka et al., 2002; Carpintero et al., 2005; Sorensen et al., 2006), *T. gondii* (Jungersen et al., 1999), Porcine Reproductive and Respiratory Syndrome (PRSS) (Asai et al., 1999), *B. bronchiseptica*, *P. multocida* type D (Francisco et al., 1996b) and pigs with haemorrhagic dysentery (Jacobson et al., 2004) or Postweaning Multisystemic Wasting Syndrome (Stevenson et al., 2006). Petersen and coworkers (2002) found lower average haptoglobin concentration in animals from specific-pathogen-free (SPF) herds when compared with animals of the same ages from conventional herds. A herd infected with *A.*

pleuropneumoniae serotype 5 had higher haptoglobin concentration than a SPF herd (Hall et al., 1992). Stress caused by managing conditions may also affect APP concentration. In piglets at high density, limited feeder space has been associated with higher haptoglobin concentration (Francisco et al., 1996a). Studies from our own group have shown elevated levels of Pig-MAP and haptoglobin associated with mixing of animals at entry to the fattening barn (Piñeiro and coworkers, 2004), changes in the pattern of food administration (Piñeiro et al., 2007a) or transport-related stress (Piñeiro et al., 2007b).

4.1 Differences between sexes were found in adult animals.

As previously reported (Ritcher, 1974; Clapperton et al., 2005), the concentration of haptoglobin was higher in sows than in boars (1.45 vs. 0.94 mg/mL). The opposite effect was observed in the case of Pig-MAP, which showed higher values in adult males (0.81 vs. 1.23 mg/mL, in females and males, respectively). Blood sampling of sows was made in the middle of gestation to avoid possible physiological changes due to farrowing, or lactation. Preliminary studies from our group indicate that a significant increase of Pig-MAP can be observed in the first week post-partum, and that levels during lactation may remain higher than in gestation (Piñeiro et al. 2006). In a recent study, Verheyen and coworkers (2006) established reference values for haptoglobin in gestating and lactating sows. Our results are in agreement with values reported by these authors (mean of 1.36 mg/mL at 3 months of gestation), although they found slightly higher haptoglobin levels in sows at first parity that were not found in our study. Differences observed between the two studies could reflect variations in health status on the farms. This would have particular significance in the case of replacement gilts that have to adapt to a new environment. Parity number did not affect Pig-MAP concentrations in sows, indicating no effect of aging on the levels of this protein. In the case of haptoglobin, sows at fourth parity showed lower concentrations, although the differences were small, and probably had no

physiological significance. In reproductive boars, APP levels were not modified in any of the four controls over the course of a year, and no differences were found between the two insemination centers, suggesting that these parameters show few variations in adult animals kept under optimal conditions. In the case of growing pigs, differences were observed depending on the age of the animals. Pig-MAP showed minimum mean concentrations in the finishing period and higher concentrations at 4–12 weeks of age, whereas haptoglobin concentrations increased with age to reach a maximum at 12 weeks of age, then falling to intermediate levels in the finishing period. The higher levels observed in the nursery period might be due to stress caused by weaning. An acute phase response is often observed after weaning, and increased expression of proinflammatory cytokines in the gut has been described at d 0–2 post weaning (Pié et al., 2004). Stressors acting at entry to the fattening barn, including transport, regrouping, change of environment and diet, might all affect APP concentration, resulting in elevated APP levels, compared to values obtained at the end of the fattening period. Increases in haptoglobin concentration, as a function of age in apparently healthy pigs during the fattening period (from about 35–100 kg live weight), were reported by Lipperheide and coworkers (1998). However, these findings could not be extended to all the farms analyzed, so the authors concluded that differences were unlikely to reflect an age effect, and more probably indicated subclinical disease or stress caused by less than optimal hygiene (Lipperheide et al., 1998). Petersen and coworkers (2002b) also found increases in haptoglobin concentration with age in conventional herds, but not in SPF herds. Le Floch and coworkers (2006) reported lower haptoglobin values when pigs were 41 d old than when around 70 d (0.39 vs. 1.54 mg/mL). In the same study, after weaning (13 d post weaning), pigs raised in a low sanitary status environment had higher haptoglobin concentrations than those growing in a high health status environment (Le Floch et al., 2006). Haptoglobin mean values obtained in our study in the finishing period were comparable, although slightly lower than those reported by Clapperton and coworkers (2005) (approximately 1.2 mg/mL in animals at 18 and 24 weeks of life), or

Chen and coworkers (2003) (1.47 mg/mL, for clinically normal 6–7 months old pigs).

Overall results show a clear time effect that could be related to some factors during the raising period. At that time, weaning was performed generally at 21 d, and the first samples in our study were taken at 28 d to avoid a weaning stress effect. Concentrations of acute phase proteins grow during the raising period, peaking at 12 weeks of age when animals enter the fattening barn, usually known as a sensitive period because of the concurrence of different stressors (mixing, housing, feed, and also vaccination against aujeszky disease).

The use of common species specific standards for calibrating the assays is essential for the direct comparison of concentration results (Eckersall et al., 1999). Data included in this study were calibrated according to the European Reference Serum for Pig Acute Phase Proteins, recently available as a result of the European Concerted Action QLK5-CT-1999-0153 (Skinner, 2001). Extension of the use of this standard should allow future comparison of data obtained in different studies provided that validated acute phase proteins assays are used.

5. Conclusions

Although establishing a reference value for biological measures is not easy because of the difficulty in discarding individuals with altered values due to subclinical disease, the mean concentrations and reference intervals described here can be considered a reasonable approach to establishing values that can be expected in pigs from conventional commercial farms under normal growth conditions and in the absence of disease outbreaks. The age and sex of the animals should be taken into account when analyzing Pig-MAP or haptoglobin concentrations. Thus, in the case of Pig-MAP, mean concentrations of around 0.80 mg/mL could be expected for sows, whereas for boars the reference average concentration was 1.23 mg/mL. For haptoglobin the average values determined for sows and boars were 1.47 mg/mL, and 0.94 mg/mL, respectively. Mean values of around 1 mg/mL were observed for Pig-MAP in nursery

piglets (4–8 weeks of age), and lower mean concentrations in the fattening period (0.7–0.8 mg/mL). With haptoglobin the concentration increased with age between 4–12 weeks of age (from mean values of 0.58–1.42 mg/mL), and mean values between 16–20 weeks of age were around 0.8– 0.9 mg/mL. Herd differences can be expected due to differences in management or subclinical disease, and higher Acute phase proteins concentration should reflect a worse herd health status.

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Tables

Table 1. Pig-MAP and haptoglobin (haptoglobin) mean concentration in sows from the different herds included in the study

	Farm								
	1	2	3	4	5	6	7	8	9
Pig-MAP (mg/mL)									
Mean	0.91[ab]	0.96[a]	0.77[bc]	0.66[c]	0.96[a]	0.74[bc]	0.67[c]	0.78[bc]	0.80[abc]
SD	0.26	0.57	0.39	0.19	0.61	0.28	0.20	0.38	0.28
CV	28	59	51	29	64	37	30	49	36
Haptoglobin (mg/mL)									
Mean	1.65[ab]	1.56[abcd]	1.72[a]	1.26[d]	1.33[cd]	1.36[bcd]	1.28[d]	1.29[d]	1.59[abc]
SD	0.63	0.78	0.67	0.73	0.66	0.56	0.37	0.64	0.60
CV	38	50	39	58	50	41	29	50	38

SD, standard deviation; CV, coefficient of variation (%).

[a–d] In the same row, data lacking of a common superscript differ ($P < 0.05$).

Table 2. Productive performance parameters in the different experimental periods†

	Pig-MAP (mg/mL)			Haptoglobin (mg/mL)		
Parity[a]	Mean	SD	Range[b]	Mean	SD	Range[b]
1	0.85	0.51	.44–1.97	1.58	0.84	0.22–3.15
2	0.74	0.24	.34–1.12	1.65	0.72	0.34–2.92
3	0.79	0.45	.45–1.77	1.48	0.51	0.38–2.38
4	0.82	0.44	.40–2.13	1.44	0.56	0.13–2.30
5	0.76	0.35	.42–1.63	1.17	0.61	0.06–2.24
6	0.88	0.39	.47–1.95	1.47	0.55	0.39–2.60
Total	0.81	0.26	.52–1.47	1.35	0.60	0.02–2.17

SD, standard deviation.

[a] 45 sows per parity were sampled (five sows per parity in each of the nine herds included in the study).

[b] 2.5th to 97.5th percentiles interval.

Table 3. Pig-MAP and haptoglobin concentration in boars from the two artificial insemination centres

	Centre 1	Centre 2
Pig-MAP (mg/mL)		
Mean[a]	1.26	1.22
SD	0.66	0.39
CV	38	32
Haptoglobin (mg/mL)		
Mean[a]	0.86	1.02
SD	0.55	0.60
CV	64	58

SD, standard deviation; CV, coefficient of variation (%).

In each center 20 boars were sampled four times over a period of 1 year, at intervals of 3 months.

[a] n = 80

Table 4. Reference values for Pig-MAP and haptoglobin concentration in reproductive boars[a]

	Pig-MAP (mg/mL)			Haptoglobin (mg/mL)		
Season[b]	Mean	SD	Range[c]	Mean	SD	Range[c]
1	1.23	0.26	0.83–1.75	1.08	0.58	0.13–2.46
2	1.14	0.32	0.71–1.81	0.84	0.53	0.15–1.70
3	1.33	0.52	0.79–1.87	0.87	0.59	0.09–1.87
4	1.22	0.50	0.70–1.80	0.96	0.60	0.19–2.21
Total	1.23	0.42	0.71–1.86	0.94	0.57	0.11–2.06

SD, standard deviation.

[a] n = 40

[b] Boars were sampled four times over a period of 1 year, at 3 monthly intervals. First sampling-point was on the month of February.

[c] Range: 2.5th to 97.5th percentiles interval

Table 5. Pig-MAP and haptoglobin mean concentrations in growing pigs from the different herds included in the study

	Farm								
	1	2	3	4	5	6	7	8	9
Pig-MAP (mg/mL)									
Mean	0.79[cd]	0.99[bc]	1.10[b]	0.81[cd]	0.76[d]	0.84[cd]	1.58[a]	1.10[b]	0.80[cd]
SD	0.29	0.54	0.52	0.42	0.39	0.41	1.12	0.65	0.19
CV	36.2	54.6	47.5	51.7	51.5	48.3	70.8	59.7	23.5
Haptoglobin (mg/mL)									
Mean	0.70[cd]	1.13[ab]	1.10[bc]	1.04[bc]	1.01[bc]	0.84[bcd]	1.47[a]	1.17[bc]	0.43[d]
SD	0.75	1.00	0.84	0.90	0.87	0.77	1.32	1.02	0.43
CV	107.1	88.7	75.9	86.0	86.4	91.7	90.0	87.1	98.8

SD, standard deviation; CV, coefficient of variation (%)

[a–d] In the same row, data lacking of a common superscript differ ($P < 0.05$)

Table 6. Pig-MAP and haptoglobin concentration reference values in growing pigs depending on age

	Pig-MAP (mg/mL)				Haptoglobin (mg/mL)			
Age (weeks)	Mean	SD	CV	Range[d]	Mean	SD	CV	Range[d]
4	0.92ab	0.34	37	0.51–1.73	0.58c	0.73	126	0.02–2.60
8	0.94ab	0.42	45	0.46–2.24	0.85b	0.84	99	0.02–3.07
12	1.10a	0.65	60	0.46–2.87	1.42a	0.96	67	0.03–3.37
16	0.69c	0.26	38	0.44–1.58	0.85cb	0.66	78	0.02–2.60
20	0.83bc	0.44	53	0.46–2.36	0.89b	0.86	96	0.02–3.00

SD, standard deviation; CV, coefficient of variation (%).

[a–c] Within a column, means without a common superscript letter differ ($P < 0.05$)

[d] 2.5th to 97.5th percentiles interval. Data on pigs from Farm 7 were not included in the calculations shown in this table

CHAPTER 3

PIG ACUTE PHASE PROTEIN LEVELS AFTER STRESS INDUCED BY CHANGES IN THE PATTERN OF FOOD ADMINISTRATION

ABSTRACT

A total of 240 pigs, 74 d old, half boars and half females, were included in a trial designed to assess the effect of stress caused by changes in the pattern of food administration on the concentration of acute phase proteins (APP) and productive performance parameters. Half of the animals (pigs fed ad libitum, AL group) had free access to feed, while the rest were fed following a disorderly pattern (DIS group), in which animals had alternating periods of free access to feed and periods of no feeding, when food was removed from the feeder. The periods of free access to feed (two daily periods of 2-h duration) were randomly assigned, and varied from day to day. Total feed supplied per day was identical in both groups, and exceeded the minimal amount required for animals of these ages. Pen feed intake, individual body weights and the main positive pig APP pig major acute phase protein (Pig-MAP), haptoglobin, serum amyloid A (serum amyloid), C-reactive protein (C-reactive protein), and the negative APP apolipoprotein A-I (ApoA-I) and transtherytin were determined every 2 weeks during the period 76 to 116 d of age. Animals fed ad libitum had better average daily gain (ADG) than DIS animals in the whole experimental period ($P < 0.01$) but the differences in ADG were only produced in the two first experimental sub-periods (60 to 74 and 74 to 116 d of age), suggesting that the stress diminished when the animals got used to the DIS feeding. Interestingly differences in ADG between DIS and AL pigs were restricted to males, no differences were observed between females. The same differences observed for ADG were found for APP. DIS males had higher Pig-MAP concentration than AL males at 74 and 116 d of age, lower ApoA-I concentration at 74 d of age and higher haptoglobin and C-reactive protein concentration at 116 d of age ($P < 0.05$). The results obtained in this trial show an inverse relationship between weight gain and APP levels, and suggest that APP may be biomarkers for the evaluation of distress and welfare in pigs.

Keywords: acute phase proteins, animal welfare, performance, pigs, stress

1. Introduction

Stress can be defined as a state of threat to homeostasis, caused by psychological, environmental or physiological stressors (Chrousos and Gold, 1992). During this state, the hypothalamic pituitary adrenal axis (HPA) and the sympathetic nervous system are activated, resulting in physiological changes required to deal with the threat and to restore the internal equilibrium (Black, 2002). It is well-known that pigs are sensitive to stress, and that the growth potential of the current genetic pig lines is often limited by the presence of stressors in the productive system. Previous studies have investigated the influence of stressors such as hot temperatures or restricted space on pig performance, finding depressed growth and poorer performance in these animals under the effect of such stressors (Hyun et al., 1998a,b; Le Bellego et al., 2002). However, attempts to define measures of stress have been difficult and controversial, and no physiological parameter has been successfully used to evaluate all the stress situations studied (Moberg, 1987). The endocrine system was thought to be an appropriate indicator of stress, but plasma cortisol was not consistently changed by different acute stressors (Hicks et al., 1998). Therefore, finding alternative parameters reacting to stressors may be of great value for the objective evaluation and optimization of productive systems.

A number of studies indicate that stress alone can induce an acute phase response (for a review see Black (2002)). The acute phase response is the body's answer to the presence of tissue damage or infection, and consists of a series of physiological responses encountered to repair the damage, recruit the host defense mechanism to fight against the threat and finally restore the internal equilibrium. During the acute phase reaction, the concentration of some plasma proteins, named acute phase proteins (APP) is modified. APP are mainly of hepatic origin, and their synthesis is regulated by pro-inflammatory cytokines, mainly interleukin-6 (Baumann and Gauldie, 1994; Gabay and Kushner, 1999). Recent studies suggest that APP may be used to assess stress in farm animals (Murata et al., 2004; Piñeiro et al., 2007a). In animals

for experimentation, physical and psychological stress elevate APP concentration (Morimoto et al., 1987; Deak et al., 1997); increases of APP have been also observed in cattle after physical stress (Alsemgeest et al., 1995). An APP response was observed in pigs after road transport, with the magnitude of the APP concentration change apparently related to the quality of transport conditions (Piñeiro et al., 2007b). In this study, the effect of a changing pattern of food administration was used as a model to induce stress in growing pigs. The concentration of the main pig APP and productive performance were assessed as markers of distressful situations and welfare of pigs.

2. Materials and methods

2.1. Animals and experimental design

Two hundred and forty pigs (LW x LR), 74 d of age with a body weight of 26.3 - 0.39 kg at the beginning of the experimental period, half boars and half females, were used in the trial. Pigs were moved to the experimental facilities the day before the beginning of the trial, and housed in 24 pens (10 animals per pen), 12 pens of males and 12 pens of females, in an environmentally controlled building. The flooring was fully slated, made of concrete slats. Pen dimensions (length x width) were 2.54 x 3.00 m, being the space allowance of 0.76 m^2 per pig, and each pen was equipped with one three-place concrete feeder, which provided 60 cm of total trough space. Feeders were located in the front of each pen, on the opposite side of the drinker location. Replicates were randomly divided into two experimental groups. The experimental treatments consisted of pigs fed ad libitum (AL) or disorderly (DIS). Two identical contiguous rooms of 12 pens each were used for the experiment, with the same experimental treatments (feeding pattern and sex) in each room. The AL group always had free access to feed, while DIS feeding consisted of feed administered in a disorderly pattern, with two periods each day

(2-h duration each) of free access to feed alternating with periods in which food was removed from the feeder. Each day the times when feed was freely available to DIS animals varied randomly. Total feed supplied per day was identical in both groups, and exceeded the minimal amount required for animals of these ages. Diet composition is shown in Table 1. Pen feed intake and individual body weight (BW) were recorded every 2 weeks (at 74, 88, 102 and 116 d of age). Average daily gain (ADG, g/d), average daily food intake (FI, kg/d), and feed:gain ratio (FGR, kg/kg) were calculated for the 2-week sub-periods and the total experimental period (74 to 116 d of age). At each weight control time-point, blood was sampled from 24 animals in each group (half boars and half females, two samples per pen) by venipuncture of vena cava. Blood sampling lasted about 0.5 min per animal, including both holding with a snout rope and sampling. Sera was immediately removed after centrifugation, and kept frozen until analysis. The animal care and experimental procedure used in this study conformed to regulations and guidelines of the Real Decreto Español 223/88 BOE 67: 8509–8511, about the protection of animals used for scientific research.

2.2 APP determination

The concentrations of pig major acute phase protein (Pig-MAP), haptoglobin, and apolipoprotein A-I (ApoA-I) were determined by radial immunodiffusion (Mancini and coworkers, 1965), in 1% agarose gel containing specific antiserum. Antisera were raised by subcutaneous injection of the purified proteins in to rabbits, as previously described (Lampreave et al., 1994; González-Ramón et al., 1995; Carpintero et al., 2005). A serum previously calibrated with the purified proteins was used as standard. C-reactive protein was determined by ELISA as follows: microtiter plates (high bound, Nunc) were coated with phosphoryl choline coupled BSA, and blocked with milk-powder in saline. Samples and standards were diluted with 50 mmol/l Tris, 0.9% NaCl, 10 mmol/l CaCl2, 0.1% Tween 20 (TBS-CT buffer), and then

bound C-reactive protein was detected using an in-house anti pig C-reactive protein monoclonal antibody, followed by a peroxidase-labelled goat anti mouse IgG antiserum (Jackson Immunoresearch Laboratories). All washings and additions of secondary reagents were done in TBS-CT buffer. The ELISA was developed using 50 mmol/l citric acid, pH 4.0, 0.1 mmol/l ABTS (2,2 Azino-bis (3-ethylbenzthiazoline6-sulphonic acid), 0.01% H_2O_2 as a color substrate and the absorbance was read at 405 nm. A porcine serum was used as an in-plate standard; the serum was validated using both a home-made purified porcine C-reactive protein preparation as well as the Porcine Acute Phase test kit from Tridelta (Tridelta Development Ltd). Serum amyloid A (serum amyloid) was assayed using the Phase serum amyloid Assay kit (Tridelta Development Ltd), according to the manufacturer's instructions. The concentration of transthyretin was determined by in-house ELISA, as previously described (Campbell et al., 2005).

2.3 Statistical analysis

Statistical analysis was performed using the pen as a statistical unit for feed intake and feed efficiency, and the animal as a statistical unit for body weight evolution, average daily gain and APP concentrations. Productive performance and APP concentration data were analyzed by the GLM procedure of Statistical Analysis Systems Institute (1990). Feeding treatment and sex were included in the model as main effects and their interaction (feeding treatment x sex) was also studied. In the case of productive performance data, the initial body weight was introduced as a covariate and all means were corrected by least squares according to initial weight.

3. Results

3.1 Productive performance parameters

The productive performance parameters for the whole experimental period (74 to 116 d of age) and for each 2-week subperiod are shown in Table 2. BW evolution is outlined in Table 3. Initial BW was similar for both groups of pigs (AL/DIS), but the BW of AL animals was higher than the BW of DIS animals at every subsequent weight control performed. Animals fed AL had better ADG than DIS animals for the whole period ($P < 0.05$) and between 74 to 88 d ($P < 0.01$), and a tendency to have better ADG between 88 to 102 d ($P < 0.1$). Differences in ADG were associated with both, FI and FGR. AL animals had higher FI between 74 to 88 d and better FGR between 88 to 102 d. Significant interactions between treatment and sex were found (Table 2). Differences in ADG between AL and DIS animals between 74 to 88 d were due to males (523 v. 398 g), since females of both groups had similar ADG.

3.2 Acute phase proteins

On the first analysis day (74 d of age) the mean concentrations of APP were similar between treatments. In the AL animals, the highest concentrations of the positive APPs Pig-MAP, haptoglobin and serum amyloid showed occurred on this day (74 d of age), decreasing thereafter (Figure 1a). Changes in APP concentrations differed between DIS males and females. In the case of DIS males, the concentration of Pig-MAP remained elevated compared with AL animals between 88 and 102 d of age, whereas there were no significant differences between treatments in the females (Figure 1a). Serum amyloid concentration was also higher in DIS males at 88 d of age than in AL males (Figure 1b), but the differences were not significant due to the wide distribution of values for this protein at all sampling times apart from 116 d of age. DIS males showed higher C-reactive protein and haptoglobin mean concentrations than AL males at 102 d of age (Figures 1c and 1d). No significant differences between treatments where observed in the case of females. The concentration of the negative APP ApoA-I increased

slightly with time in AL animals (Figure 2a). Significant differences between groups were observed for males at 88 d of age, having DIS concentration being lower than for AL. As in the other APP studied, no differences between treatments were found for females. No difference was found in TTR concentration between the AL and DIS groups (Figure 2b)

4. Discussion

Novelty has been demonstrated to be a very strong stressor, especially when an animal is suddenly confronted by it (Dantzer and Mormede, 1983), and thus the lack of food in the feeder was expected to cause a stress response in animals used to having food permanently available. In our trial, the alteration in the feeding pattern resulted in a loss of weight gain, but although the effect was observed for the whole experimental period (74 to 116 d of age), analysis for 2-week sub-periods showed that the overall differences in ADG were only due to differences in the two first experimental sub-periods, suggesting that the stress was reduced once the animals got used to the new conditions. Previous studies have shown that pig response to repeated stress diminishes with time as the animal adapts to the new situation (Jensen et al., 1996; Schrader and Ladewig, 1999).

Interestingly, significant differences were found in productive performance depending on sex. Males were affected by the disorder feeding, whereas the growth of females was not significantly different between treatments. Studies with induced stressors have not generally included the effect of sex on productive performance, however indicators exist that suggest pig stress responses may differ between sexes (Ruis et al., 1997; De Groot et al., 2001). The alterations in the feeding pattern also resulted in changes in the APP concentrations, and differences between sexes for APP were similar to those observed for growth efficiency. DIS males showed higher APP levels between 88 and 102 d of age when compared with the control group, whereas no significant differences were observed for females. In the final period (102 to

116 d of age), ADG was similar between control and DIS animals, while the APP concentration had returned to normal levels, with no difference between treatments.

The strong inverse correlation between daily gain and APP concentration levels can be explained by the fact that during the acute phase reaction, pro-inflammatory cytokines do not only affect the liver, so inducing changes in APP production, but also affect other different targets that lead to a systemic reaction that includes profound metabolic changes. For example, appetite is suppressed and food intake diminishes, while muscle catabolism is accelerated. An animal in this state of immunological stress is in negative energy balance, and so loses rather than gains weight (Johnson, 1997; Spurlock, 1997). Some studies have found an association between elevated pig APP concentration and decreased weight gain (Eurell et al., 1992; Dritz et al., 1996; Clapperton et al., 2005). In this trial, a wide picture of the APP response was obtained, as the study included the main pig positive APP Pig-MAP (Lampreave et al., 1994; González-Ramón et al., 1995; Heegaard et al., 1998; Hulten et al., 2003) as well as the negative APP Apo A-I (Carpintero et al., 2005) and transthyretin (Campbell et al., 2005). Some differences were observed in the behaviour of the APPs studied. Pig-MAP was the most sensitive protein in the detection of the stress caused by changes in the feeding pattern, as its concentration remained elevated at both, the 88 and 102 d of age sampling points. In the case of serum amyloid, DIS males had a tendency (P, 0.10) to show higher values at 88 d of age, but there was a wide distribution of values at this date and on the first sampling day (74 d), which contributed to the inconclusive nature of the results. In the case of haptoglobin and C-reactive protein, significant differences were only observed at 102 d of age. These results suggest the presence of different regulation patterns between APP in response to stress. The mechanism of induction of APP after stress has not been completely elucidated.

Many studies have shown that psychological stressors alone can induce pro-inflammatory cytokine secretion (Nukina et al., 2001; Black, 2002), but the mechanisms and target cells involved in such cytokine production are not known for certain. Neuropeptides, as well as stress

hormones, might have a central role in the activation of the APP response elicited by stress (for a review see Black, 2002). This sex difference, clearly shown in this paper, might help in the future to elucidate the physiological mechanisms of the APP response. This outcome leads us to think that a further study of similar design, but including the sex factor with barrows or even immuno-castrated animals (castrated only from a certain time point in their lives), could provide valuable information for this purpose.

The concepts of acute, chronic or intermittent stress have been largely discussed in the literature (Burchfield, 1979). In general, the understanding of long-term stress is poor, as is how they affect the various stress responses of the organism. Consequently, we have great difficulty in identifying animals that are experiencing chronic stress, since no behavioral or adrenal challenge tests have been successfully applied, nor have any physiological tests been run. The probably reason for this is because we tend to treat chronic stress as a constant, unvarying state, rather than a succession of repeated acute stressors, which seems to be more correct, (Moberg, 2000) and could match better the type of stress induced in this experiment. Another reason could be that an organism subjected to long-term intermittent stress, changes its responses to the stressors over time. This phenomenon has been defined as sensitization and desensitization, or the response to a stressor that initially increases the intensity of the response and later decreases the intensity, due to habituation (Ladewig, 2000).

Both concepts match well with the stress induced in this paper, where the animals didn't perceive stress all the time, but only in those moments where they saw pigs in other pens being fed while they did not have their feed delivered. On the other hand, it is important to highlight that this study supports the notion that non-inflammatory, psychophysical stress can induce a discernible APP response in healthy domestic animals, whereas previously the APP response had been considered almost an exclusive marker of inflammation and / or infection. Our group demonstrated the same fact in a previous study, where the stress was generated by different qualities of transport (Piñeiro et al., 2007b). Both the previous and current studies confirm the

stress – APP linkage in pigs under commercial conditions, suggesting that the APP response is can be induced by a stressful event, to which domestic animals are ubiquitously exposed during daily management. This point of view, highlighting the inconspicuous but essential linkage between stress and the APP response, has been proposed by Murata (2007). However, the mechanism by which the APP response is induced in stressed animals remains unknown.

Acute phase protein concentrations on the first analysis day were slightly elevated in all treatment groups compared with the normal levels reached at the end of the trial (decreased in the case of the negative APP Apo A-I). These initial APP levels may have been caused by the stress due to the movement to the new installations, including transport, change of environment and regrouping. An earlier study showed similar elevations in the APP concentration 1 day after pigs entered the fattening barn, with greater elevations when pigs were mixed with previously unknown animals (Piñeiro et al., 2004). Previous studies have also shown that stressors have generally an additive effect (Hyun et al., 1998b); thus, the presence of these initial stressors probably enhanced the effect caused by the disorderly feeding. Environmental conditions and handling prior to or during the trial, apart from management of food, were identical for all the experimental groups. Thus, since initial stressors would be acting on all the groups, including controls, the differences observed between DIS and AL males can be attributed to the management of feeding. The reason why the disorderly feeding significantly affected males and not females cannot be elucidated from this trial. It is possible that males experienced more stress when exposed to a treatment that may involve dominance and competition, and it is possible that the disorderly feeding might have resulted in an increase of aggressive behavior that affected more males than females. We could not register data about agonistic behavior, which would have thrown some light on this subject. However, other studies have found no difference between boars and females in the aggressive behavior observed after regrouping of animals of the same sex (Giersing and Andersson, 1998), and one study even recorded higher aggressive response in females (Stookey and Gonyou, 1994), although this study was performed with

barrows and not with intact males, as in our trial. There are however some indicators that the stress response may differ between sexes in pigs, independent of aggressive behavior. De Groot and coworkers (2001) found depressed immune function in barrows after mixing, but not in gilts, even though agonistic behaviors were similar between sexes. Ruis and coworkers (1997) showed that after 4 h isolation the amplitude of the cortisol circadian rhythm was increased in barrows but was unchanged in gilts. Differences in HPA activation in response to stress have been also found in other species, including rats, humans and sheep (Turner et al., 2002; Kajantie and Phillips, 2006, Kudielka and Kirschbaum, 2005; Renard et al., 2005). Attempts to define measures of stress have been difficult and controversial. Failure to establish an acceptable and general measure of stress has arisen from the inability to solve problems, such as lack of a stress response that characterises all types of stressors, inter-animal variability in the biological responses to stress, and no clear correlations between stress measures and meaningful impact on animal welfare (Moberg, 1987). In this sense, it has been suggested that only the stress conditions that lead to pathological states, such as immunodepression, failure of reproduction or abnormal growth, clearly affect animal well-being (Moberg, 1987). According to these criteria APP may be valuable to measure, as they are elevated in distress situations that involve growth loss or activation of the immune system. Further studies will be performed to gain more insight about the potential of APP as markers of different causes of stress.

5. Conclusion

This study shows that psychological stressors induced by disordered feeding can trigger the acute phase reaction that modifies the serum concentration of acute phase proteins, mainly Pig-MAP, being most apparent in entire males. This has important implications for producers to avoid this kind of stressor in order to maintain animal health and performance.

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- **Tables**

Table 1. Composition and nutrient content of the experimental diet

Ingredients	
Barley	464.2
Maize	150.0
Wheat	120.0
Soya-bean meal 44	183.4
Sunflower meal 35	30.0
Lard	28.0
$CaCO_3$	10.3
Dicalcium phosphate	1.40
Salt	4.30
L-Lysine liquid	4.80
Methionine liquid	0.60
Vitamin/mineral mix †	3.00
Nutrient analysis (g/kg DM)	
Net energy (kcal/kg)	2250
Crude Protein	160.0
Total Lysine	9.40
Crude fat	29.0
Crude fibre	46.0
Ash	45.0
Gross energy (kcal/kg)	3973

†Vitamin/mineral mix provided the following per kg diet: retinol, 1.575mg; cholecalciferol 0.013mg, alpha-tocopherol, 26.8mg; phytylmenaquinone, 1mg; thiamine, 1 mg; riboflavin, 4mg; pyridoxine, 2mg; cyanocobalamin, 20μg; biotin, 10μg; niacin, 18 mg; Ca-D-pantothenic acid, 10mg; choline, 175mg. Minerals: Fe, 80mg; Zn, 110mg; Cu, 90mg; Mn, 50mg; Co, 0.1mg; I, 1mg; Se, 0.2mg.

Table 2. Productive performance parameters in the different experimental periods†

	Experimental period (d of age)											
	74 - 88			88 – 102			102 – 116			74 - 116		
	ADG	FGR	FI	ADG	FGR	FI	ADG	FGR	FI	ADG	FGR	FI
AL group												
Male	523[a]	1.96	0.95[a]	577	1.91	1.05	652	2.28	1.42	591	2.06	1.13
Female	439[b]	2.36	0.99[a]	627	2.05	1.25	681	2.26	1.5	593	2.19	1.24
DIS group												
Male	398[b]	2.61	0.82[b]	513	2.37	1.09	676	2.32	1.51	537	2.36	1.12
Female	445[b]	2.11	0.91[ab]	574	2.03	1.13	656	2.4	1.52	559	2.19	1.18
s.e.	22.0	0.263	0.032	32.2	0.129	0.056	31.3	0.106	0.062	21.1	0.096	0.032
Significance												
Treatment	**		**	‡	‡					*		
Sex			‡	‡		*						*
Interaction	**				‡							

[a,b] Means within a column having a different superscript letter differ significantly (P < 0.05)

†Abbreviations are: AL: control pigs fed *ad libitum*, DIS: pigs fed following a disorderly pattern, ADG: average daily gain (g/d), FGR: feed:gain ratio (kg/kg), average daily food intake (kg/d)

‡ Approaching significance (P < 0.1) * Significance P< 0.05, ** Significance < 0.01

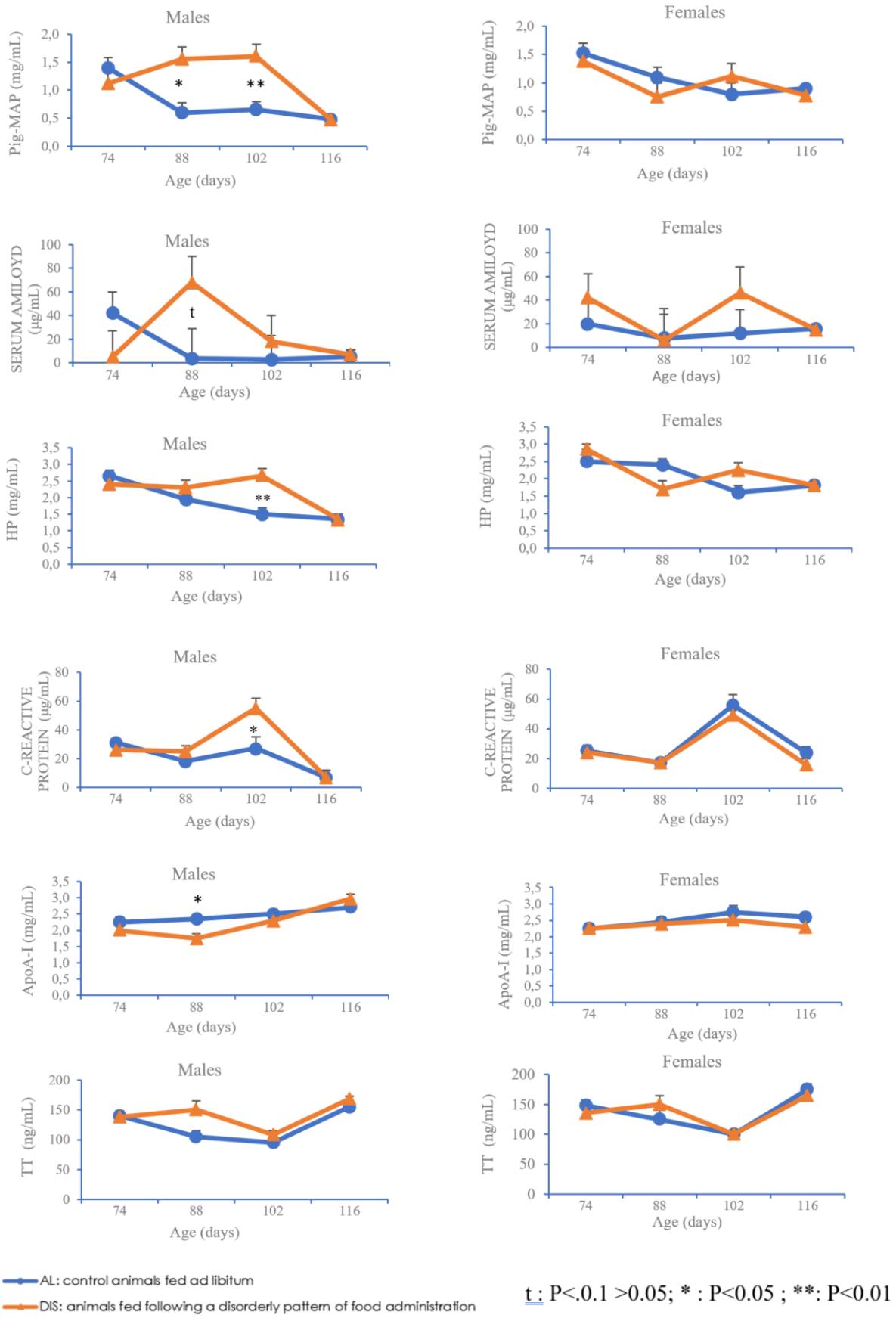
Table 3. Body weight evolution (AL: control pigs fed ad libitum, DIS: pigs fed following a disorderly pattern)

	Body weight (kg) at age in d			
	74	88	102	116
AL group				
Male	25.1[b]	33.7[a]	41.8[a]	51.2[a]
Female	28.2[a]	32.5[b]	41.3[a]	51.3[a]
DIS group				
Male	26.3[b]	31.9[b]	39.2[b]	48.9[b]
Female	25.4[b]	32.6[b]	40.6[ab]	49.9[b]
s.e.	0.500	0.308	0.629	0.886
Significance				
Treatment		**	**	*
Sex	*			
Interaction	***	**		

[a,b] Means within a column having a different superscript letter differ significantly (P < 0.05)

* Significance P< 0.05, ** Significance < 0.01, *** Significance < 0.001

Figure 1 Time course of Pig-MAP, serum amyloid, haptoglobin, C-reactive protein, apolipoprotein-I and transthyretin concentrations



t : P<.0.1 >0.05; * : P<0.05 ; **: P<0.01

CHAPTER 4

GESTATION AND LACTATION PARITY AFFECTS PRODUCTIVE PERFORMANCE AND IMMUNE RESPONSE OF THE OFFSPRING

ABSTRACT

A total of 20 sows, 10 primiparous (PP) and 10 multiparous (MP; from 3rd to 5th parity), were used to study the effect of parity of the gestation sow and lactation sow on the growth and health status of piglets from birth to slaughter. There were 4 treatments in a 2 x 2 factorial arrangement design, with piglets from PP sows suckled by PP or MP sows, and piglets from MP sows suckled by PP or MP sows. Piglets were allotted to treatments immediately after farrowing, before any colostrum intake. Piglets were weaned at 28 d of age and housed in pens of 9 pigs. Growth performance was controlled from weaning to 144 d of age, and concentrations of IgG and major acute phase protein (Pig-MAP) were measured as markers of health status throughout the study. The parity of the gestation and lactation sow affected pig growth performance to different extents depending on the experimental period considered (28-76 d, 76-116 d, 116-144 d). No interactions were found among types of gestation and types of lactation sows in any variables studied. Total ADG was higher in piglets born from MP sows than in those born from PP (669 vs. 605 g/d; P = 0.001) and in piglets suckled by MP sows than in piglets suckled by PP sows (655 vs. 620 g/d; P = 0.037). Total ADFI was higher for pigs born from MP sows than for those born from PP sows between 76-116 d (2019 vs. 1812 g/d; P = 0.003) and 116-144 d (2384 vs. 2063 g/d; P = 0.002). Total G:F tended to be higher for pigs suckled by MP sows than for those suckled by PP sows (0.43 vs. 0.41 g/d; P = 0.076). At weaning, IgG serum concentration was higher (P = 0.013) in pigs suckled by MP sows than in piglets suckled by PP sows. However, IgG concentrations were also higher for pigs born from PP sows than for pigs born from MP sows on d 116 (P < 0.001) and 146 (P = 0.088) of age. Pig-MAP tended to be lower in pigs suckled by MP sows than in pigs suckled by PP sows on d 40 (P = 0.070) and 60 (P = 0.089) of age. The results suggest that both gestation and lactation sows are important in growth performance and health status of the offspring.

Key words: acute phase protein, fattening pigs, immunoglobulin, multiparous sow, primiparous sow

1. Introduction

First parity sows (PP) have higher nutrient requirements and fewer piglets born than multiparous sows (MP). Usually, PP sows are bred before they reach mature body size and when the amounts of back fat are still limited. As a result, part of their nutrient intake during the reproductive cycle is still used for their own tissue growth (Young et al., 2005). Piglet birth weight and growth performance during lactation are also lower for piglets to PP sows than to MP sows (Mahan, 1993; Carney-Hinkle et al., 2013), and these differences may affect piglet growth during the nursery and finishing phases. However, the impact of sow parity on pig growth during the whole life has not been evaluated.

Pigs do not obtain any Ig through the placenta, and therefore they depend on Ig obtained from colostrum for specific immune reactions during the first stages of life (Kim and coworkers, 1996). Because of differences in the composition of colostrum and milk, PP sows confer less nutrients and immune components to their litters than MP sows (Klobasa and Butler, 1987; van de Ligt et al., 2002). A reduced Ig concentration during lactation may result in reduced health status and poorer growth performance of nursery pigs (Ferrari et al., 2014).

Before specific immune reactions take place, pigs respond to threats with a series of physiological changes known as acute phase response, which induces profound metabolic alterations such as anorexia and increased muscle catabolism (Murata et al., 2004). When a disease occurs, concentrations of acute phase proteins (APP) increase and can be used as a non-specific marker of the health status of the animal (Petersen et al., 2004). The current study investigated the effect of sow gestation or lactation parity (PP or MP) on the growth performance of piglets from birth-weaning to slaughter. In addition, serum concentration of piglets' Pig-MAP and Ig were evaluated as markers of health status from weaning to slaughter and immunoglobulin transmission to the piglets, respectively

2. Materials and methods

All the experimental procedures used in this study were in compliance with European Union Guidelines (European Parliament, 2010).

2.1 Animals Husbandry Diets and Experimental Design

A total of twenty sows (Large White x Landrace) at 107 ± 1 d of gestation, comprising 10 PP sows and 10 MP sows (from third to fifth parity), were selected for the experiment. All sows had the same adaptation program. Briefly, they arrived on the farm at 120 d of age, were immunized against the main pathogens that they might be exposed to in the feces of reproductive sows, and were vaccinated against major diseases (Parvovirus, Erysipela, Rhinitis, Mycoplasma, Aujeszky, Influenza, and porcine reproductive and respiratory syndrome (PRRS) according to standard protocols. At the second estrus, the sows were moved to the gestation barn and then were inseminated 21 ± 1 d later, coinciding with the third estrus. At 107 ± 1 d of gestation, the experimental sows were randomly housed in an environmentally controlled farrowing room containing 20 pens (2.2 x 3.0 m) with plastic-slatted flooring, and kept in farrowing crates (1.0 x 2.5 m) equipped with a trough feeder with a sow nipple drinker. The pens also had a piglet nipple drinker. Barn temperature was maintained at 23ºC, and supplementary heat was supplied to the piglets for the first week of lactation to keep the temperature at piglet height at 30ºC.

2.2 Diets

During gestation, sows were fed twice a day with approximately 1.5 kg of a cereal-soybean meal-based diet, formulated to contain 2,045 kcal NE/kg, 14.0% CP, and 0.60% Lys.

During lactation, sows were fed a diet based on the same ingredients, but that contained 2,150 kcal NE/kg, 17.0% CP, 0.90% Lys. For the first 7 d after farrowing, sows were offered increasing amounts of feed until they reached their ad libitum feed intake. Individual daily feed intake of sows was calculated by subtracting the daily amount of feed supplied from the amount of feed remaining in the feeders at the end of feeding.

2.3 Experimental design

Piglets were ear tagged, weighed and allotted to treatment immediately after farrowing, before any colostrum intake. The experiment followed a completely randomized design with four treatments organized as a 2 x 2 factorial design, with two parity groups, and the parity of the gestating sow and parity of the lactating sow as main factors. Half of the piglets born to a PP sow were also suckled by a PP sow, while the other half were suckled by a MP sow. Equally, half of the piglets born to a MP sow were suckled by the same parity sow, while the other half were suckled by a PP sow litters were balanced to ensure 10 and 11 piglets for PP sows and MP sows respectively, in order to have similar kg of litter suckled per kg of sow.

Litters were balanced to ensure 10 and 11 piglets for PP sows and MP sows respectively, in order to have similar kg of litter suckled per kg of sow.

The experiment was divided in 3 periods; lactation (from birth to weaning at 28 ± 2 d of age), nursery (from weaning to 74 ± 2 d of age), and growing-finishing (from nursery to 144 ± 2 d of age). After weaning, piglets were allocated by previous treatments and sex to 20 pens of 10 pigs each (5 pens per treatment). The ten animals were removed randomly from pigs born from MP sows and were suckled by PP sows (5 pigs) and MP sows (5 pigs) to balance the pen density. Piglets were housed in an environmentally controlled barn. The temperature was set at 28 ºC for the first week after weaning and was then decreased by 1ºC per week until reaching 20 ºC at 74 d of age, after which it was kept constant. Pens (2.5 x 3.0 m) were provided with

plastic-slatted floors, except for a non-slatted central area (1 m wide). Pens were equipped with floor heating, and each pen was provided with 60 cm of through with 3 holes and a nipple drinker. Pigs had ad libitum access to water and pelleted diet throughout the trial. The feeding program was the same for all the pigs, and consisted of 4 diets based on barley, corn, wheat and soybean meal (Table 1) supplied respectively from 28 to 38 d of age (pre-starter diet), 38 to 74 d (starter diet), 74 to 116 d (growing diet), and 116 to 144 d of age (finishing diet). All diets met or exceeded the nutritional requirements of the pigs (NRC, 1998). Animals remained in the same pens from weaning until they were sent to the slaughter house.

2.4 Data Collection

Sow feed intake was controlled individually during the lactation period, and piglets were individually weighed at birth and at 28 ± 2 d of age to calculate the average daily gain (ADG) during lactation. After weaning, feed intake per pen and individual body weight (BW) of the pigs were recorded every 2 weeks, with mortality recorded and weighted. The ADG, average daily feed intake (ADFI), and gain: feed (G:F) were calculated for each period (nursery, growing, and finishing) and for the entire experimental period (28-142 d of age) from these data.

Blood samples (7 mL) were obtained from vena cava of 3 piglets per sow chosen at random (15 piglets per treatment) at 14, 28, 38, 60, 90, and 144 d of age using 10 mL vacutainer tubes (BD, San Agustin de Guadalix, Madrid, Spain). Serum was immediately removed after centrifugation at 3,500 × g for 5 min at room temperature and was kept frozen (-20ºC) until their analysis for serum concentrations of Pig-MAP and IgG.

2.5 Pig-MAP and IgG Determination

Serum Pig-MAP and IgG concentrations were determined by following the methods

of Tecles and coworkers (2007) and Broom and coworkers (2006), respectively. Briefly, the concentration of Pig-MAP was determined using a commercial sandwich ELISA kit based on two monoclonal anti Pig-MAP antibodies (Pig-MAP Kit ELISA, PigCHAMP Pro Europa SL, Segovia, Spain), according to the manufacturer´s instructions. Serum IgG quantification was performed using sandwich ELISA, using commercial kits (Bethyl Laboratories Inc, BioNova, Madrid, Spain).

2.6 Statistical Analysis

Statistical analyses were performed as a completely randomized design with 4 treatments arranged as a 2 × 2 factorial design, using the pen as the experimental unit for growth performance and the animal for Pig-MAP, and IgG concentrations in serum. Data on growth performance were analyzed by repeated measures, with parity of gestation sow and parity of lactation sow, time point, and their interactions as main effects, by using the MIXED procedure of SAS 9.2 (SAS Inst. Inc., Cary, NC). When the parity of gestation or lactation sow had a significant interaction with time, p-values for each time point were obtained. Data on Pig-MAP and IgG concentrations in serum were analyzed by 2-way ANOVA at each time point. Mortality data showed lack of normality and therefore data was analyzed using the GLIMMIX procedure of SAS for generalized linear mixed models. Tukey's test was used for multiple mean comparisons. Alpha level for determination of significance was 0.05 and trends were discussed using an alpha level of 0.10.

3. Results

The average size of the litter at farrowing was of 11.9 ± 2.46 piglets born and 11.1 ± 2.35 piglets born alive, with no differences detected between MP sows and PP sows. Also, there was

no difference between treatments in the BW of the pigs at birth (1.7 ± 0.05 kg BW) and at weaning at 28 d of life (7.9 ± 0.25 kg BW). During the second week of lactation, MP sows tended to have higher ADFI than PP sows (6.08 vs. 4.99 kg/d; P = 0.090). Animals born from MP sows tended to have higher pre-weaning mortality than pigs born from PP sows (5.0 vs. 1.1; P = 0.077).

No interactions were found between the parity groups of gestation and lactation sows for any of the variables studied (P > 0.2 in all cases), and therefore the results are presented as main effects and changes with time (Table 2). An interaction (P = 0.002) was found for BW between the parity groups of gestation sows and time. Also, a trend (P = 0.069) to the interaction between parity of lactation sow with time was found for BW. Parity of the gestation or lactation sow did not affect BW at weaning. However, pigs born to MP sows showed higher BW than those born to PP sows at 116 d (62.4 vs. 57.3 kg, P = 0.015) and 144 d (87.0 vs. 79.4 kg, P = 0.002) of age. Also, pigs suckled by MP sows showed higher BW than piglets suckled by PP sows at 76 d (32.6 vs. 30.2 kg, P = 0.083), 116 d (61.7 vs. 58.0 kg, P = 0.065) and 144 d (85.4 vs. 80.9 kg, P = 0.052) of age.Total ADG was higher in piglets born to MP sows than in piglets born to PP sows (669 vs. 605 g/d; P = 0.001). Also, piglets suckled by MP sows showed higher ADG than piglets suckled by PP sows (655 vs. 620 g/d; P = 0.037). Cumulative ADFI was only affected by the parity of the gestation sow depending on the time (P of the interaction = 0.002). From 76 to 116 d (2,019 vs. 1,812 g; P = 0.003) and from 116 to 144 d of age (2,384 vs. 2,063 g; P = 0.002). ADFI was higher for pigs born to MP sows than for pigs to PP sows. Also cumulative G:F tended to be higher for pigs suckled by MP sows than for those suckled by PP sows (0.43 vs. 0.41; P = 0.076). Parity of lactation sow affected mortality between 28 and 144 d, being lower for pigs suckled by MP sows than for pigs suckled by PP sows (4.0 vs. 10.3 %; P = 0.035).

An interaction trend (P = 0.083) was found between the gestation and lactation sow effects for Pig-MAP at 14 d of age; for pigs born to MP sows, those suckled by MP sows tended to have lower serum Pig-MAP concentrations than those suckled by PP sows (0.45 vs. 0.77

mg/mL; Table 3). After weaning, Pig-MAP tended to be higher in pigs that were suckled by PP sows than in pigs suckled by MP sows (1.01 vs. 0.74 mg/mL; p = 0.070 at 40 d of age and 0.80 vs. 0.63 mg/mL; P = 0.089 at 60 d of age). Also, at 116 d of age, an interaction trend (P = 0.098) was detected between gestation and lactation sow; for pigs born to PP sows, those suckled by PP sows had higher levels of Pig-MAP in serum than those suckled by MP sows (1.14 vs. 0.51 mg/mL).

At 28 d of age, Ig concentration was higher in pigs suckled by MP sows than in pigs suckled by PP sows (30.0 vs. 17.8 mg/mL; P = 0.013; Table 4). At 40 d of age, pigs born to MP sows tended to have higher levels of IgG than pigs born to PP sows (15.4 vs. 7.4 mg/mL; P = 0.084), and pigs suckled by MP sows tended to have higher levels of IgG than pigs suckled by PP sows (15.8 vs. 7.8, P = 0.052). However, at 60 d of age, pigs suckled by PP sows had higher IgG concentration compared to pigs suckled by MP sows (6.7 vs. 4.2 mg/mL; P = 0.010). Also, pigs born to PP sows had higher IgG concentrations in serum at 116 d of age than pigs born to MP sows (30.9 vs. 17.0 mg/mL, P < 0.001), and concentrations still tended to be higher at 144 d of age (45.0 vs. 35.5 mg/mL, P = 0.088).

4. Discussion

Pigs born to PP sows tend to be less viable and to have lower growth rates than those born to MP sows (Carney-Hinkle et al., 2013; Ferrrari et al., 2014). The reason for these differences is a subject of debate and may be related to innate factors of the piglets born to PP sows, such as fewer muscle fibers, or to differences in composition of colostrum and milk between PP sows and MP sows. This observation might be important under practical conditions, because growth performance of litters from PP sows could be improved by cross-fostering with MP sows, or through nutritional changes. Results from the current study demonstrate that both the gestation sow parity and lactation sow parity can affect pig growth performance during

throughout its productive life, resulting in important differences by the time pigs reach market weight. In the current experiment, BW at slaughter was 9% lower for pigs born to PP sows than for pigs born to MP sows, and 7% lower for litters suckled by PP sows than for litters suckled by MP sows. Consequently, fostering extra pigs produced by PP sows with MP sows might mitigate BW growth delay to some extent, with effects that are apparent during the whole productive life.

The reproductive cycle and hormonal system of PP sows are naïve and their development is still competing for nutrients with their muscle development. In contrast, mature MP sows have a well-established reproductive cycle (Whittemore, 1996). Under these circumstances, fetal nutrient supply might differ between PP sows and MP sows, thus affecting fetus development. Averette and coworkers (1999) and Moore (2001) have reported a lower birth weight for pigs born to PP sows compared to pigs born to MP sows, indicating that this difference could be due to retardation in fetal growth as well as to fewer skeletal muscle fibers, which cannot be compensated for during the postnatal growth (Rehfeldt and Kuhn, 2006), even when no differences in BW are detected at birth (Moore and Davies, 2005).

Primiparous sows might not be well adapted to the new environment, including acquisition of specific immunity against pathogens present on the farm. The differences in growth observed between piglets from PP sows and MP sows might be explained by transmission of pathogens from the mother to the fetus (Feng et al., 2001) or due differences in prenatal stress (Tuchscherer et al., 2002; Ruiz and Avant, 2005). When a pig perceives a threat, it responds with a series of physiological changes aimed at fighting against the threat and restoring internal homeostasis. These changes are known as acute phase response and are responsible for profound metabolic alterations such as anorexia and increased muscle catabolism (Murata et al., 2004). The concentration of acute phase proteins (APP) is increased, and this increase can be used as a non-specific marker of the health status of the animal (Murata et al., 2004; Petersen et al., 2004). In our study, Pig-MAP concentration was increased after weaning in pigs suckled by PP sows as

compared to pigs suckled by MP sows, which might be indicative of a more pronounced challenge suffered at weaning by these PP sow suckled pigs.

Pigs are also born with limited reserves of fat and stored glycogen (Friend, 1974; Seerley et al., 1974; Boyd et al., 1978). Thus, an adequate colostrum intake by the newborn pig is key for its survival, providing nutrients for growth and development. Ferrari and coworkers (2014) have recently shown that a minimum of 200-250 g of colostrum is necessary to avoid growth retardation. Also, Decaluwé and coworkers (2014) have shown an association between colostrum intake and growth and survival of the piglet. Colostrum also provides passive immunity derived from maternal immunoglobulin transmission (Le Dividich et al., 2005). In pigs, placental transmission of Ig from mother to fetal circulation is not possible, and so Ig uptake from colostrum is very important for the protection of the new-born pig. Colostrum and milk also contain high amounts of bioactive components (e.g. insulin-like growth factors, epidermal growth factor, lactoferrin, leptin, nucleotides) which play a role in organ maturation, growth, and disease resistance (Jensen and coworkers, 2001; Wolinski and coworkers, 2003, Donovan and coworkers, 2004; Danielsen and coworkers, 2011). Because milk composition is linked to mammary development (Kensinger and coworkers, 1986), components of colostrum and milk such as Ig, total fat content, or energy and growth factors differ between PP sows and MP sows (Carney-Hinkle et al., 2013; Declerck et al., 2015). Previous studies have reported lower Ig concentrations in blood from PP sows than from MP sows (Inoue and coworkers, 1980; Klobasa et al., 1986; van de Ligt et al., 2002; Declerck et al., 2015). Moreover, the concentration of Ig in neonatal pig serum has been found to be directly proportional to the concentration found in colostrum and in sow serum (Klobasa et al., 1986; Damm et al., 2002; Krakowski et al., 2002; van de Ligt et al., 2002). However, there is some controversy about the effect of colostrum IgG concentration on piglets. Some studies have not observed any effect of IgG concentration in colostrum nor IgG colostrum intake on the health and performance of the litter (Bland et al., 2003). However, other studies have found a positive effect of colostrum Ig and milk

concentration intake on piglet health (Salmon, 1999; Krakowski et al., 2002). Values obtained in our trial showed a difference of 44% in IgG concentrations in piglets at weaning between those suckled by PP sows and MP sows, which corresponds with results by van de Ligt and coworkers (2002), and Klobasa and coworkers (1986), who reported respectively 46%, and 42% lower IgG concentration in pigs from PP sows compared to pigs from MP sows. Pigs suckled by MP sows showed better growth rate and lower Pig-MAP concentrations in the nursery period than pigs suckled by PP sows, which indicates a better health status of this group of animals.

Pig growth immediately after weaning was affected by the parity of the lactation sow. However, the parity of the gestation sow had less effect in this early phase of the growing period, although it did affect growth later. This difference in effects may be due to innate traits in maternal immunity. Maternal immunity provided by the lactation sow to the offspring decayed from IgG levels around 30 mg/mL at 14 d of age to around 6 mg/mL at 60 d of age. Then the pig developed its own immunity, and serum IgG concentrations in pigs increased again with time to reach levels around 35 mg/mL for pigs born to MP sows and 45 mg/mL in pigs born to PP sows at 142 d of age. There is no obvious reason for the difference in the IgG concentrations reported although it could be related to the reduced growth rate reported for pigs born to PP sows. The higher IgG concentrations in pigs born to PP gilts might reflect a higher incidence of disease in this group, a less effective innate immunity, or differences in the regulatory components of the immune system with predominance of the humoral response.

5. Conclusion

In conclusion, the research indicates that the growth performance and health status of the offspring of PP sows is improved by cross-fostering with MP sows. These results open the possibility of an interesting strategy for improving the growth of litters from PP sows, a strategy that is easy to apply compared with current programs based on parity segregation. However, it is important to note that the results obtained in the current study might have also been affected

by factors such as the health status of the studied herd, as well as the management conditions and feeding programs. Further studies are needed to gain more knowledge on the possibilities of this strategy. Pig-MAP and IgG concentrations in serum help explain some of the differences found in production data.

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Tables

Table 1. Ingredient composition and calculated nutrient composition of the experimental diets (% DM unless indicated otherwise)

	Prestarter (28 - 38 d)	Starter (38 - 74 d)	Growing (74 - 116 d)	Finishing (116 – 144 d)
Ingredients				
Corn	450.0	450.0	84.0	87.7
Sweet dried milk whey	150.0	50.0	-	-
Extruded soybean	114.0	30.0	-	-
Barley	110.0	220.0	250.0	300.0
Soybean meal 44 % CP	100.0	186.0	188.7	93.7
Wheat	-	-	290.0	297.0
Corn DDGS	-	-	100.0	150.0
Glycerin, 85%	-	-	30.0	30.0
Sugar beet molasses	-	-	15.0	15.0
Soybean oil	39.7	24.5	15.7	-
Dicalcium phosphate	16.9	18.7	-	-
Mono-calcium phosphate	-	-	4.0	2.0
Calcium carbonate	0.7	2.5	11.7	13.3
Sodium chloride	2.0	3.1	2.3	2.3
L-Lysine HCl, 78%	5.7	5.1	3.7	4.3
L-Threonine	2.0	2.0	1.1	1.2
Methionine-OH, 88%	2.9	2.3	0.8	0.5
L-Tryptophan	1.1	0.9	-	-
Vitamin-mineral mix[1]	5.0	5.0	3.0	3.0
Nutrients content,				
NE, MJ/kg	10.79	10.26	9.83	9.61
CP	191	170	173	150
Digestible Lys	13.5	11.5	9.4	8.0

[1]Vitamin/mineral mix prestarter and starter diets provided the following quantities per kilogram: vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E, 100 IU; vitamin K_3, 2 mg; thiamine, 4 mg; riboflavine, 10 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.03 mg; biotin, 0.4 mg; niacin, 50 mg; choline, 300 mg ; Ca-d-pantothenic acid, 30 mg ; folic acid, 1.8 mg ; Fe, 60 mg; Zn, 100 mg; Cu, 10 mg; Mn, 50 mg; I, 0.6 mg; Se, 0.2 mg. Vitamin/mineral mixes for growing and finishing diets provided the following quantities per kilogram: vitamin A, 6,000 IU; vitamin D_3, 1,500 IU; vitamin E, 20 IU; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavine, 3 mg; vitamin B_6, 1 mg; vitamin B_{12}, 0.02 mg; niacin, 15 mg; choline, 100 mg ; Ca-d-pantothenic acid, 12 mg ; Fe, 110 mg; Zn, 110 mg; Cu, 150 mg; Mn, 45 mg; I, 0.8 mg; Se, 0.2 mg.

Table 2. Growth performance of pigs from weaning to slaughter[1] :28 to 144 d of age

Gestation	Multiparous (MP)		Primiparous (PP)		SEM	P-value[2]	
Lactation	MP	PP	MP	PP	(n = 5)	Gestation	Lactation
BW, kg							
28 d	8.4	7.9	7.9	7.7	0.53	0.512	0.454
74 d	33.5	30.7	31.6	29.6	1.29	0.273	0.083
116 d	63.5	61.2	59.8	54.8	1.84	0.015	0.065
144 d	88.0	86.0	82.7	75.7	2.15	0.002	0.052
ADG, g							
28-144 d	675	663	634	576	15.5	<0.001	0.037
28-76 d	524	476	494	457			
74-116 d	750	760	707	625			
116-144 d	816	816	763	712			
ADFI, g							
28-144 d	1602	1582	1474	1401	42.0	<0.001	0.358
28-76 d	799	746	773	747			
74-116 d	2004	2033	1854	1770			
116-144 d	2386	2381	2120	2005			
G:F							
28-144 d	0.42	0.42	0.43	0.41	0.006	0.526	0.076
28-76 d	0.66	0.64	0.64	0.61			
74-116 d	0.37	0.37	0.38	0.35			
116-144 d	0.34	0.34	0.36	0.36			

[1] Gestation indicates the type of sow from which piglets were born, and lactation type indicates the type of sow which suckled the piglets.

[2] All variables were analyzed by repeated measures. The model included type of gestation (G) and lactation (L) sow, age of the piglet (A), and their interactions GxL, GxA, LxA, and GxLxA. Age was always significant ($P < 0.001$), while other interactions were not significant for any variable ($P > 0.10$) except for BW. The P values of the interactions (GxA, LxA) for BW were 0.001 and 0.069, respectively.

Table 3. Pig-MAP serum concentration in pigs, mg/mL[1]

Gestation	Multiparous (MP)		Primiparous (PP)		SEM	P-value		
Lactation	MP	PP	MP	PP	(n = 15)	Gestation	Lactation	Interaction
d of age								
14	0.45	0.77	0.77	0.76	0.093	0.095	0.096	0.083
28	0.93	1.12	0.80	0.77	0.170	0.174	0.646	0.513
40	0.74	1.12	0.75	0.91	0.146	0.500	0.070	0.455
60	0.58	0.88	0.69	0.73	0.101	0.837	0.089	0.210
90	1.02	0.88	0.72	1.17	0.198	0.960	0.432	0.137
116	0.50	0.52	0.51	1.14	0.182	0.088	0.077	0.098
142	0.59	0.64	0.79	0.681	0.082	0.143	0.724	0.309

[1] Gestation indicates the type of sow piglets were born from and lactation indicates the type of sow which suckled the piglets, defined as multiparous sows (from 3 to 5 parities) and primiparous sows.

Table 4. Ig serum concentration in pigs during lactation, nursery, and growing-finishing phases, mg/mL[1]

Gestation	Multiparous (MP)		Primiparous (PP)		SEM	P-value		
Lactation	MP	PP	MP	PP	(n = 15)	Gestation	Lactation	Interaction
d of age								
14	33.4	34.4	29.8	40.8	7.15	0.847	0.406	0.487
28	23.7	18.9	37.0	16.4	4.98	0.282	0.013	0.119
40	23.0	7.7	8.6	7.0	4.25	0.084	0.052	0.113
60	5.0	6.9	3.4	6.6	0.95	0.300	0.010	0.495
90	21.1	26.6	17.3	20.4	3.68	0.175	0.242	0.736
116	19.5	13.9	30.8	30.9	3.68	0.001	0.451	0.445
142	34.8	36.4	49.0	43.5	6.24	0.088	0.751	0.564

[1] Gestation indicates the type of sow piglets were born from and lactation indicates the type of sow which suckled the piglets, defined as multiparous sows (from 3 to 5 parities) and primiparous sows.

CHAPTER 5

Individual Pig Care program improves productive performance and animal health in nursery-growing pigs

ABSTRACT

Individual Pig Care (IPC; Zoetis, Paris, France) is a new management tool for swine farmers, based on daily keen observation of pigs, early detection of health problems, and prompt reaction to them. In this study, IPC improved production performance both in the nursery period, with higher ADG for IPC group (368 vs 326 g/d) and a tendency to a higher BW (20.85 vs 19.42 Kg) and higher homogeneity (87.1 vs 82.9) and in the growing period, with higher ADG (439 vs 391 g/d), ADFI (696 vs 637 g/d), FCR (1.590 vs 1.634, g/g), BW (32.71 vs 29.97 Kg) and homogeneity (85.9 vs 82.6). Besides of this field trial, the IPC protocol was used in a larger number of farms, involving a total of 169 commercial farms from 17 countries observing and caring a total of 1.140.069 pigs from nursery and finishing phases. Classical and new key performance indicators were defined to assess health impact and use of medication, such as the ratio treated / scored, early detection rate, treatments per animal, under or overdosing ratio, percentage of mortality and fall-out and health index. All of them were very useful to assess and understand the positive impact of the protocol implementation. These results suggest that IPC protocol properly applied not improve performance but as well a more effective management with more targeted use of medication.

Keywords: swine, Individual Pig Care, health, management, antibiotic judicious use

1. Introduction

Antimicrobial-resistant zoonotic bacteria may be transmitted from pigs to the human population, potentially resulting in human disease that may not respond efficiently to antimicrobial treatment (Khanna et al., 2008, De Jong et al., 2009). In an attempt to reduce antimicrobial resistance in zoonotic pathogens, the pig-production industry is promoting a more judicious use of antibiotics either via national regulations (The Netherlands, France, and Germany are the latest countries involved in this initiative) or through the demands of customers (retailers and supermarkets). However, animal health and welfare require a highly efficient and economically sustainable system for disease control, including a need for both vaccination and antibiotics.

To fulfill those objectives, a new management tool has been developed for swine farmers in Europe. It is called Individual Pig Care (IPC; Zoetis, Paris, France) and is based on daily observation of individual pigs, early detection of husbandry and health problems, and prompt and accurate reaction to these problems, enabled by rapid and effective data collection and processing.

The IPC program is a commercial service delivered by coaches called husbandry educators. To determine if IPC positively affects swine productivity in nursery-growing pigs, a study was conducted at a commercial swine production facility. Productive performance and health status outcomes for a group monitored by a dedicated on-site IPC educator (IPC group) were compared against a group raised according to the standard care protocol (Control group).

2. Materials and Methods

Animal care and experimental procedures used in this study followed the regulations and guidelines of the Spanish government for the protection of animals under scientific research

(Real Decreto Español 223/88 BOE 67: 8509-8511).

2.1 Study facilities

The study was conducted on a commercial, 700-sow, farrow-to-finish farm in Segovia, Spain. The health status of the farm was medium-low; the herd was positive for porcine reproductive and respiratory syndrome (PRRS) and there was a high incidence of colibacillosis in the nursery phase.

A total of 24 pens (2.5 m x 2.8 m) distributed in four nursery rooms were used for the study. Environmental conditions during the trial (temperature and ventilation rate) were automatically controlled and assessed as appropriate for the age of the pigs. Each pen was equipped with one six-hole self-feeder and two nipple waterers, allowing ad libitum access to feed and water.

The IPC system was used on 169 commercial farms from 17 countries (Belgium, Denmark, Germany, Estonia, Spain, France, United Kingdom, Hungary, Italy, Lithuania, The Netherlands, Philippines, Poland, Portugal, Romania, Thailand and USA) during the period 2012-2014. The IPC system used in our study was the same as the system used on these farms. Data was entered in to a smartphone with a digital pen, where it was sent to the cloud and automatically processed and visualized on a cloud-based dashboard in real-time. The replicate was the batch, defined as a group of animals entered in the batch in one or several times and moved or sold one or several times, but always in the same physical place (room or barn). Table 2 shows examples of batches on the dashboard (identification, properties and status). The age at entry is defined for each batch, that is associated to a date to be able to perform overtime controls. The daily weight of every animal was based in a literature review and in our 20 years old field trials database, by regression. A total of 1377 nursery, 295 finishing and 6 wean-to-finish batches were included.

2.2 Study animals and housing

A total of 368 pigs, with equal numbers of females and entire males, were selected for the study at 23 d of age (weaning day) and were observed until they were 90 d of age. The pigs were randomly assigned (by random number generator) to the four nursery rooms. Each room had four pens per room, two for males and two for females, with 23 pigs per pen. There were two additional pens per room, initially empty, that were used as hospital pens. A total of 1,140,069 pigs were individually observed and cared for, from the nursery stage to the finishing phase.

2.3 Experimental design

The main effect assessed was management of the pigs in the standard (Control) and IPC models. Animals and pens were equally distributed in both treatment groups. Each hospital pen accommodated pigs from only one IPC pen in order to maintain pen integrity. The control group was managed according to the traditional methodology used on this farm, which provided one observation of the pigs per day. Sick pigs were marked with a spray and treated according to the standard operating practices on the farm. Briefly, clinical signs were treated according to the preexisting treatment protocols at the site. When clinical signs affected several pigs in a pen, treatment was applied in feed or via drinking water to the entire pen. In addition, severely ill pigs were treated, removed from the pen, and left in the corridor, with feed and water available, until they died naturally or their health status improved and they were returned to their pens.

In the IPC group, the IPC guidelines were followed for health and husbandry management of pigs (Pantoja and coworkers, 2013). A different caregiver from the standard care protocol, previously trained in the IPC guidelines by an IPC veterinarian educator, also monitored the IPC-trained farmer during the study. Management consisted of one daily visit to the pigs by the caregiver, treating sick pigs according to clinical signs and the preexisting treatment protocols

at the site, but individual pig treatment was emphasized in this group. The same intramuscular (IM) antibiotics were used in both groups.

All data was recorded using paper forms and a digital pen paired with the SIM card of a commercial smartphone. These devices sent data to a database prepared to automatically process data, which was delivered to a Web-based dashboard or control panel, enabling people to check and monitor data and information generated immediately after collection. Sick pigs were scored, and clinical signs were quantified according to severity (A, mild signs of disease; B, medium; C, serious; and D, very serious or near death) and type of disease (digestive, respiratory, lameness, neurological, bite wounds, or other). Pigs with diseases categorized as B or C were placed in the hospital pens in each room, with males and females accommodated in separate pens. Pigs that did not recover within 3 to 4 d remained in the hospital pen for the duration of the study. Dying pigs (category D) were immediately euthanized.

2.4 Measurements and observations

Pigs were individually weighed and feed intake was measured by pen at d 0 (23 d of age); at day 40, the end of the nursery period (63 d of age); and at d 67, the end of the growing period (90 d of age). Parameters calculated in each of these three phases were average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). Body weight (BW) homogeneity within each pen was also calculated, using the equation "100 minus the coefficient of variation" in each pen. Deaths and incidence of diseases were recorded daily on forms traditionally used on the farm (Control) or on digitalized paper forms using a digital pen (IPC). The use of this digital technology allowed the real-time control of pigs' health in terms of:

- Monitoring the number of pigs that receive a treatment daily
- Monitoring daily the number of pigs scored for a certain disease or treated for it
- Monitoring distribution of each disease and its severity

- Monitoring the products used, their types, dosages and time of use
- Monitoring in real-time fallen out, dead or euthanized pigs
- Calculate new or known ratios and KPIs of interest for the veterinarian and health authorities such as:
    - **Ratio treated / scored**, and therefore the pigs that really need treatment. Indicates how many sick animals are observed vs how many of them are treated. The higher this ratio, the better. This is a new KPI for health control proposed from this project.
    - **Early detection ratio**. Where a value of 1 is perfect early detection and a lower value means late detection. It is calculated by Number of A sickness severity pigs / divided by the sum A+B+C+D. It ndicates how soon an observer detects sick animals. The closer to '1', the better since it indicates that there is a low number of animals detected with more severe degrees of disease. This is a new key production indicator (KPI) for health control proposed from this project.
    - **Treatments per animal**. The number of treatments a pig receives in its batch, either water (a day with water is a treatment) or feed (a day with feed is a treatment).
    - **Ratio UDD/ADD**. The ratio between the dose received and the adequate dose for that weight. Since the age each day is known for every batch, the expected average weight is automatically associated with the pigs and therefore, the system calculates the ratio between the dose recorded (received) and the ideal one based on label recommendations for every antibiotic. This allows us to determine when pigs are being over or under dosed and the over time evolution. The closer to '1' indicates a more accurate dosage.
    - **Percentage mortality**. Number of pigs that died or were euthanized in a batch divided by the initial census.

- **Percentage fall out**. Number of pigs sold as fall out in a batch divided by the initial census.
- **Health index**. Number of animals that are healthy (not scored or treated) divided by total census that day. Varies from 0-100.
- **Dosing ratio** for overdosing or underdosing, comparing doses administered vs doses needed. Once scored, a decision to treat or not, was taken for the antibiotic recommended by the vet and applied. The system takes into account the commercial product, its active compound concentration, and the weight of the piglet based on its age, calculated by regression of the average vales of daily weights of pigs from the literature and our company field trials database of the last 20 years. Therefore, the accuracy of dosing for every pig can be determined based on the ratio between used doses and administered doses (UDD / ADD) and its change over time.

The sickness severity categories were defined based on the following scale:

- **'A' pigs**. Characteristics:
    - From above, full flesh or "bloom"
    - From side, may have slightly gaunt flanks
    - Commonly depressed, may be feverish
    - Listless ears
    - Dull or weepy eyes
    - Might have increased breathing or even thumping
- '**B' pigs.** Characteristics:
    - Definite gauntness
    - Thinner, slab-sided

    - Beginnings of flesh loss
    - Uncomfortable posture: Stiff or rounded back
    - Depressed or feverish
    - Rough hair - Soiled coat
    - Exudate around eyes - Listless ears
- **‘C’ pigs.** Characteristics:
    - Severe gauntness
    - Advanced flesh loss
    - Depressed, commonly no fever
    - Rough hair
    - Soiled coat
    - Dull listless eyes, black exudate
    - Listless ears
- **‘D’ pigs**. Characteristics:
    - A pig that is not recovering and needs to be humanely euthanized.
    - Pigs that show inadequate improvement or have minimal prospect for improvement after two d of intensive care.
    - Severely injured or non-ambulatory pigs with inability to recover.
    - Any pig immobilized with a body condition score of 1.

All of them were qualified for different types of symptoms (respiratory, enteric, brain , lameness, biting, or other -undefined-).

- The cost of medicines, labor, and pigs market prices were used to simulate a return on investment for every farm conditions.

Data was automatically processed and available in real-time on a dashboard of a web-based program specially designed from our team for the project.

2.5 Statistical analyses

Data were analyzed as a randomized complete-bock design using SAS 9.2 (SAS, Cary, North Carolina). Productive performance data were analyzed by ANOVA (PROC GLM), and mortality and incidences of diseases were analyzed as binary variables using the chi-square test. The pen of 23 pigs was the experimental unit. The model included treatment and gender as fixed effects and room as a random effect. Least-squares means were calculated for each treatment, and the effect of treatment was considered significant when $P < 0.05$ and as a trend when $P < 0.10$. No statistical treatment of data of the large trial has been performed until now, only the visual presentation in a web-based dashboard of the KPI's defined.

3. Results

Growth performance is presented in Table 1. Both in the nursery and in the growing periods, ADG and ADFI were higher and FCR was lower in IPC pigs than in the control group ($P < 0.05$). Body weight homogeneity tended to be higher in IPC than in the control group at d 40 and 67. Final BW was higher in the IPC group than in the control group.

Mortality did not differ between treatments in the nursery period (Table 1). Three pigs died, one in the control group and two in the IPC group. Both deaths in the IPC group were D pigs which were humanely euthanized. In the growing phase, pigs were clinically affected by PRRS at approximately 80 d of age, when typical signs were observed (lack of appetite, lethargy, respiratory signs, and blue discoloration of the skin on the ears). Mortality (which was approximately 1%) increased above the average in this phase on this farm and tended to be higher in the control group than in the IPC group (Table 1). One of the seven pigs that died in the control group was placed in the corridor with neurological signs and died approximately 24 hours later.

In the nursery period, morbidity did not differ between treatments: it was high immediately after weaning (43.4% and 52.7% of Control and IPC pigs, respectively, presented some type of

clinical sign) and then decreased progressively up to the first week after weaning when incidence was < 10% in each group. In the 7-d period after weaning, all clinical signs observed were digestive disorders. In the IPC group, sick pigs (52.7%) were individually treated by IM injection of antimicrobials. In this group, 78 sick pigs were scored as A pigs (42.4%), 17 as B pigs (9.2%), and two as C pigs (1.1%). All B pigs were moved to the hospital pen within the first week after weaning, and were treated and returned to their pen within 2 to 3 d, after showing signs of recovery. Both C pigs were moved to the hospital pen and remained there until they died during the growing period. In the Control group, mass antibiotic treatment was the treatment of choice when 20% to 30% of pigs per pen showed clinical signs of a digestive disorder. Within the first 7 d after weaning, all pens in the Control group received colistin sulphate via drinking water (100,000 IU colistin per kg BW daily for 3 consecutive d) and zinc oxide in the feed (2500 g per tonne for 14 d). In addition, more seriously affected pigs (43.4%) received individual IM antibiotic treatment.

In the growing phase, no mass treatments were used, and the percentage of pigs individually treated with antibiotic did not differ between treatments (4.5%). The overall results of the trial for the IPC and control groups are shown in Charts 1 and 2, respectively. Overall disease prevalence and severity by disease is shown in Charts 3 and 4. It is very interesting to see how a higher percentage of IPC group animals are clearly identified for enteric disease (54,9 vs 28,5, $P < 0.05$). Regarding the accuracy of dosage, it is interesting to see how IPC animals are much more accurately dosed over time (much closer to '1') than the Control animals that are overdosed most of the time, in particular at the beginning of the nursery period (Charts 5 and 6). The IPC pigs received a lower number of treatments throughout the whole trial.

The results of the epidemic curves for animals treated for respiratory and enteric disease for the whole data base of 160 farms are shown in Chart 7. There are different patterns for how these two diseases appear, including clear differences in the number of animals treated for each disease. Mortality patterns are different for every disease, as shown in Charts 8, 9, 10, 11 and

12. The percentage deaths and euthanized pigs are shown in Image 1, together with the distribution of their respective causes. It is interesting to note that 70% of all the deaths on all the farms studied occurred by 70 d of age (Charts 8-12)Further studies and analysis are required to confirm this value, but at the moment it could be considered as a possible reference for farms in terms of mortality distribution over time.

Finally, a return on investment calculator based on pigs' performance, mortality and products used was designed to support farmers in decision-taking. The calculator included other variables such as market prices of weaners, nursery piglets and finishers, inspection time related to labor costs and other fixed costs to show the benefits of the use of the protocol. These benefits included the extra price per pig sold, the extra costs per pig sold, total extra incomes and finally benefits per pig out and return on investment per € invested. The dashboard is interactive, web-based and very easy to use (Chart 7).

All these preliminary results related to the whole database should be analyzed in depth to describe properly the effects mentioned above, but this work is beyond of the scope of this thesis.

4. Discussion

The present study demonstrated that the use of the husbandry and health-management program proposed in this study (the IPC program) improved productive performance (both ADG and FCR). In the UK, the Responsible Use of Medicines in Agriculture (RUMA) Alliance of farming, animal-health industry, food-retailing, and associated groups have as their goal the promotion of a coordinated and integrated approach to best practice in the use of medicines. The Pig Working Group of the RUMA Alliance has published guidelines for responsible use of antimicrobials in pig production (RUMA Alliance, 2014). In this document, the importance of early recognition and treatment of disease is considered essential to protect animal welfare, and

is also a cornerstone of responsible medicine use, which is completely in line with IPC principles. The IPC program trains the caregiver to identify sick pigs at an early stage of disease (categorized as “A”).

As a result, the IPC pigs in this study received individual treatment early in the disease process, which may have allowed them to recover quickly with minimal treatment. While they did not receive mass medication, their productive performance was better than that of the control pigs, and, in the growing phase, mortality tended to be lower than that of the control pigs, which had all received mass medication. Injectable antibiotics provide the most effective treatment of outbreak infections, mainly because they do not depend on water or feed consumption, which are usually reduced in sick pigs (Gemus, M.E., 1996).

This protocol is strongly aligned with the recent position of the European Commission in their ‘Guidelines for the prudent use of antimicrobials in veterinary medicine’ from 2015, where they stated that antimicrobial resistance (AMR) is a priority. They recommend aghering to the following principles about when it is necessary to use antimicrobials:

- *‘Prescription should be based on a diagnosis made following clinical examination of the animal by the prescribing veterinarian’*. This is considered as an important basis of the IPC protocol since the starting point is the close observation of the pigs.
- *‘Antimicrobial metaphylaxis should be prescribed only when there is a real need for treatment. In such cases, the veterinarian should justify and document the treatment on the basis of clinical findings regarding the development of a disease in a herd or flock.’* The differentiation of ‘scored’ and ‘treated’ animals in the IPC protocol supports only the treatment of animals that really need it; moreover clinical findings based on scoring ABCD and by disease supports the need to document treatment on clinical findings.
- *‘Routine prophylaxis must be avoided. Prophylaxis should be reserved for exceptional case-specific indications’*. No animal is treated until there is an individual clinical scoring that supports it.

- *'Administering medication to an entire herd or flock should be avoided whenever possible. Sick animals should be isolated and treated individually (e.g. by administrating injectables).'* IPC by definition is individual.
- *'All information relating to the animals, the cause and the nature of the infection and the range of available antimicrobial products must be taken into account when making a decision regarding antimicrobial treatment.'* The IPC protocol promotes close observation from general to specific things, such as observing housing and barn conditions, then the pen and group behavior and finally the individual pigs within the prior context.
- *'Use of antimicrobial agents prone to propagate transmissible resistance should be minimised.'* Using individual treatments and only when required, minimizes the amounts used. In addition, the dashboard IPC report of 'overdosing / underdosing ratios over time' offers valuable information to see what antibiotics are given and if they are properly dosed.
- *'The need for antimicrobial therapy should be reassessed on a regular basis to avoid unnecessary medication.'* Automatic cloud processing of data collected on farm when using the IPC protocol allows the vets to check what is going on real-time and to change the treatment immediately if no results are achieved
- *'The pharmacovigilance system should be used to obtain information and feedback on therapeutic failures, so as to identify potential resistance issues in the case of use of existing, new or alternative treatment options.'*. Exactly the same as in the prior bullet. Pharmacovigilance is easier than ever with these protocols and the cloud dashboard designed to monitor and analyze it.
- *'Whenever possible, individual treatment of the affected animal(s) (e.g. injectable treatments) should be preferred to group or mass treatment.'* It is one the basic principles of IPC.

- *'The quantities of antimicrobials administered in feed or water should be monitored and documented on a continuous basis, especially in intensive food production systems.'* The system is designed to control every antimicrobial administered, including its real dose, timing and type and age of the pig treated. The system leaves hard copies on farm for audit purposes.
- *'In particular, when administering antimicrobials to a group of animals, farmers or any other person administering antimicrobials, should ensure that the correct group of animals is treated, at the required dosage, and for the specified duration of the treatment.'* Every group of animals is identified in a batch with a unique code, with farm name, code, number of animals and entry / exit dates.
- *'The appetites of diseased animals can be depressed, so farmers or any other person administering antimicrobials should monitor whether all animals ingest the adequate/full quantity of the medicated feed containing the therapeutic dose, to avoid under-dosing. Where there is a risk of this occurring, farmers should inform the prescribing veterinarian who should assess the need to modify the treatment regime (e.g. by switching to parenteral treatment).'* The IPC protocol allows feed and water intake to be registered if those data are available, and to link them to health data for instant alert or further analysis
- *'Those who administer antimicrobials should also:*
    - *Cooperate with the veterinarian who regularly visits the animals and knows the history and current health status of the herd, flock or animal, to allow him/her to put in place disease prevention measures that also take account of animal welfare.*
    - *Ensure that the correct dose, treatment duration and dosing schedule is followed.'* Since the dashboard is a web-based application, vets can check if

treatment and doses are correct, anywhere and in real-time, including checking if clinical symptoms evolve properly

- *'Universities and other research facilities should give priority to research in the area of AMR. In veterinary medicine, focus should be given to:*
    - *Developing alternative, preferably preventive, tools for infection control;*
    - *Evaluating the impact of the use of antimicrobials in animals on public health and the environment.'* Our group, as a Contract Research Organization, developed this tool to assess the impact of antimicrobials and minimize their use, the results of which are presented in Chapter 5.
- *'Further investigating pharmacokinetic and pharmacodynamic data and using models to simulate the effects of different dosing schedules (based on different combinations of: disease, pathogen, target tissue and animal species). The results from modelling should provide a scientific background for setting effective dosing schedules in practice. Scientific publications should promote the principles of prudent use.'* The dataset generated can support further pharmacokinetic and pharmacodynamic studies and this paper clearly supports the benefits of prudent use, by means of another dashboard to calculate the return on investment based on different scenarios and market conditions.

It is increasingly necessary to adopt new approaches to food safety and pork quality. One way to describe the quality of pork production might be to collect information about medications used, proportion of pigs needing treatment, and management of herd health. In two studies, antimicrobials used in the different phases of swine production were registered and associated with production, sales, and trade information (Espinasse, 1993, Blocks et al., 1994). However, this kind of data gives little information about how, where, when, and why antimicrobials are used (Dunlop **et al.**, 1998). The current study proposes a new protocol, the IPC program, to generate these records properly, accurately, and quickly. Health and growth

performance might improve considerably with more comprehensive control of disease. In addition, records obtained with this program provide evidence of the timing and amount of medications used and the results of treatment.

5. Implications

Under the conditions of this study, on farms with low health status, IPC training enables the caregiver to identify and treat sick pigs at an early stage of disease, which may contribute to better growth and productivity during the nursery and growing periods. Emphasizing individual treatment of sick animals through the IPC program, rather than mass medication, may result in less overall antibiotic usage and improved productivity. This is reinforced by the monitoring performed under the same protocol applied in a bigger number of farms, since overall results confirm the trends and the feasibility of the system designed for real-time control

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Tables

Table 1. Mean (standard deviation) of growth performance in pigs managed under the Individual Pig Care (IPC) program or a standard care system (Control)*

	Control (n = 8 pens)	IPC (n = 8 pens)	P†
Nursery period (23 to 63 d of age) ‡			
Initial BW (kg)	6.78 (1.122)	6.73 (0.867)	0.91
ADG (kg/d)	0.326 (0.030)	0.368 (0.031)	0.01
ADFI (kg/d)	0.467 (0.041)	0.511 (0.042)	0.048
FCR (kg/d)	1.434 (0.032)	1.392 (0.033)	0.047
BW at 63 (kg)	19.42 (1.333)	20.85 (2.303)	0.07
Homogeneity at 63 (kg)	82.9 (4.142)	87.1 (3.142)	0.06
Mortality (%)	0.54	1.09	0.56
Growing period (64 to 90 d of age)			
ADG (kg/d)	0.391 (0.017)	0.439 (0.044)	0.03
ADFI (kg/d)	0.637 (0.036)	0.696 (0.042)	0.04
FCR (kg/d)	1.634 (0.058)	1.590 (0.047)	0.04
BW at 90 (kg)	29.97 (1.304)	32.71 (2.448)	0.04
Homogeneity at 90 (kg)	82.6 (3.879)	85.9 (3.003)	0.07
Mortality (%)	3.83	1.10	0.09
Total nursery - growing period (23 to 90 d of age)			
ADG (kg/d)	0.3481 (0.011)	0.399 (0.031)	0.004
ADFI (kg/d)	0.532 (0.032)	0.595 (0.048)	0.049
FCR (kg/d)	1529 (0.029)	1.486 (0.032)	0.049
Mortality (%)	4.35	2.17	0.24

* A total of 368 pigs weaned at 23 d of age were used for the experiment, randomly allotted to 16 pens (23 pigs per pen), resulting in eight pens and 184 pigs per treatment group

† One – way ANOVA for productive performance comparisons (ADG, ADFI, FCR, BW and BW homogeneity) and chi-square test for mortality comparisons.

‡ In the IPC group, 19 pigs were moved to the hospital pens within the first week after weaning: 17 returned to their pens in 2 to 3 d; two pigs remained in the hospital pen and died during the growing period. Average daily feed intake was controlled in the hospital pen and included in final calculations.

BW= body weight; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.

Table 2. Tables showing the identification, properties and status of every batch on a farm

Alerts threshold

Select farm / batch

(All)

Vaccines

Batch(es) general information

Husbandry indicators

Batch name	Batch type	Date start	Date end (last form)	Batch duration	Initial inventory	Batch status	Form used
BEAAA-2-2012-107	2	15/11/2012	24/12/2012	39	224	Closed	Form I
BEAAA-2-2012-108	2	15/11/2012	21/12/2012	36	224	Closed	Form I
BEAAA-2-2012-116	2	09/11/2012	18/12/2012	39	320	Closed	Form I
BEAAA-2-2012-117	2	09/11/2012	21/12/2012	42	320	Closed	Form I
BEAAA-2-2012-118	2	09/11/2012	21/12/2012	42	159	Closed	Form I
BEAAA-2-2012-216	2	28/12/2012	04/02/2013	38	304	Closed	Form I

Chart 1. Main screen of IPC dashboard for the trial for the IPC group, showing key performance indicators and ratios

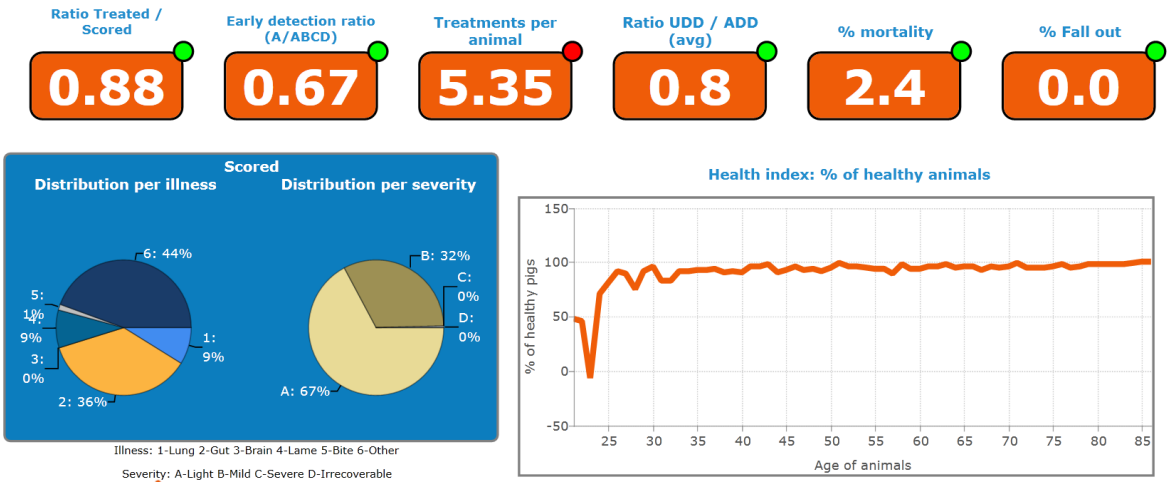


Chart 2. Main screen of IPC dashboard for the trial for the Control group, showing key performance indicators and ratios

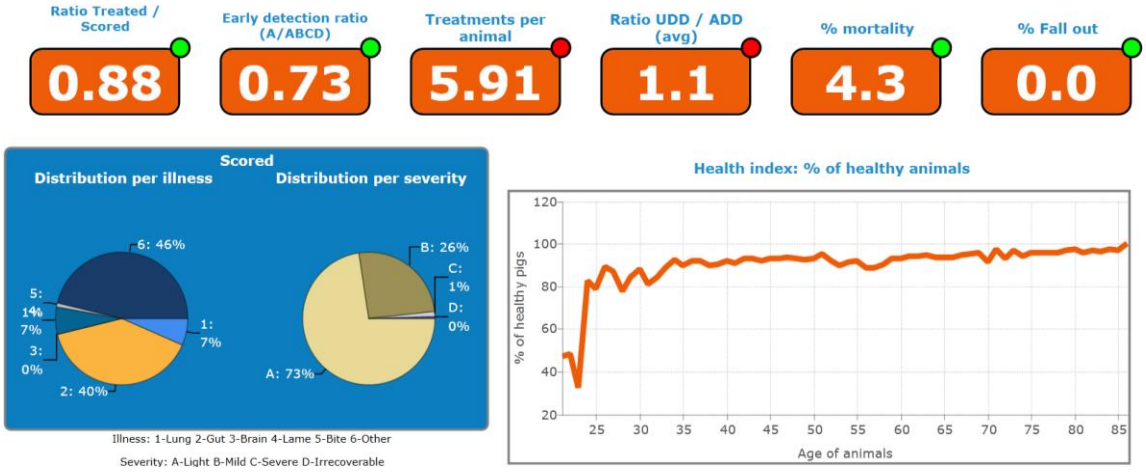


Chart 3. Dashboard for treated and severity distribution for the IPC group

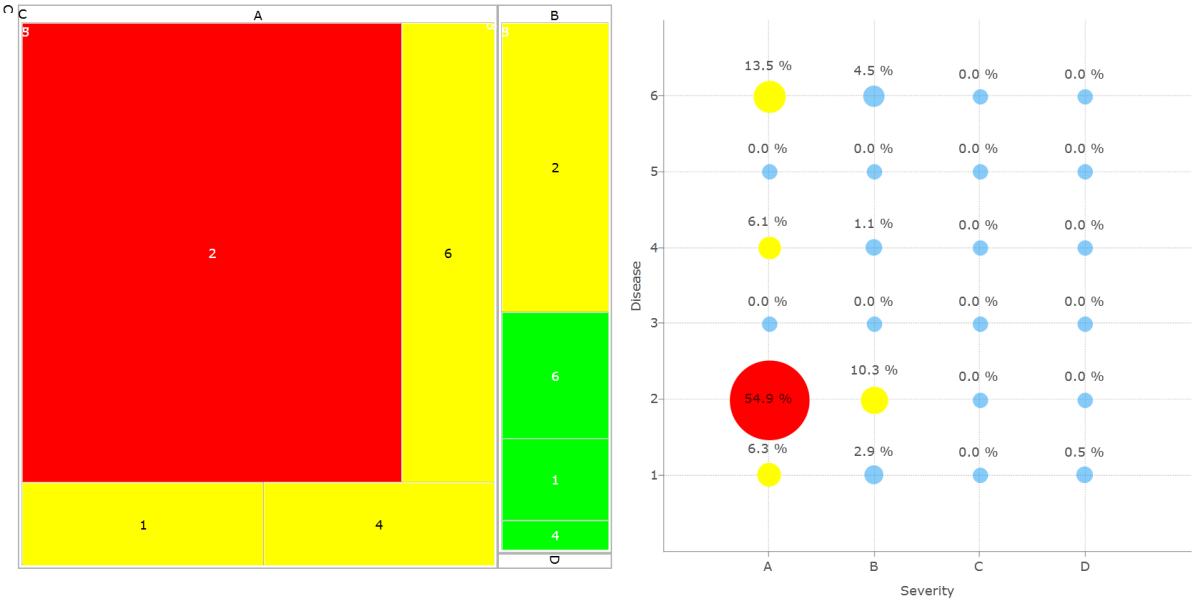


Chart 4. Dashboard for treated and severity distribution for the Control group

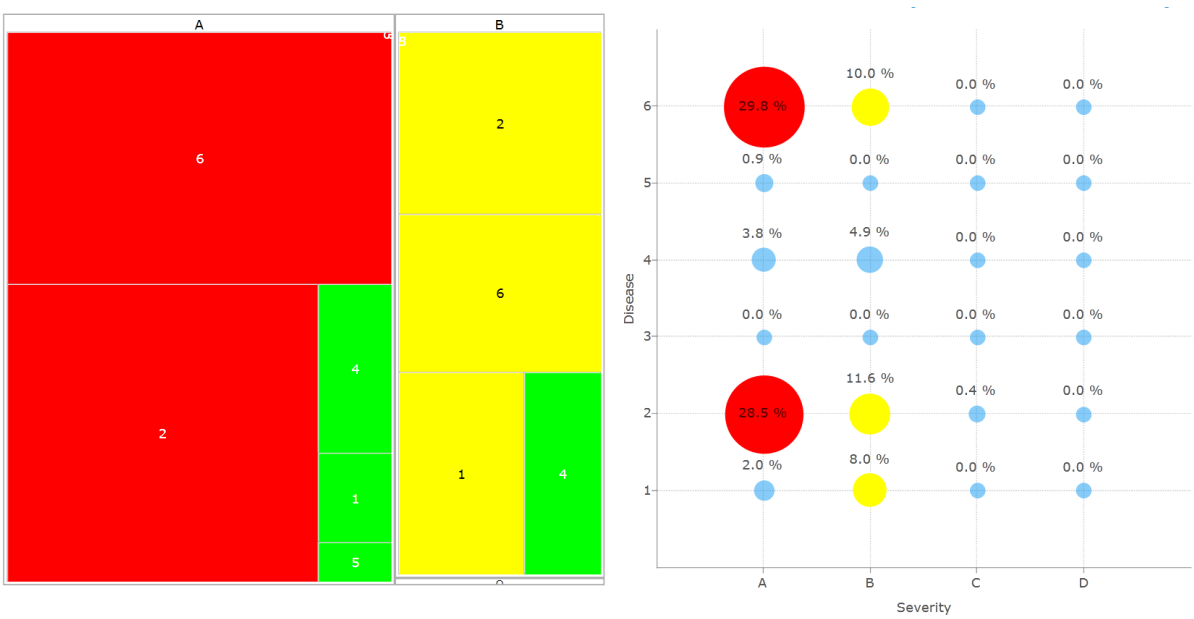


Chart 5. Dashboard for evolution of treatments over time and proper dosing in the IPC group

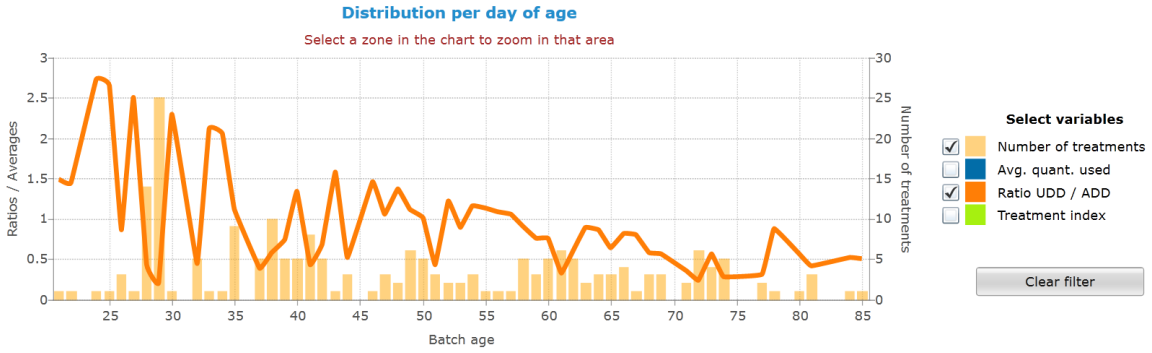


Chart 6. Dashboard for evolution of treatments over time and proper dosing in the Control group

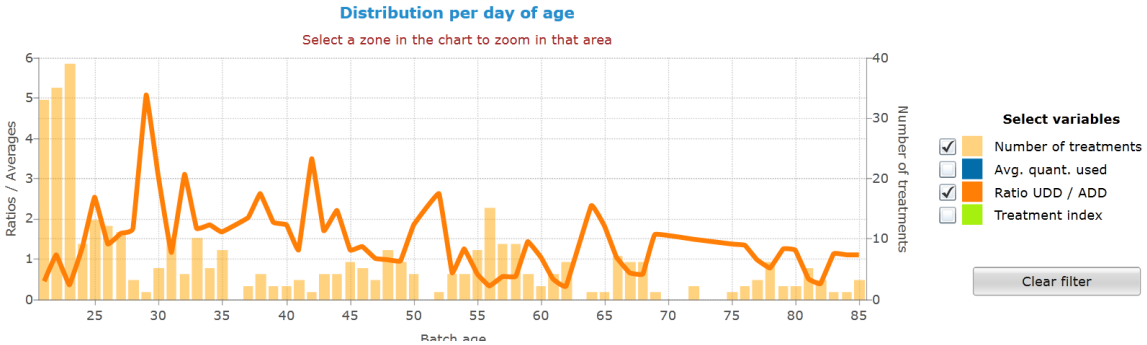


Chart 7. Dashboard for the epidemic curves for animals treated for respiratory (blue) and enteric (orange) disease of the whole database

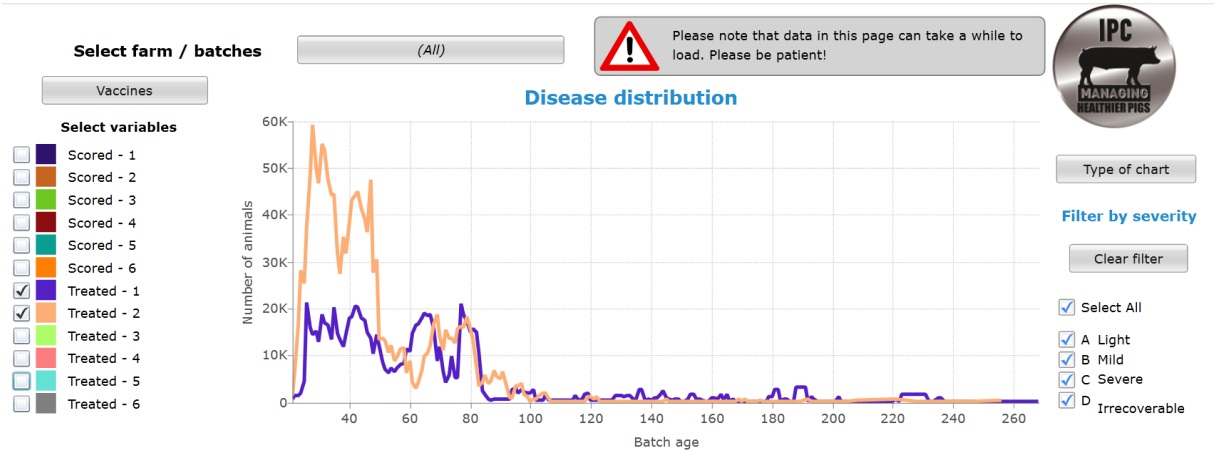


Chart 8. Mortality distribution for respiratory disease by age in the whole database of IPC protocol.

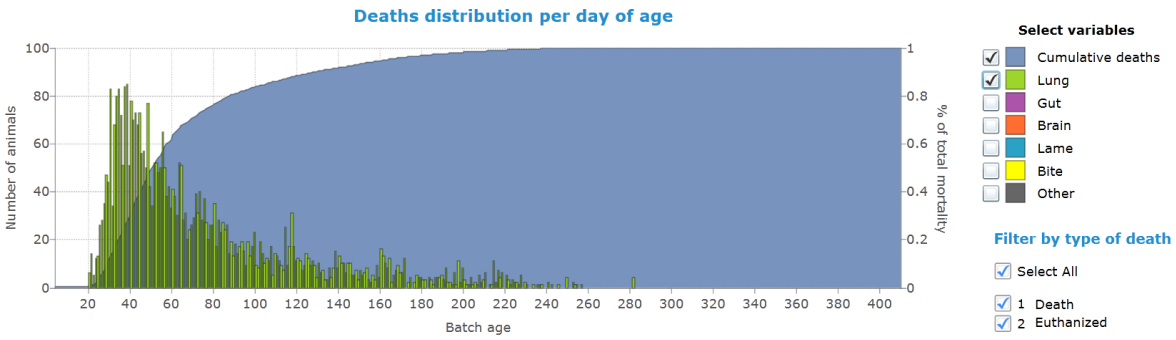


Chart 9. Mortality distribution for enteric disease by age in the whole database of IPC protocol.

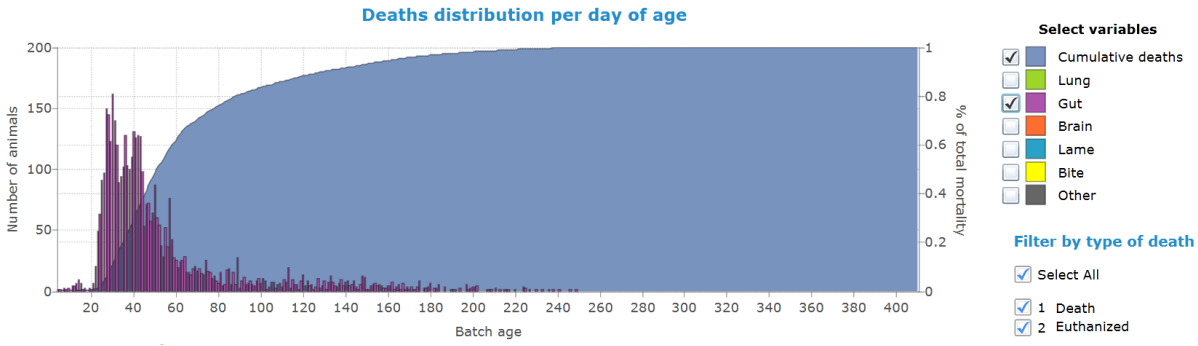


Chart 10. Mortality distribution for brain disease by age in the whole database of IPC protocol.

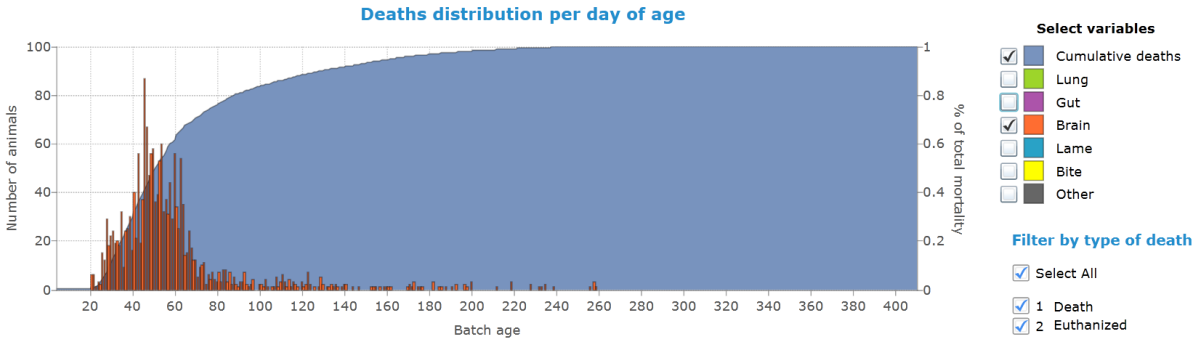


Chart 11. Mortality distribution for lame disease by age in the whole database of IPC protocol.

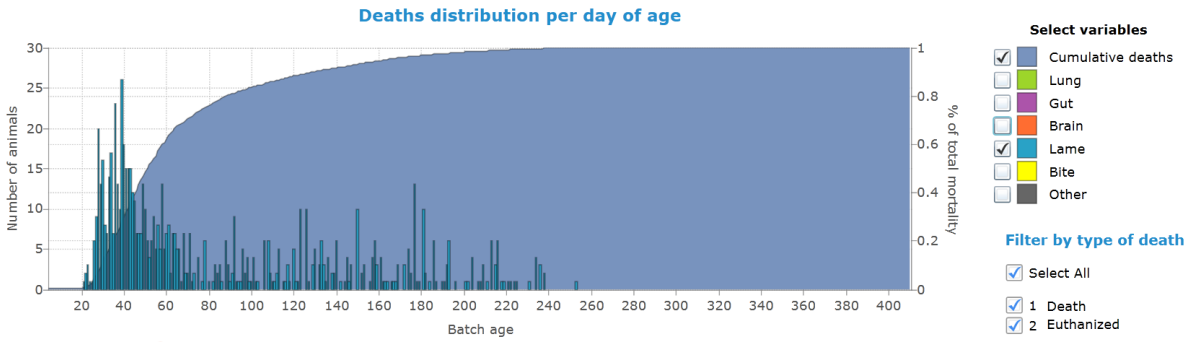


Chart 12. Mortality distribution for bite disease by age in the whole database of IPC protocol.

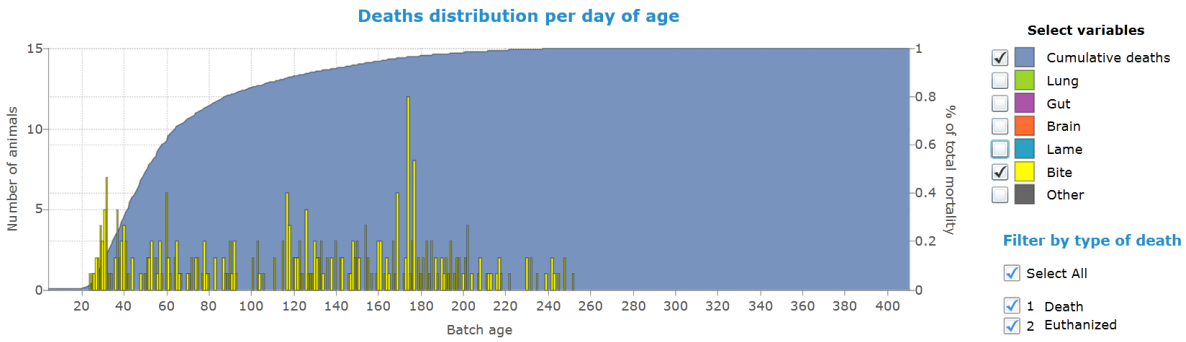


Chart 13. Dashboard for the calculation of the return on investment (ROI) based on the trial and under different scenarios

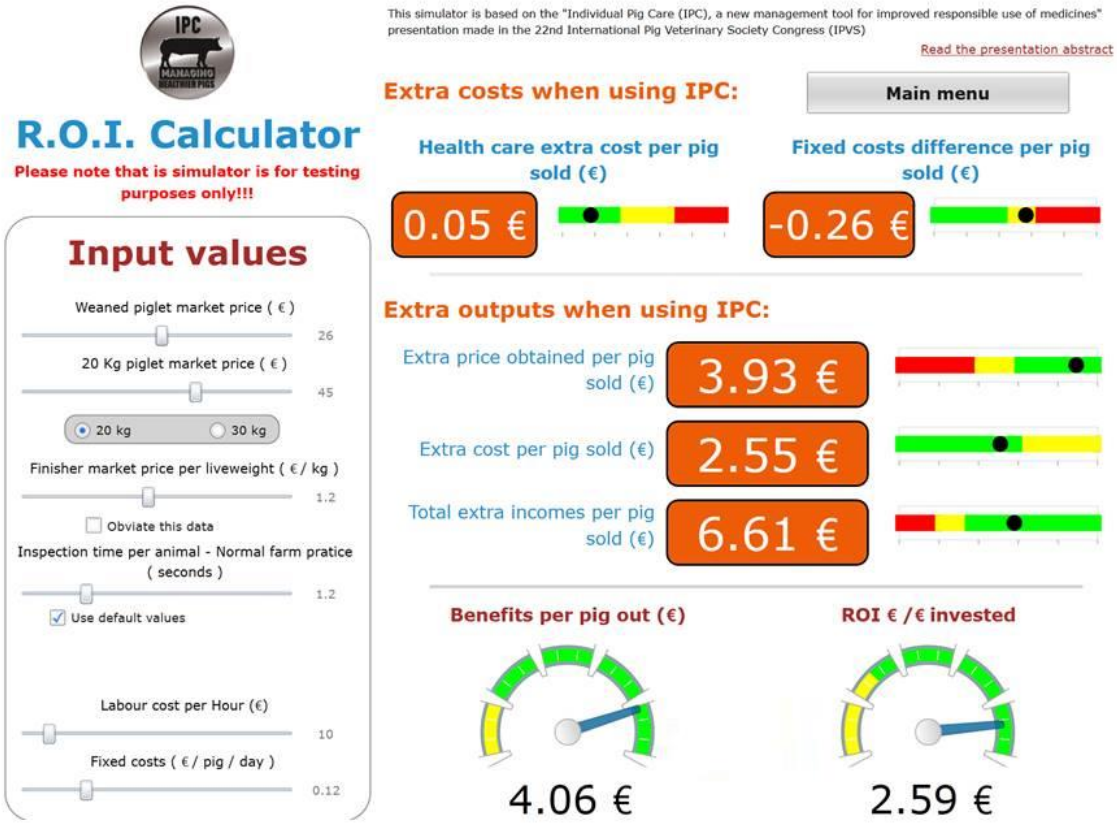
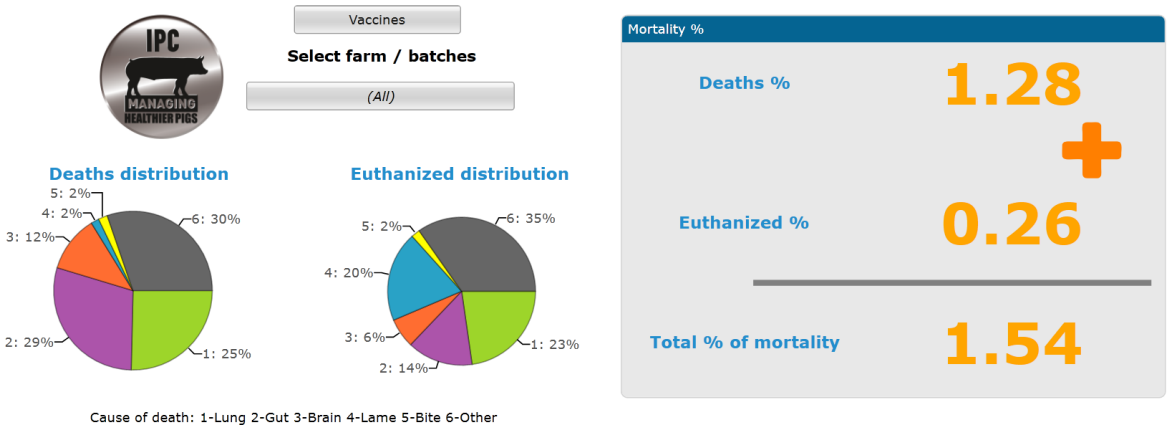


Image 1. Deaths and euthanized pigs distribution and percentage of them in the whole database of IPC protocol.



OVERALL DISCUSSION

Current swine production is led by competitiveness worldwide. This must be understood as achieving a high productive performance under the highest quality standards required by the market and regulations, including compliance with welfare, control of pollutant emissions and food safety assurance including antibiotic restriction or even free of them.

Customers are changing the way they eat. There is a growing number of conferences and lectures on trends in food consumption worldwide. Main topics are related to health concern, importance and variety and new experiences, food safety, ethical and environmental issues. Of course, meat is a central element in our eating, and its role is continuously discussed when discussing about food, but probably animal welfare, food safety and environment are the most relevant topics for the consumers about pig farming. Animal welfare is of growing importance for customer's concern. Eurobarometer 2015 reported that almost all Europeans consider the welfare of farmed animals to be important and that their welfare should be better protected than it is now, and that they are prepared to pay more for products sourced from animal welfare-friendly production systems. Moreover, one of the major concerns for consumers is antimicrobial resistance (AMR). Abuse in food animals has important consequences for public health, as it promotes the development of antibiotic-resistant bacteria and resistance genes that can be passed on to people. Ultimately, this can result in human infections with antibiotic-resistant bacteria that can be difficult or impossible to cure. AMR is not only an animal health and economic concern because it decreases the efficiency of antimicrobial treatment in animals, but it is also a public health concern due to the transmission of antimicrobial-resistant bacteria through the food chain and the transmission of resistance from animal bacteria to human bacteria.

Efforts should focus on reducing the unnecessary use of antibiotics and reducing the spread of antibiotic-resistant bacteria. These general guidelines related to animal welfare and AMR tackling, published from very prestigious world institutions as the WHO and EC, coherent

with customer and market trends are strongly aligned with the objectives and working principles of this thesis.

Understanding, detecting, as early as possible, and controlling the acute phase reaction is a way to detect risks and losses of performance, even before they are clinically evident, since acute phase reaction can be understood as a biological thermometer of stress. To understand the contribution of the most relevant acute phase proteins, pig major acute phase protein and haptoglobin were studied to determine respective reference levels in pigs under commercial conditions on farms, standardizing results for parity, sex and season. Once this was established, a study was performed to determine the influence of psychological stress induced by means of a disordered feeding pattern on (APP) levels and productive performance. The study is closely related with the concept of chronic-intermittent stress, that has been largely discussed in the literature. Novelty has been demonstrated to be a very strong stressor, especially when an animal is suddenly confronted by it, and thus the lack of food in the feeder was expected to cause a stress response in animals used to having food permanently available. This concept matches nicely with the stress induced in this study, where the animals didn't perceive stress all the time, but only in those moments where they saw pigs in other pens being fed while they did not have any feed delivered. However, it is also important to highlight that this study supports the notion that non-inflammatory, psychophysical stress can induce a discernible APP response in healthy domestic animals, since the APP response has been considered almost an exclusive marker of inflammation and / or infection. Our group demonstrated the same fact in other study where the stress was generated by transport and the different quality of it. Both studies of our group confirm the stress – acute phase reaction linkage in pigs under commercial conditions, suggesting that the acute phase response is inducible to a considerable extent by stressful events to which, domestic animals are ubiquitously exposed during daily management. This point of view, highlighting the inconspicuous but essential linkage between stress and the acute phase

response has been proposed. In any case, the mechanism by which the acute phase response is induced in stressed animals still remains unknown.

Parity segregation of the progeny is a technique, progressively and intuitively used by producers, since some of its effects are evident them in a daily use, and this thesis presents a way to modulate and fine tune the technique by properly managing colostrum intake between gilts and mature sows, to avoid later problems up to the end of the finishing period where its effects remain. A practical application of this protocol will be the implementation of a segregation of the piglets from gilts, to raise them with adequate health or management plans that control the negative impacts.

Finally, the IPC protocol has similar objectives qualifying individual pigs on early symptoms and treating them accordingly, with the right antibiotic, at the right time and at the right dose. The application of the protocol demonstrated positive effects on productive performance and homogeneity, and the methodology for its application on a large scale (more than one million pigs controlled) delivered new insights on type of disease prevalence, dynamics epidemiological curves for every disease and antibiotics use and dosage. These two protocols, individually or combined, are effective tools to improve the efficiency of production and ensure the highest production standards required from customers and regulations.

In conclusion, this Thesis shows that biomarkers present in the acute phase reaction, Pig-MAP in particular, are useful tools to assess the health and welfare status of pigs on commercial farms, and even to predict later risks of losses of performance and carcass quality. Furthermore, two working protocols to avoid health and performance problems or to promote their early detection, were successfully assessed under commercial conditions, being a good practical option for farmers, veterinarians and production managers. This has important practical implications since the objectives are to improve productive performance and at the same time respect animal welfare and decrease antibiotics use can be achieved by producers in

in an easy, practical and affordable way.