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Risk based approach towards more sustainability in European pig production

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ABSTRACT

Risk based approach towards more sustainability in European pig production

Major aim of this thesis was to demonstrate how the use of a HACCP concept, a risk based approach, improves the sustainability of added value chains in European pig production. The thesis is featured as a pseudo-cumulative work with general introduction and conclusions and five independent chapters. Sustainability comprises nine themes. This thesis concentrates on animal health, meat quality and meat safety. 127 pig producing farms from five European countries and 15 different farming systems were investigated to design a catalogue proposing checklists for sustainability evaluation. This catalogue was used to assess farm specific risks in relation to animal health and meat safety, using principal component analysis. Only in the case of low risks due to diseases and failures of management, pig producing farms and the whole meat production chain can be categorized as sustainable. High intra- and inter-system differences of present risks were identified by this procedure. A combination of results from audits based on checklists and results from monitoring measures increase the certainty of risk assessment. A method for a continuous control and management of these sustainability aims was developed based on the principles of the HACCP concept. Unspecific and sensitive inflammatory markers take a key role regarding these monitoring measures. The innate immune system is affected by many factors like lesions, diseases, infections and permanent psychological stress and responds by increased concentrations of so called acute phase proteins. During a life cycle study with 99 pigs from rearing to slaughter, resulting in a data set of more than 18000 individual data records, these indicators of increased risks were investigated in detail. The correlation analyses of serum concentrations of these indicators measured at an age of 13 weeks presented the most significant coherences with parameters of meat and carcass quality. A direct coherence of the sustainability themes animal health and meat quality was proved for the first time. The risk of organ abnormalities was 16 times higher in cases of increased serum concentrations of one of these indicators measured directly before slaughter. The results proved these indicators in combination with further information to improve efficiency in terms of risk assessment and attendant measures. Implementation to practice was supported by the development of a rapid measurement method for the indicators based on a biosensor system.

KURZFASSUNG

Risikobasierter Ansatz zur Steigerung der Nachhaltigkeit der Europäischen Schweineproduktion

Das übergeordnete Ziel der Arbeit war es, zu demonstrieren, wie durch das HACCP Konzept als Risiko basierter Ansatz für die gesamte Wertschöpfungskette die Nachhaltigkeit der Europäischen Schweineproduktion gesteigert werden kann. Die Arbeit ist als pseudo-kumulative Schrift gestaltet mit allgemeiner Einleitung und Zusammenfassung sowie fünf in sich geschlossenen Publikationen. Nachhaltigkeit umfasst neun Themengebiete, von denen im Rahmen dieser Arbeit schwerpunktmäßig Tiergesundheit, Fleischqualität und Lebensmittelsicherheit bearbeitet wurden. Zur Gestaltung eines Checklistenkatalogs standen insgesamt 127 Schweine haltende Betriebe aus 5 EU Ländern und 15 verschiedenen Haltungssystemen zur Verfügung. Der entwickelte Checklistenkatalog ermöglichte es, unter Anwendung der Hautkomponentenanalyse, betriebsspezifische Risiken für die Gesundheit der Tiere zu bewerten und die Sicherheit, der von diesen Tieren stammenden Lebensmittel, einzuschätzen. Nur, wenn bezogen auf beide Bewertungskriterien die Risiken für das Auftreten von Krankheiten oder Managementfehler gering sind, können tierhaltende Betriebe sowie die gesamte Wertschöpfungskette Fleisch als nachhaltig eingestuft werden. Sowohl innerhalb als auch zwischen verschiedenen Haltungssystemen konnten sehr unterschiedliche Risikolagen identifiziert werden. Die Sicherheit der Risikobewertung wird durch eine Kombination von Checklisten-gestützten Audits mit Ergebnissen aus Monitoringmaßnahmen erhöht. Hier wurde eine Methode zur kontinuierlichen Überwachung und Steuerung dieser Nachhaltigkeitsziele in Anlehnung an die HACCP-Methode entwickelt. Eine zentrale Rolle im Rahmen eines Monitorings nehmen dabei unspezifische, sensitive Entzündungsmarker ein. Das Immunsystem reagiert auf eine Vielzahl von Faktoren, wie Verletzungen, Infektionen oder andauernden psychischen Stress, durch eine vermehrte Ausschüttung der sogenannten Akute Phase Proteine. Diese Risikoindikatoren wurden, im Rahmen eines Versuchs zur Beschreibung des Lebenszyklus von 99 Schweinen mit mehr als 18.000 erfassten Einzeldaten, weiter untersucht. Korrelationsanalysen der Serumkonzentrationen dieser Indikatoren wiesen im Lebensalter von 13 Wochen die meisten signifikanten Zusammenhänge zu Parametern der Fleisch- und Schlachtkörperqualität, die nach der Schlachtung bewertet wurden, auf. Es wurde erstmals nachgewiesen, dass zwischen den Nachhaltigkeitsaspekten Tiergesundheit und Fleischqualität ein direkter Zusammenhang besteht. Für das Auftreten von Organveränderung lag bei erhöhten Konzentrationen eines Indikators, ermittelt kurz vor der Schlachtung, ein 16fach erhöhtes Risiko vor. Es wurde gezeigt, wie diese Indikatoren in Verbindung mit Informationen aus Audits, zu einer Effizienzsteigerung im Rahmen der Risikobewertung und sich daran anschließenden Folgemaßnahmen genutzt werden können. Die Entwicklung einer Schnellmethode zur Messung der Risikoindikatoren, basierend auf einem Biosensorsystem, unterstützt eine mögliche Implementierung der entwickelten Methode.

Content

List of abbreviations

AGP	acid glycoprotein
APP	acute phase protein
APR	acute phase reaction
CAC	Codex Alimentarius Commission
CCP	critical control point
CMD	carboxymethyl dextran
CRP	C reactive protein
CV	coefficient of variation
DE	German large white
DL	German landrace
EC	European Commission
e.g.	exempli gratia
ELISA	Enzyme Linked Immunosorbent Assay
EU	European Union
FMEA	Failure Mode and Effects analysis
GHP	Good Hygiene Practices
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
Hp	haptoglobin
IQR	interquartile range
ME	metabolizable energy
O	occurence rating
OR	odds ratio
PBS	phosphate buffered saline
Pig-MAP	pig major acute phase protein
PPP	Private Public Partnerships
QAS	Quality assurance system

RPN	risk priority number
S	severity rating
SAA	serum amyloid A
SAW	surface acoustic wave
SD	standard deviation
SME	small and medium sized enterprises
SPR	surface plasmon resonance
TR-IFM	time-resolved immunofluorimetry
WCED	World Commission on Environment and Development

1 General introduction

1.1 Introduction

The expected doubling in global food demand within the next 50 years will be the major challenge for sustainability both of food production and of terrestrial and aquatic ecosystems and the services they provide to society. Agriculturalists are the managers of useable lands and they will mainly shape the surface of the Earth in the coming decades. New incentives and policies for ensuring the sustainability of agriculture and ecosystem services will be necessary if we are to meet the demands of improving yields without compromising environmental integrity or public health (Tilman *et al.*, 2002). Since the World Commission on Environment and Development published the Brundtland report “Our Common Future” (WCED, 1987), academic, scientific and policy-making communities were focused considerably on the concept of sustainable development. A general definition of sustainable agriculture was formulated by Harwood (1990):

> *“Sustainable agriculture is a system that can evolve indefinitely toward greater human utility, greater efficiency of resource use and a balance with the environment that is favorable to humans and most other species.”*

Brown *et al.* (1987) already named the three general definitions of sustainability ecological, social and economic. Bloksma and Struik (2007) supported farmers to redesign their farms in accordance with these three sustainability aims, now called “people, planet and profit“. In 2011 Bonneau *et al.* defined nine themes for an enquiry to evaluate the overall sustainability of fattening pig farms. However, there is an absolute necessity of effective information systems along the whole production chain and a high potential of quality management methods to control and improve chain wide sustainability (Schmitz, 2006; Lehmann *et al.*, 2011; Wever *et al.*, 2011, Wognum *et al.*, 2011). Quality management systems are wide spread, well known and even required by law in food production chains (EC, 2002). This fact even enhances the recommendation of quality management methods for sustainability assessment.

A very well known quality management system heading for consumers protection is the Hazard Analyses and Critical Control Point (HACCP) system. This concept originally focuses on hazards of food origin for human health (CAC, 1997). But this established approach is not only suitable to cope with hazards, but also with other aspects of food production, like nonconformity with defined sustainability criteria. HACCP is implemented in pork chains in all stages of the production, except the farm level (figure 1.1).

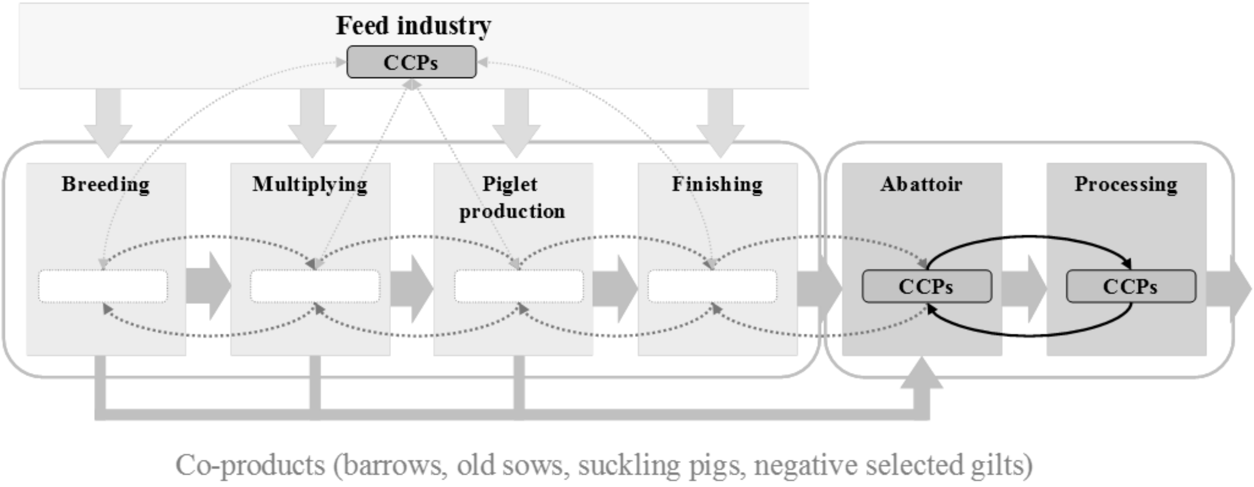

Figure 1.1 Product and co-product flow on farm-level in pork chains – HACCP gap on farm level

In pork chains the implementation of HACCP is advanced in large companies on feed production, slaughter and processing level according to EC (2002, 2004). At this stages of a chain SMEs often have problems, because of a deficit of essential human resources. This is more distinctive on farm-level. Here a gap still exists because a practicable way of implementing HACCP has not been found, yet. Hence, primary production was excluded from the legal requirement to implement HACCP in the self control of farms for now, the member states shall motivate primary production to develop concepts for the HACCP implementation. The EU aspires to have a coherent HACCP in the whole food chain (EC, 2002). For some pathogens the use of a HACCP system is well described in publications, especially *Salmonella* ssp. (Noordhuizen and Frankena, 1999; Borell *et al.*, 2001). But the application of quality management tools to improve and control farm sustainability was not tested, yet. Within current literature a high interest of farmers to participate in monitoring programs for pig health is reported (Schütz, 2010, Ellebrecht, 2012). The willingness of farmers to attend such monitoring activities seems to be lower than the willingness to improve their biosecurity measures (Vaaleva *et al.*, 2011). A combination of both improved biosecurity measures and monitoring programs to control the effectiveness of taken actions would be advantageous from the point of quality and animal health management (Petersen *et al.*, 2002). Therefore, Brinkmann *et al.* (2011) defined a chain coordination model to encourage quality management strategies of pork supply chains. The model highlights the importance of service organizations (Network coordinators) supporting all enterprises along the production chain sharing their data (collection, analyses, communication) and by this enables a joint decision making. Besides these supporting organizations, the use of incentive mechanisms for food safety and animal health control is recommended (van Wagenberg, 2010).

In herd health and production management programmes it is common use to make an inventory of the herd performance status (Petersen *et al.*, 2002). The activities comprised under “inventory” are often called “monitoring”. Monitoring is an important component of quality risk management programmes following the rules of a HACCP concept as well. Monitoring is an act of conducting a planned sequence of observations or measurements of certain control parameters to assess whether a certain point in the production process is under control or functioning correctly or shows conformity with market or society demands. It is highly indicated to conduct also an inventory (i.e. monitoring) regarding the prevailing risk conditions on the farm in animals, their surroundings, the management and the farm records (Berns, 1996; Mack, 2007). Such risk conditions can be found through a strengths- and weaknesses assessment on the farm. Preventive quality management methods proposed in ISO 9000 and 22000 have been introduced to support management in decision making, to reduce failure and costs, to assure conformity with demands and thereby increase income (Petersen und Nüssel, 2013).

1.2 Research aims and underlying hypotheses

Major aim of this thesis is to design a conceptual approach for a risk based quality management system towards more sustainability in pig production. Therefore, several steps have to be implemented. First, it is necessary to identify whether there is a need for a continuous assessment of farm sustainability aspects. The status quo of on-farm situations of important sustainability themes must be evaluated. Furthermore, it has to be determined on which level the assessments should be performed (e.g. single farms, production systems).

Based on the results from a survey on farm level following hypotheses were tested:

1. Is it possible to design a risk based approach towards more sustainability?
2. Can parameters with high potential for on-farm risk assessment be identified?
3. Can a rapid method for the measurement of these risk indicators be developed?

To reach the aims and prove the hypotheses, the following outline of the study was set up.

1.3 Outline of the thesis

The research design of this study was organized following a three level approach. In figure 1.2 the conceptual structure of the thesis is presented showing the empirical and methodological steps that were taken to achieve the results.

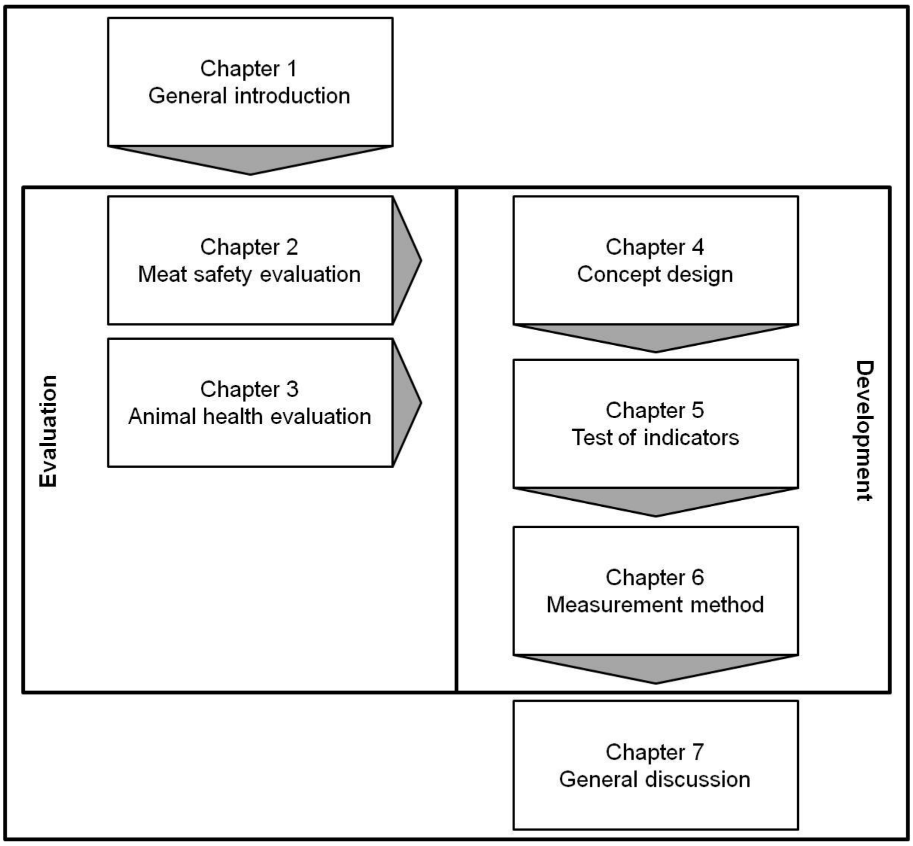

Figure 1.2 Outline of the thesis

In chapters 2 and 3, tools for the assessment of the animal health situation and the compliance with specific meat safety aspects on farms were developed and tested based on a survey of farmers from five European countries. The tools were described in detail and the variability of farms was shown on the level of investigated production systems. Using statistical tools, the practicability of the developed tools were checked and possibilities for future improvements explained.

Based on the results presented in chapters 2 and 3, a risk based approach towards more sustainability in pig production was created. This approach is presented in chapter 4. Well known quality management tools were applied to identify those sustainability themes having the biggest impact on overall sustainability of farms. Indicators for the assessment of these themes were revealed and critical limits were defined. Furthermore, monitoring measures and corrective actions were presented. Thus, a risk based approach towards more sustainability of fattening farms was presented.

Within an experimental study performed with 99 pigs the identified indicators were tested to give practical indications for an optimization of the testing strategy (chapter 5). Therefore, the experimental animals were observed during their whole life. Samples were taken and analyzed to show the potential of these indicators for the prediction of certain sustainability aspects. The study focused on sustainability aspects for which the coherence to these indicators was not reported in literature.

As the results of the experimental study proved the practicability and potential of the identified indicators, modern technology was applied for their measurement (chapter 6). Surface acoustic wave biosensor technology was tested in comparison with results from the current common measurement methodology. Therefore, the upset of the chip-surface was adapted to the investigated parameter. The results indicated this technology could fasten and cheapen the measurement of the identified sustainability indicators. Besides, a combination of different parameters could be enabled using this technology.

Chapter 7 was concentrated on general discussions of the results, conclusions, the practical usage of the results and the need for further research.

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2 Evaluation of meat safety management in contrasting pig husbandry systems in Europe

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2.1 Abstract

Meat safety, animal health and welfare issues are becoming increasingly important to all sectors of pig production. Improvements of these issues will determine the sustainability of European pig production in the next years. Within this study 127 farmers representing 15 different husbandry systems from five European countries were interviewed using a standardized quantitative questionnaire. The objective of the enquiry was to evaluate differences regarding meat safety aspects between and within husbandry systems using a simple and comprehensive tool. The study was part of a bigger attempt to evaluate and compare the overall sustainability of different European pig husbandry systems within the European Integrated Project ‘Q-PorkChains’ (FOOD-CT-2006-036245) of the 6th EU Framework Programme. Farms of ‘conventional’ or ‘adapted conventional’ categories of husbandry systems achieved a higher standard concerning meat safety measures than farms of the categories ‘organic’ and ‘traditional’. However, a high variability between farms was observed within the investigated husbandry systems likewise, except for the ‘traditional’ systems where most farms achieved a low level of accordance with the requested meat safety parameters. It can be concluded, that the used approach is suitable to point out differences between pig husbandry systems regarding meat safety. It is possible to produce fattening pigs in conformity with the requested meat safety parameters, but for traditional systems this seems to be more difficult. The developed tool enables fattening farmers to benchmark with others and it is suitable for administration by non-specialist.

Keywords: Meat safety, enquiry; pig fattening; sustainability; Europe

2.2 Introduction

Over the last years biosecurity at farm level as well as preventive animal health and hygiene measures became key parameters to improve and obtain meat safety in primary pork production (Food and Agriculture Organization, 2010; European Food Safety Authority, 2011; Wilke *et al.* 2012). A rapid data acquisition and timely communication along the whole chain are important to apply measures to eliminate possible hazards on the following stage of pork production chains when necessary (Petersen *et al.*, 2002; Lehmann *et al.*, 2011). Therefore, simple and comprehensive tools are needed to collect data identifying possible risks.

From 2000 on, different tools for hazard analyses have been designed to assess and reduce overall risks for meat safety or to prevent risks caused by specific pathogens (Howell and Hutchison, 2009; Baptista 2011; Van der Wolf *et al.*, 2011). The Codex Alimentarius Commission (2003) set guidelines for pre-harvest food safety, being instructions for good hygiene practices, mainly. They do not aim at assessing a food safety status (Siekkinen *et al.*, 2006). To benchmark or compare meat safety issues of abattoirs and cutting plants, tools such as the Hygiene Assessment System (HAS) (Pinillos and Jukes, 2008) or the Hygiene Risk Assessment Model (HYGRAMR) (Tuominen *et al.*, 2003) are available. But since the commission enacted the EU Regulation 178/2002 (European Commission, 2002) the farmers are forced to minimize foodborne risks for consumers, too. Siekinnen *et al.* (2006) presented a first tool concentrating on hygiene proficiency in Finish farms. Tools assessing food safety for the whole chain, which did not resort to laboratory analysis, have also been recorded (Jacxsens *et al.*, 2010; Baert *et al.*, 2011). A sanitary risk index (SRI) to assess the prevalence of *Salmonella* in pig farms has been developed by Hautekiet *et al.* (2008). Besides, internet based tools like “Biocheck” from Belgium (*http://www.biocheck.ugent.be/v4/about/pig/*) and the tool created in the United Kingdom (Howell and Hutchison 2009; *www.ukmeat.org/FSAMeat/NewMethod.aspx*) concentrate on risk assessment and minimization on the farm level. These tools are internet based, as very much and detailed information is requested (Howell and Hutchison, 2009; Van der Wolf *et al.*, 2011).

For meat safety management on farm level physical hazards like broken needles or bone fragments are not of major importance, as these hazardous materials or objects can be detected and removed during slaughter and cutting due to the private food safety systems (Aladjadjiyan, 2006; Knura *et al.*, 2006) and applied technologies (Chen, 2003; Diaz *et al.*, 2011). However, these materials affect animal health and welfare. Thus, the compliance with good farming practices is very important (von-Borell *et al.*, 2001). Chemical hazards (e.g. dioxin contamination) often result from defects in animal feed production and should be detected already at this stage of production, by effective private food safety systems (De

Meulenaer, 2006; Heres *et al.*, 2010). Chemical hazards can also result from incorrect or criminal dispensing of medical drugs (Andree *et al.*, 2010). Hence, there is a strong linkage between the prevention of biological hazards and the occurrence of chemical hazards caused by medical drugs (Rovira *et al.*, 2006; Laanen *et al.*, 2011). Thus, biological hazards are of major interest for quality management at fattening farms (Sofos, 2008; Fosse *et al.*, 2009; Rostangno and Callaway, 2012).

Biological hazards are *“biological [...] agents in, or condition of, food or feed with the potential to cause an adverse health effect”* (EC, 2002). The motto of the EU Veterinary Week 2010 “*Animals + Humans = One health*” makes clear that biological hazards are not only related to animals’ but also to consumers’ health.

The three aims of this study were to develop and test a comprehensive tool to identify weaknesses in the potential of farms to meet a required level of meat safety (1), to identify structural differences between farms, systems and categories (2) and to check the tool for possible simplifications by elimination of redundancies (3).

2.3 Material and methods

To limit possible costs and making the tool an additional risk at farm level by extra farm visits, a questionnaire suitable for administration by non-specialist was developed. Figure 2.1 shows the schematic approach of the designed questionnaire. The questionnaire was tested in a wide variety of pig husbandry systems over Europe and the potential for further simplifications by elimination of redundancies was investigated.

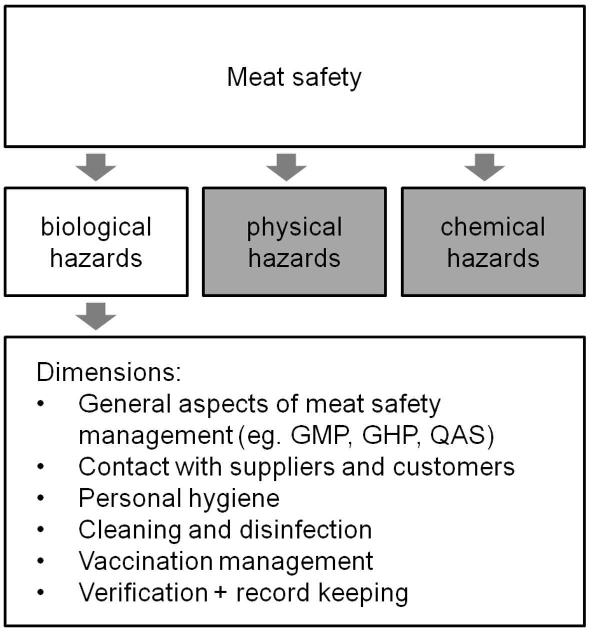

Figure 2.1 Schematic development of the dimensions used within the questionnaire

2.3.1 Development of a tool to assess on-farm meat safety management

Several available tools, mentioned above, for the assessment of meat safety risks were combined to design the questionnaire, covering important aspects of meat safety assessment in primary production (figure 2.1) and suitable to be administered by experienced people that are not specialist on this theme. The questionnaire was developed following the ideas of quality management (Stretch, 2005), where hazards are addressed as aspects of food safety management on the stage of food preparation. The six dimensions of the questionnaire were designed following earlier reports by others (von Borell *et al.*, 2001; Norrung and Buncic, 2008; Fosse *et al.*, 2011; Doyle and Erickson, 2012; Jenson and Sumner, 2012). The meat safety tool covered six dimensions, each being addressed with a different set of questions (table 2.1) regarding a meat safety aspect.

Table 2.1 Questioned dimensions and aspects of meat safety

no.	**General aspects of meat safety management**
1	Do you follow requirements of guidelines like “Good Hygiene Practice” etc.?
2	Do you produce under a quality system? (Ex. QS, IKB, QSG, Certus; if yes please comment the name)
3	Do you attend to a brand meat programme? (if yes, please comment)
	Contact with suppliers/customers
4	Do you demand cleaning and disinfection of the vehicles, loading ramp, drivers etc. (time and manner)?
5	Do you instruct how long the last contact (of vehicle and driver) with a pig farm has to date back, to enter your farm?
6	Do you have determined a special routing, that each supplier/customer has to follow with his vehicle/by foot on the farm-site?
7	Is it obvious to anyone that it is forbidden to get into contact with the animals?
8	Do you make visual inspection if the transport vehicles etc. have been cleaned?
9	Do you make sure that the haulier uses the required ways on the farm-site?
10	Do you make sure that only your employees and your equipment is used inside the stable?
	Personal hygiene
11	Do you have a documentation of all persons entering the stable?
12	Do you have a hygiene sluice for each stable? (If only for the whole farm, please comment)
13	+ Is the room divided into a black and a white area? (for example with a bench)
14	+ Is there a shower?
15	+ Is a wash-basin with disinfection for the hands installed?
16	+ Is there in your hygiene-sluice a disinfection-bowl for the boots?
17	+ Are there fresh work clothes for everyone entering the stable/farm in your hygiene sluice?
	While entering the stable/farm, does everyone…
18	+ change boots and clothes?
19	+ clean and disinfect the whole body/ hands using the shower/ wash-basin?
20	+ disinfect the boots when leaving the stable by using the disinfection-bowl?
21	+ use all materials (buckets, force-boards etc.) only inside or outside the stable/ in one stable section?
22	+ disinfect the boots between the sections?
23	Are the hygiene sluice, the work clothes and the boots cleaned and disinfected every week?
24	Do you make sure that the hygiene sluice is clean?
25	Do you make sure that there are enough clean clothes and boots available for everyone entering the stable/farm?
26	Do you document when visitors enter the stable/farm?
27	Do you make sure that all entering persons use the hygiene sluice?
28	Do you document when the hygiene-sluice is cleaned and disinfected?
29	Do you make sure by this list that the hygiene sluice is cleaned regularly?
	Cleaning and disinfection
30	Do you apply the all-in/all-out-method? (discharging the whole stable-section, cleaning and disinfection of the empty-section)
31	Do you clean the stable-section, the corridors and all used equipment after the pigs left the stable?
32	Do you use an adequate disinfectant?
33	Do you follow the manufacturer’s instructions of the cleaning agent regarding the use and maintenance of equipment and use of chemicals for cleaning and disinfection?
	Proceeding of the cleaning and disinfection:
34	+ Is an automatic system to soak the dirt used on your farm?
35	+ Do you remove the coarse dirt and feed remains with a high pressure cleaner?
36	+ Do you always clean the section from the ceiling down to the floor?
37	+ Do you clean the used equipment (buckets, boards etc.)?
38	+ Do you drain the liquid-manure channels?
39	+ Do you wait until all planes and surfaces are completely dry before new pigs enter the section?
40	+ Do you disinfect all planes and surfaces?
41	+ Do you always adhere the affecting period of the used cleaning agent?
42	+ Do you change the active ingredients of the disinfectants sometimes to avert resistances?
43	Do you make sure that the stable is completely dry before disinfection?
44	Do you inspect the stable as well as the equipment after cleaning?
	Vaccination management
45	Do you instruct your personnel about the legal requirements to handle and use vaccines?
46	Do you have a vaccination-plan designed from you and the veterinarian?
47	+ Do you document with which pathogens/diseases the pigs have contact?
48	+ Have you determined against which pathogens a vaccination is suggestive?
49	+ Have you defined which vaccination methods should be used?
	Implementation of the defined vaccinations:
50	+Knows everyone, accomplishing the vaccinations, about the vaccination plan and what do they have to pay attention for while injecting the pigs?
51	+ Do you make sure that the vaccination is done according to the vaccination plan?
52	Do you keep a list of personnel that are trained to carry out vaccinations?
53	Do you make sure that all involved persons are familiar with the necessary requirements?
54	Do you keep a document with the conducted vaccinations, the group of pigs, the used serum and the date?
55	Do you make sure on the basis of this document that the vaccination plan is complied with?
56	Do you weekly check if the adequate amount of the required serums and equipment is available?
57	Do you weekly check together with the veterinarian, if the vaccination plan is still current?
58	Do you make sure that the waiting times after vaccination are kept?
	Verification and record keeping
59	Do you and your employees record all monitoring activities and corrective actions you perform?
60	Is this documentation kept on your farm for a determined period of time?

Each of the 60 questions represented one meat safety aspect and could be answered with 'Yes', 'No', 'Don't know' or 'Not applicable', where in all cases a 'Yes' answer indicates a conformity with a desired situation (table 2.1). For subsequent analysis, answers were replaced by scores, as presented in table 2.2.

Table 2.2 Scoring of possible answers

Answer	Score
Yes	1.0
No	0.5
Don't know	0.2
Not applicable	1.0

The scores can take values of 02., 0.5 and 1.0. A score of 0.0 was avoided, as there are additional measures available to prevent biological hazards which were not addressed in the questionnaire. The answer 'Not applicable' was given a score of 1 in order to avoid penalizing the farms where this question did not apply. It has been given e.g. for questions dealing with specific management methods which could not be applied because an activity was not present of due to the special requirements of a husbandry system (for instance hygiene sluice in free-range farming). The lowest score 0.2 was given for the answer 'Don't know'. Knowing that only responsible employees or the owners of the farms were interviewed, 'don't know' implies a lack of interest for the meat safety aspect. This is worse than the decision not to take specific measures to improve the meat safety situation, due to a special farm situation (e.g. location of the farm, market demands or husbandry system).

The following formulae were applied to calculate the conformity with requested aspects of meat safety on the dimensional level (formula 1) and also to calculate the overall level of conformity (formula 2) of single farms:

Formula 1: Level of conformity of meat safety aspects within single dimensions

$$L_j = 10 \times \frac{1}{n_j} \sum_{i=1}^{n_j} x_{ij}$$

L_j = Level of conformity of dimension j (1-6); n_j = Number of questions for dimension j (1-6);
x_{ij} = *Score for* answer to question i within dimension j

Formula 2: Overall level of conformity of dimensions of meat safety aspects

$$C = \frac{1}{n_d}\sum_{j=1}^{n_d} L_j$$

C = Overall conformity; L_j= Level of conformity of dimension j (1-6);
n_d = Number of dimensions (6)

For each farm, the level of conformity for each dimension was calculated as the average of the scores given to the questions within the given dimension, transformed to a minimum of 2.0 and a maximum value of 10.0 points.

2.3.2 Data acquisition

127 farmers from five European countries were interviewed using a standardized quantitative questionnaire. The questionnaires were administered on the levels of single farms (n=127), husbandry systems (n=15) and pre-defined groups of husbandry systems, the categories (n=4). Details of the different husbandry systems and the factors for the grouping of systems in categories were described by Edwards *et al.* (2013). From each country three different pig husbandry systems, represented by between 3 and 13 farms, were investigated and assigned to defined categories (table 2.3).

Table 2.3 Categories, husbandry systems and number of investigated farms

Category	Systems	No. of investigated farms
Conventional	C-1	9
	C-2	10
	C-3	10
	C-4	12
	C-5	3
Adapted Conventional	AC-1	4
	AC-2	9
	AC-3	11
	AC-4	9
	AC-5	10
Organic	O-1	4
	O-2	5
Traditional	T-1	13
	T-2	11
	T-3	7

All interviews were performed by trained researchers face to face with the farmers or responsible employees.

2.3.3 Statistical analyses

In all analyses, the farm was the statistical unit. The conformity with investigated meat safety aspects was quantitatively analyzed using a linear scoring model as described above. Boxplots were created to show the distribution of single farm scores for each dimension. Mann-Whitney-U-Test was applied to evaluate significant differences between the various categories of husbandry systems. Differences were considered as significant at $p \leq 0.05$. Each farm was considered as a statistical unit within a category. To check for redundancies within the questionnaire, correlations between the different dimensions and the overall assessment were tested by the Kendall Tau method and a principal component analyses (PCA) was performed. The levels of conformity for the 6 individual dimensions were included in the PCA as active variables whereas the overall conformity was included as additional, inactive variable. A hierarchical cluster analysis (Ward’s method) was performed to search for farms grouping by the level of conformity with the requested meat safety aspects. All statistical analyses were performed using SPSS 19 (IBM, USA) and Excel 2007 (Microsoft, USA).

2.4 Results

2.4.1 Variability of investigated husbandry systems and categories

Variability is calculated for all dimensions separately and overall to assess whether a dimension is discriminating and thereby useful to differentiate farms and systems. To show the variability between farms within one system (intra-system), between farms from different systems (inter-system) as well as farms within one category (intra-category) and farms from different categories (inter-category), boxplots were created. The letters at the top of the figure indicate significant differences between the categories of husbandry systems ($p \leq 0.05$).

‘General aspects of meat safety management (General)’

For the dimension of ‘General aspects of meat safety management’ none investigated category of husbandry systems differs by statistical significance. The intra-system variability was low. Only for two of the fifteen investigated systems a small variability was observed. These two systems presented an extreme skewness of distribution. Three other systems show single extreme values and the rest showed no variability at all. Twelve systems contain farms reaching the maximum conformity with the requested aspects. Thus, the inter-system variability was low, as also indicated by the comparison of mean scores. Anyway, the general standard deviation of 1.82 is high compared to the other dimensions. The mean score of 8.77 for all categories shows the high conformity of most systems with the requested meat safety aspects (figure 2.2).

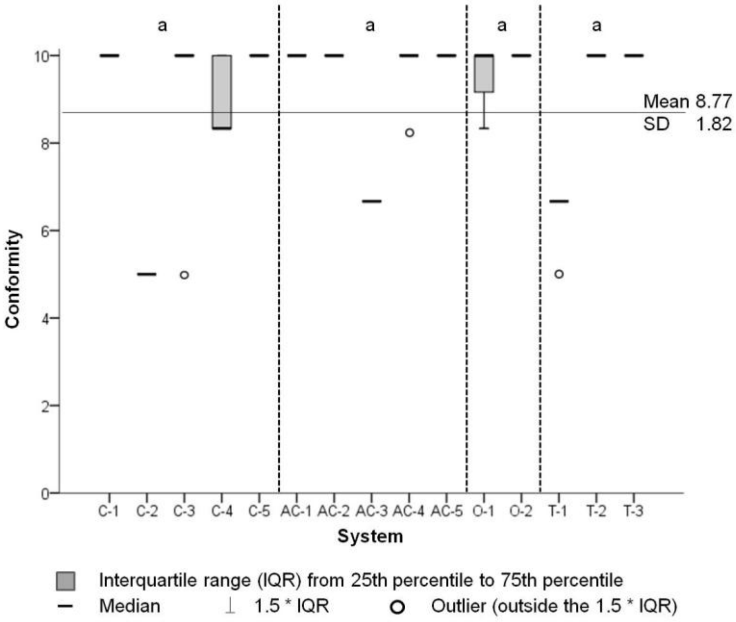

Figure 2.2 Level of conformity of farms in the investigated husbandry systems with the dimension ‘General’. Significant differences between categories of systems (C = conventional; AC = adapted conventional; O = organic; T = traditional) are indicated by lower case letters (Mann-Whitney U-Test; $p \leq 0.05$); Mean and SD were presented for all 127 farms

‘Contact with suppliers and customers (Contact)’

By the results of the Mann–Whitney U test three statistical groups were given. The ‘adapted conventional’ category can be differentiated from the ‘traditional’ one by significance, where the ‘conventional’ and ‘organic’ categories were intermediate. The intra-system variability is high with a maximum of 5 scores (AC-3). But there are also four systems without any variability and there are only four outliers. The inter-systems variability is high and ranges from a median of 4 scores up to a median of 10 scores. With 2.07 the standard deviation is the highest of all dimensions. The mean score is low (7.50). Mean score and standard deviation show the differences between the investigated husbandry systems and categories, again (figure 2.3).

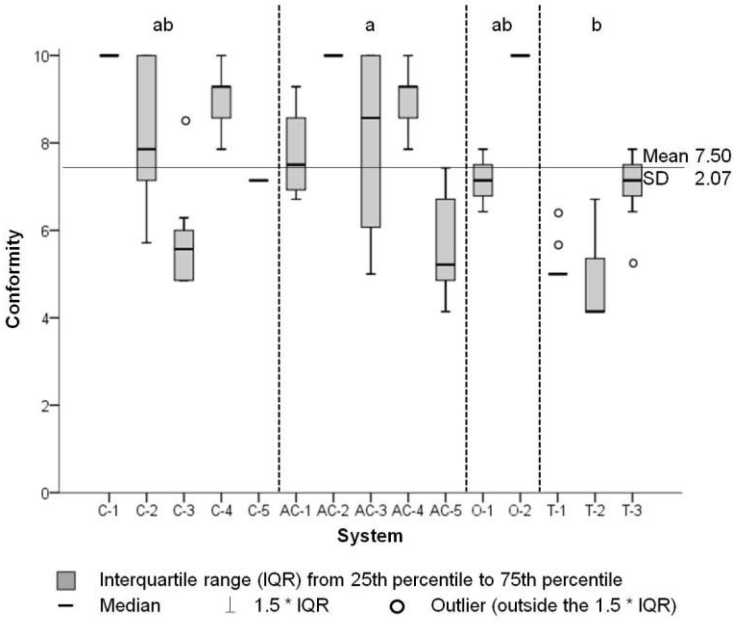

Figure 2.3 Level of conformity of farms in the investigated husbandry systems with the dimension ‘Contact’. Significant differences between categories of systems (C = conventional; AC = adapted conventional; O = organic; T = traditional) are indicated by lower case letters (Mann-Whitney U-Test; $p \leq 0.05$); Mean and SD were presented for all 127 farms

‘Personal hygiene (Hygiene)’

The categories of systems can be differentiated in three groups for the dimension ‘contact’. ‘Organic’ differs from none of the others, but the ‘traditional’ category differs from the ‘conventional’ and ‘adapted conventional’ one. Thus, there is a high conformity group represented by the categories ‘conventional’ and ‘adapted conventional’, a low conformity group represented by the farms of the ‘traditional’ category and the third group of the ‘organic’ category which is intermediate. Intra-system variability has a maximum range of 4 scores and is rather low. The medians within the boxplots indicate a skewness of distribution for many systems. Only one of the investigated systems (C1) showed no variation. Most systems had outliers. The systems medians range from 3.7 to 10.0 scores. The standard deviation of all farms together was 1.95 and the mean score (7.38) show the high inter-system variability of the dimension ‘Hygiene’ (figure 2.4).

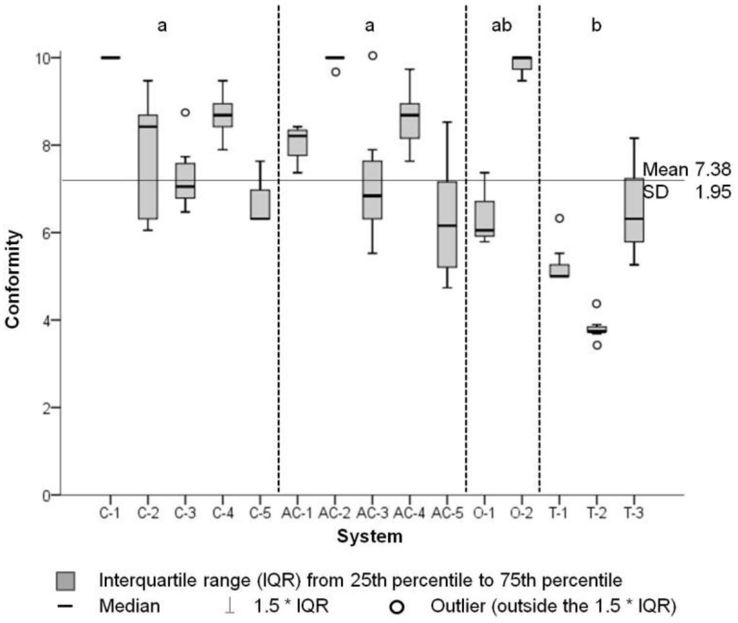

Figure 2.4 Level of conformity of farms in the investigated husbandry systems for the dimension ‘Hygiene’. Significant differences between categories of systems (C = conventional; AC = adapted conventional; O = organic; T = traditional) are indicated by lower case letters (Mann-Whitney U-Test; p ≤ 0.05); Mean and SD were presented for all 127 farms

‘Cleaning and disinfection (Cleaning)’

The ‘conventional’ category differs from the ‘traditional’ one. ‘Adapted conventional’ and ‘organic’ systems are intermediate and cannot be distinguished from any other category for the dimension ‘Cleaning and disinfection’. Intra-system variability is low. The maximum range of variability is 3.5 scores. Thus, only three outliers were found. However, the inter-system variability is rather high. The medians range from 5 to 10 scores. Anyway, the standard deviation of this dimension is low (1.67). Also the high mean score of 8.23 indicates a low intra and inter-systems variability (figure 2.5).

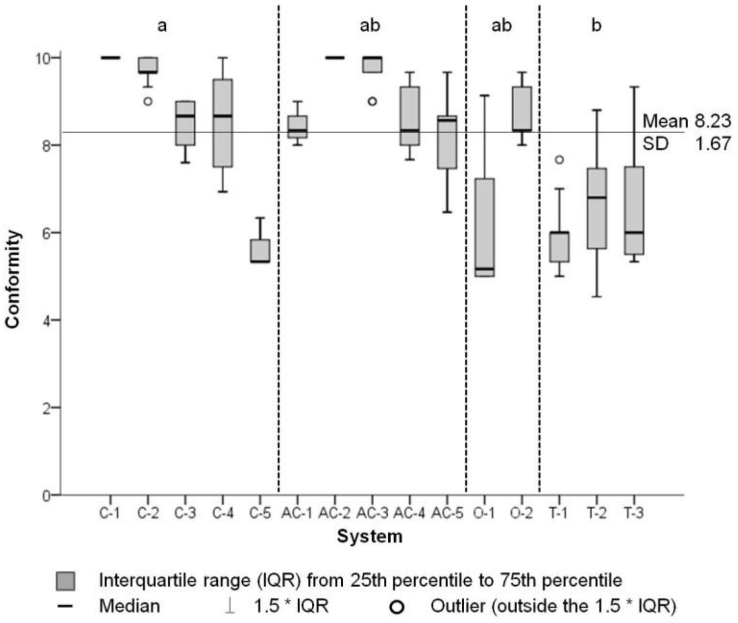

Figure 2.5 Level of conformity of farms in the investigated husbandry systems for the dimension ‘Cleaning’. Significant differences between categories of systems (C = conventional; AC = adapted conventional; O = organic; T = traditional) are indicated by lower case letters (Mann-Whitney U-Test; $p \leq 0.05$); Mean and SD were presented for all 127 farms

‘Vaccination management (Vaccination)’

The Mann–Whitney U test results in the same grouping than for the ‘cleaning’ dimension. ‘Conventional’ and ‘traditional’ differ by significance and the others are intermediate. Like for the dimension ‘contact’ the maximum range of scores within one system were 6 scores. Nearly all systems show an extreme skewness within the distribution of results per farm. Thus, six outliers were displayed. However, three of the systems showed no variation in the results of affiliating farms. To conclude, high intra-system variability was observed. The intra-category variability of farms was highest of all dimensions in this case. The inter-system variability was high, too. Anyway, the inter-category variability is rather low, as the standard deviation is lowest of all dimensions (1.64). Additionally, the very high mean score of all systems (8.49) indicates a high level of conformity with the requested meat safety aspects (figure 2.6).

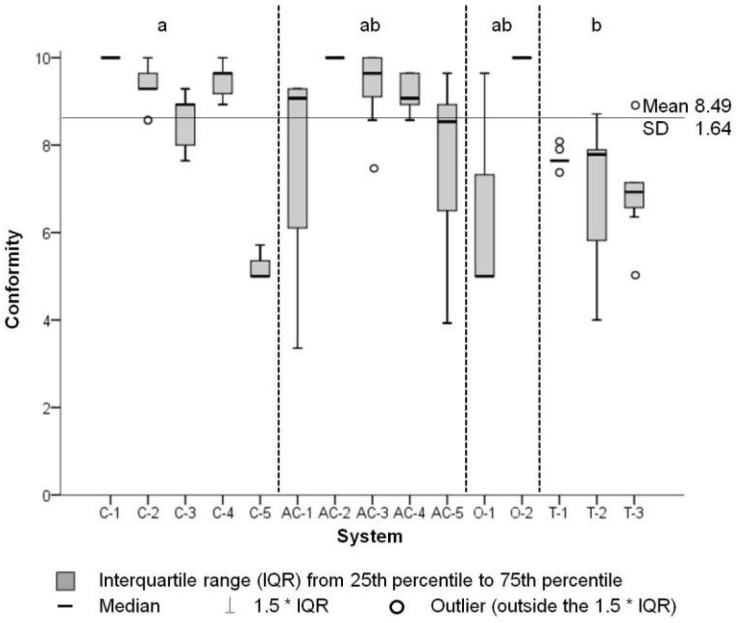

Figure 2.6 Level of conformity of farms in the investigated husbandry systems for the dimension ‘Vaccination’. Significant differences between categories of systems (C = conventional; AC = adapted conventional; O = organic; T = traditional) are indicated by lower case letters (Mann-Whitney U-Test; $p \leq 0.05$); Mean and SD were presented for all 127 farms

‘Verification’

As for dimension ‘general’ no significant differences between the categories were observed for ‘verification’. Intra as well as inter-system variability were low, due to the fact that eleven of the investigated systems showed no or very rate variability at all. Five outliers were detected and thirteen husbandry systems had at least one farm which reached the maximum score of 10. The level of conformity of a single system differed by a maximum of 2.5 points. Due to the farms which did not reach the maximum score, the intra and inter-category variability was rather high. The farm within one category ranged from 3.5 to 10.0 scores. Thus, the highest mean value of all dimensions (8.95) and the comparison of mean values between the categories indicate a low variability, the standard deviation of 1.80 shows variation (figure 2.7).

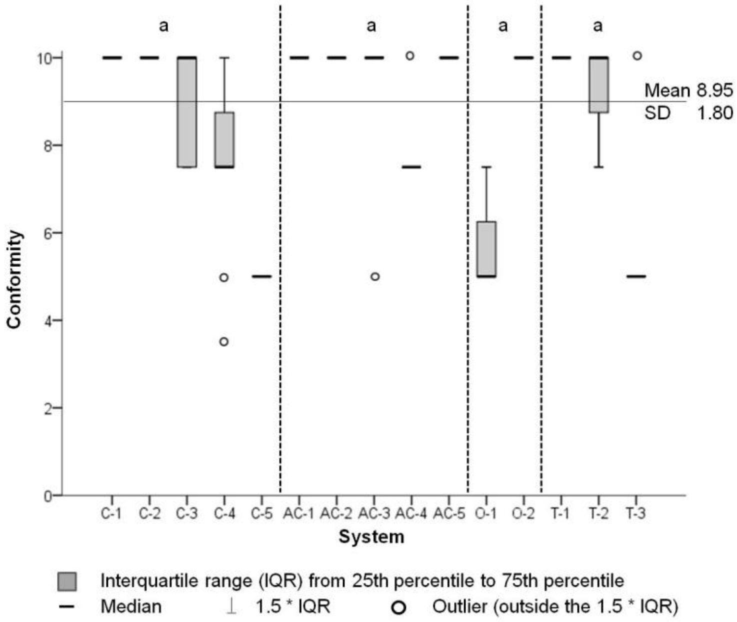

Figure 2.7 Level of conformity of farms in the investigated husbandry systems for the dimension ‘Verification’. Significant differences between categories of systems (C = conventional; AC = adapted conventional; O = organic; T = traditional) are indicated by lower case letters (Mann-Whitney U-Test; $p \leq 0.05$); Mean and SD were presented for all 127 farms

Variability of the overall level of conformity

As for all the single dimensions above, the variability of the overall level of conformity was evaluated. Intra-system variability is quite low for overall conformity. The maximum range of scores within on system is 2.5. Thus, also the inter-system variability was low some of the categories can be distinguished by significance. The ‘conventional’ category differed from the ‘traditional’ one ($p = 0.034$). ‘Adapted conventional’ and ‘organic’ systems were intermediate and could not be distinguished from any other category. Very rare outliers were shown by the boxplots. The medians of systems within a category differed by a maximum of 3.5 scores. The intra-category variance was high for the categories ‘conventional’, ‘adapted conventional’ and ‘organic’ compared to ‘traditional’. The standard deviation of the overall conformity (1.2) was lower than of each single dimension. The mean value displayed the average of all six single dimensions (figure 2.8).

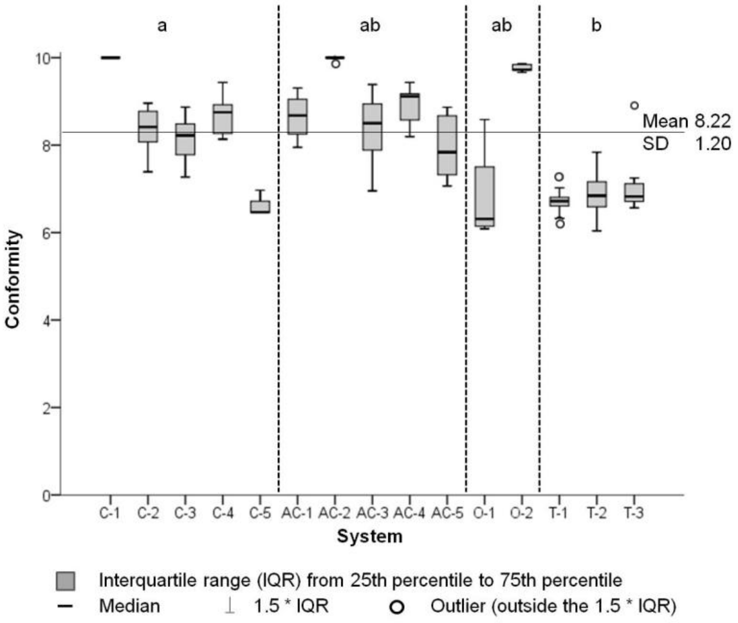

Figure 2.8 Overall level of conformity of farms in the investigated husbandry systems. Significant differences between categories of systems (C = conventional; AC = adapted conventional; O = organic; T = traditional) are indicated by lower case letters (Mann-Whitney U-Test; $p \leq 0.05$); Mean and SD were presented for all 127 farms

High variability between systems and categories were obtained for the dimensions ‘contact’, ‘personal hygiene’, ‘cleaning and disinfection’ and also ‘vaccination management’. For the dimensions ‘general’ and ‘verification’ the results were more homogenous. The effects described for the single dimensions partly eliminated or compensated each other, as the variability and standard deviation of the overall level of conformity were lower than for single dimensions.

2.4.2 Profiles of the various categories of husbandry systems

To make strengths and weaknesses of the systems and categories obvious, spider charts were created. The charts display the level of conformity for all dimensions of investigated husbandry systems and in addition the overall level of conformity. The results were given in figure 2.9. Three different major shapes of spider charts were obvious. One recurrent shape was showing homogenous results for all dimensions. The second noticeable shaped were the inhomogeneous systems. Drop-shaped systems were the third group of recurrent shapes (figure 2.9).

The homogenous systems were found in the ‘conventional’ and ‘adapted conventional’ categories only. Two conventional systems, C-1 and C-4, as well as two adapted conventional systems, AC-1 and AC-4, presented this shape. These systems performed reasonably well for all dimensions of meat safety.

Within the categories ‘conventional’ (C-2), ‘adapted conventional’ (AC-2, AC-3) and also ‘organic’ (O-2) inhomogeneous systems were found. The shapes of these systems were characterized by good results for all dimensions except one or two. This shape also indicated good farming practice regarding meat safety with one or two rather weak dimensions. In two cases the weak point was in dimension ‘general’. These systems did not produce under a quality standard or a brand meat program but stated to follow good manufacturing practices. One showed a weak point for ‘Cleaning and disinfection’ and the last one for ‘Personal hygiene’. Both could be improved by the strict obedience of cleaning work and the utilization of cleaning equipment for the employees and visitors.

Drop-shaped systems obtained a high score for only one or two dimensions, where all others were considerably lower. The group of drop-shaped systems was the only one present in all four categories of husbandry systems. Two systems of the ‘conventional’ category showed this shape (C-3, C-5), one ‘adapted conventional’ system (AC-5), one of the ‘organic’ systems (O-1) and all systems of the ‘traditional’ category. The drop-shaped systems obtained good scores for dimension ‘general’ and/or ‘verification’ but showed lower conformity with the other dimensions of meat safety. The degree of distinctness of this shape differed for system to system.

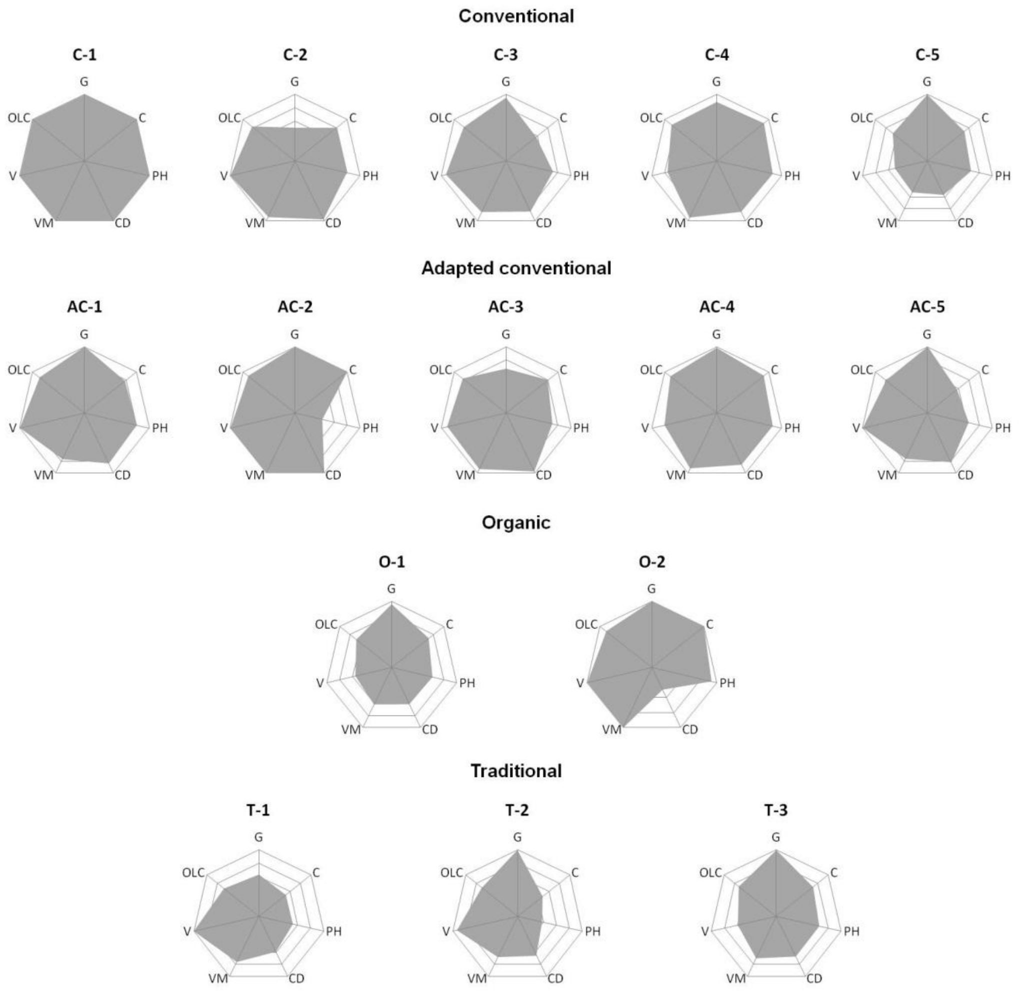

Figure 2.9 Spider charts showing the level of conformity for all dimensions of investigated husbandry systems: G = General, C = Contact, PH = Personal hygiene, CD = Cleaning & disinfection, VM = Vaccination management, V = Verification, OLC = Overall level of conformity; scale is from 0 to 10, minimum obtainable value is 2.

Compared to the other two groups each containing four systems, the drop-shaped group was biggest (7 systems). All these drop-shaped systems performed worse than the systems of the other groups. However, the different shapes are spread nearly all over the four categories.

2.4.3 Correlations among level of conformity for dimensions

To check the developed tool for redundancies, correlations between the calculated levels of conformity of single dimensions and with the overall conformity were analyzed. All dimensions showed significant correlations to the ‘overall conformity’ (tab. 2.4). The dimensions ‘contact’, ‘personal hygiene’, ‘cleaning and disinfection’ and ‘vaccination management’ showed rather high correlation with ‘overall conformity’ (r = 0.66-0.72), whereas ‘general’ and ‘verification’ were poorly related to ‘overall level of conformity’ (r ≤ 0.28).

Table 2.4 Correlation matrix between levels of conformity for individual dimensions and overall conformity

		Contact	Hygiene	Cleaning	Vaccination	Verification	Overall conformity
General	R	0.065	0.150*	-0.035	-0.002	-0.168*	0.195**
Contact	R	1.000	0.700**	0.486**	0.560**	0.062	0.651**
Hygiene	R		1.000	0.526**	0.624**	0.085	0.724**
Cleaning	R			1.000	0.606**	0.293**	0.656**
Vaccination	R				1.000	0.188*	0.699**
Verification	R					1.000	0.283**

R = rank correlation coefficient; * = P < 0.05; ** = P < 0.01.

The high number of significant correlations indicates many partial redundancies. However all coefficients of correlation were lower than 0.72, indicating that a maximum of 50 % of the variability of conformity level for a dimension (or of overall conformity) was explained by the conformity level for another dimension. To obtain more global information on redundancies and on the most discriminating dimensions, a principal component analysis was performed.

2.4.4 Principal component analysis (PCA) and cluster analysis

The percentage variability explained by the first and second components together is close to 74 % (figure 2.10.). This makes the conclusions drawn from the graph solid. The first component explaining 51.8 % of the variability is determined mostly by the dimensions ‘contact’, ‘personal hygiene’, ‘cleaning and disinfection’ and ‘vaccination management’. The second component explaining 22.1 % of the variability is mostly determined by the dimensions ‘general’ and ‘verification’. Whereas the dimensions affecting the first component are quite close to each other, the dimensions appropriated to the second component are lying opposite each other, in accordance with their negative correlation (r = -0.168; *p* = 0.039). The results from the PCA indicate that some of the dimensions are partially redundant. The dimensions ‘contact’ and ‘personal hygiene’ are close to each other. Also the dimensions ‘vaccination management’ and ’cleaning and disinfection’ are located very close together in the graph. Thus, they contribute to the explained variation somewhat tautologically. In figure 2.10 all single farms are added in the PCA graph.

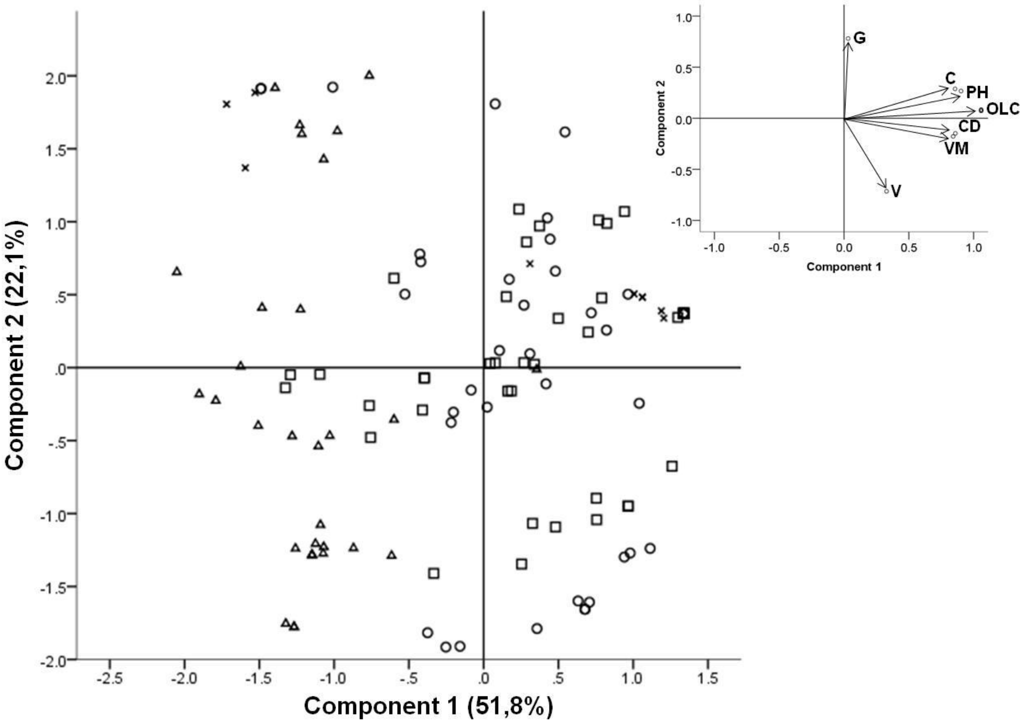

Figure 2.10 Principal component analysis with all farms; different symbols indicate the affiliation to the pre-defined categories of husbandry systems: ○ Conventional, □ Adapted conventional, ✕ Organic, **Δ** Traditional. Levels of conformity for the 6 individual dimensions were included as active variables: G = General, C = Contact, PH = Personal hygiene, CD = Cleaning & disinfection, VM = Vaccination management, V = Verification. The overall level of conformity (OLC) was included as additional, inactive variable.

The categories ‘conventional’ and ‘adapted conventional’ cannot be separated from each other with a very high variability within each of the categories. The two ‘organic’ systems are distant from each other, the one high on the left side (O-1) indicating low scores for all dimensions but "General". The farms in the ‘traditional’ systems are all on the left side of the graph, which indicated the low level of conformity with the dimensions appropriate to component 1. But they are allocated upon the whole range of component 2, indicating different status regarding "General" and "Verification".

The results of the hierarchical cluster analysis are tapestried in figure 2.11. The different symbols indicate the three distinguished clusters. It is obvious, that the clusters are mainly dedicated to the differences within component 1. The farms are the active individuals within this analysis. The systems are displayed as inactive individuals.

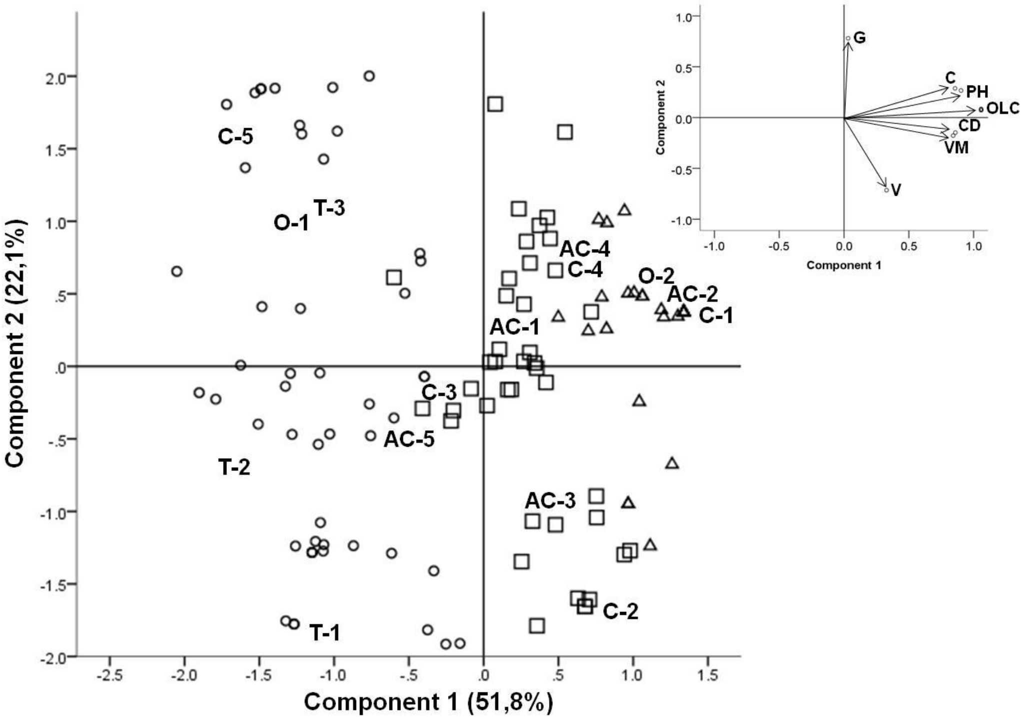

Figure 2.11 Principal component analysis with all farms; different colors indicate the affiliation to clusters determined by hierarchical clustering: ○ Cluster 1, □ Cluster 2, **Δ** Cluster 3; systems were included as inactive individuals; Levels of conformity for the 6 individual dimensions were included as active variables: G = General, C = Contact, PH = Personal hygiene, CD = Cleaning & disinfection, VM = Vaccination management, V = Verification. The overall level of conformity (OLC) was included as additional, inactive variable.

Cluster 1 includes the three ‘traditional’ systems plus one conventional (C-5) and one organic (O-1). This cluster is characterized by low conformity level for all dimensions pertaining to the first component with different status regarding "General" and "Verification". The three systems reaching almost the maximum overall conformity can be found in cluster 3 (C-1, AC-2, O-2) and the rest of the systems (3 conventional and 4 adapted conventional) is summarized in the second cluster, characterized by intermediate levels of conformity regarding the dimensions pertaining to the first component.

2.5 Discussion

The aims of this study were to develop and test a comprehensive tool to find weakness in the potential of farms to meet a required level of meat safety, to identify structural differences between farms, systems and categories and to check the tool for possible simplifications by elimination of redundancies.

The 60 different aspects addressed by the developed meat safety tool display basic requirements of preventive health management aiming at a reduction of risks for animals and consumers. The dimensions reflect ambitions to reduce the introduction and spread of infectious diseases as well as essential requirements of quality management. Thus, further development should pay attention to special prevention measures of ‘traditional’ farms and systems. The influence of pig density, farm size and bedding/ rooting material should be integrated into the tool. Besides, emerging diseases and determining factors for the spread of resistant pathogens should be addressed by the tool. These aspects of meat safety management are of gaining interest in scientific research (Cagienard *et al.*, 2005; Alt *et al.*, 2011). Therefore, a tool to assess meat safety on the farm level should not be fixed to defined aspects, but should develop continuously according to gaining knowledge.

The results of the performed analysis show a high variability of conformity for nearly all systems and farms. Nevertheless, a tendency can be derived from the analysis by categories. Farms of the category ‘traditional’ present higher risks for the investigated meat safety aspects than others. All the farms of this category were located in areas with low pig density and they were not integrated in big production chains. The traditional systems were focused on market niches mainly in local markets. Some of the farmers brought the pigs to the slaughterhouse themselves avoiding mixing of the animals. These measures lower the risks for a spread of infection diseases and thereby possible risks for meat safety (Albina, 1997). The high variability of systems especially within the ‘conventional’ and ‘organic’ categories indicates the wide range of possibilities to manage and organize such husbandry systems. Comparison of single dimensions show that differences between systems cannot be assigned to single dimensions and discernible patterns are not detectable. But the tool enables a ranking of farms and the assessment of meat safety endeavours. The assembly of the results of this study with other current research results suggests that the conclusions are coherent (EFSA, 2011). Except on case (O-2) the systems offering outdoor access to the pigs present higher meat safety risks due to the aspects of this study. From several other studies a higher risk due to outdoor access is reported as well (Jensen *et al.*, 2004; EFSA, 2011; Davies, 2011), but there are also studies reporting no increase of risks (Millet *et al.*, 2005; Mulder *et al.*, 2009; Sandberg *et al.*, 2011).

The cluster analysis displays that a general grouping by systems is possible. However, some farms are located in other clusters than the mean of the related husbandry system. The limited conformance within categories and systems and the high variability of farms within systems and categories make obvious that meat safety endeavours are not mainly determined by systems or categories. Thus, the commitment of the farmer seems to be a key factor to lower risks for the consumers, as the accessible levels of conformity with meat safety aspects are not related to the husbandry systems and even less so to categories. The high importance of the farmer is also reported within the literature (Fosse *et al.*, 2009). Therefore, a pre-categorization of systems and farms is hardly possible. The many possibilities to manage meat safety issues at farm-level make it necessary to consider meat safety aspects for every farm particular. Consequently, this tool cannot be used to compare systems or categories in detail, while here within class variation is too large, but gives useful information to benchmark individual farms on risk management of biological hazards. The PCA clarified that variance of farms is mainly associated with the dimensions ‘contact’, ‘personal hygiene’, ‘cleaning and disinfection’ as well as ‘vaccination management’.

Both, the correlations among dimensions as well as the results of the performed PCA show the outstanding positions of the dimensions ‘general’ and ‘verification’. The two dimensions concentrate on the essential requirements of quality management. Whereas the other four dimensions address more practical measures preventing meat safety risks. These are highly correlated and contribute to the explained variation of the PCA tautological. Considering the identified redundancies within the tool the number of aspects could be reduced, but this simplification should be based on a more conclusive database.

2.6 Conclusions

The developed scoring model enables rapid detection of weaknesses in meat safety management on the level of single farms, farming systems and even categories of pig farming systems. The given scores enable a clear differentiation of desired and un-desired meat safety situations. But scientific knowledge supporting the definition of these scores is limited. Therefore, validations of the tool based on additional assessments and also cross-validations with other tools to assess meat safety on farm level have to be performed. A comparison with outcome parameters of meat safety, like antibody detection against zoonotic agents, could also be used to validate the presented scoring model.

A large and conclusive database from more different husbandry systems will be needed to enable farmers, system authorities and/ or service providers to benchmark farms due to their meat safety management and to validate the developed tool. However, the developed tool performed reasonably well in terms of objectivity, practical feasibility, and applicability to individual farms of different husbandry systems and can be used to benchmark conformity with desired meat safety aspects of finishing farms.

Acknowledgement

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Disclaimer

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3 Evaluation of the quality of health management in contrasted pig husbandry systems in Europe

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3.1 Abstract

Animal health is one of the major production and welfare aspects in livestock farming and in this way contributing to sustainability of meat chains. In the Quality Pork Chains project a tool to measure the conformity to desired status of different health aspects has been developed to assess the Animal Health performance in pig husbandry systems. This tool tested in a questionnaire survey in 15 European pig production systems. One conventional and two differentiated systems were evaluated in each of five countries. The survey was conducted by different persons on several farms from each system. The questionnaire was analysed on four dimensions 'general health management', 'presence of diseases', 'parasites' and 'health situation' Mean scores of the level of conformity to desired situations in several aspects in these dimensions are presented graphically in boxplots and spider-diagrams, and summarizing values were calculated. High variability between farms of the same and different production systems was found. The farms in the category 'traditional' ranked markedly lower when compared to farms in the categories 'conventional', 'adapted conventional' and 'organic'. Due to inconsistent results of the performed analysis investigating the possibilities to reduce the tool to a more practical size, it was concluded, that a larger dataset is needed to decide about simplifications.

Keywords: Animal health, enquiry; health status; Europe, farm level, pig production

3.2 Introduction

To answer current, interregional and international, trade conditions and also political, governmental and consumers demands on sustainability, a wide knowledge of the quality of pork production is required. Sustainability of pork production is of major interest for the meat chain. This can be expressed in several, sometimes contradicting, dimensions that can be analysed in detail. This was attempted in the EU FP6 Programme Quality PorkChains project. Assessment of sustainability of pork production at farm level requires comprehensive and practical tools for each dimension that can be used on a routine basis without the presence of specialists on each aspect. It needs a uniform check list that can be read and understood by all concerned parties and to be unequivocally filled in and updated at a regular basis without much effort.

Animal health is an important dimension in the sustainability because without a desired animal health status regional and international trade is restricted and production at pig farms reduced (Sofos, 2008). The pork chain has a high commitment in reducing the use of antibiotics and transmittance of zoonosis (Coenraadts and Cornellissen, 2011).

Animal health is directly connected to meat safety aspects. To safeguard public health, the selection and dissemination of resistant bacteria from animals should be limited. This can only be achieved by reducing the amounts of antibiotics used in animals (van den Bogaard and Stobberingh, 2000; Wegener, 2003).

The three aims of this study were to develop a comprehensive tool to assess and benchmark health status and potentials of different pig husbandry systems (1), to identify structural differences in health status and potentials between systems (2) and to check the developed tool for possible simplifications (3).

In Germany, Berns (1996) developed and tested a checklist for assessment of health status of primary pig farms. This was later used and refined in an inter-organisational quality and health management pilot by van der Wolf *et al.* (2004) and Mack (2007). In Denmark the Health and Production Surveillance System HEPS (Christensen *et. al.*, 1994) was developed to provide pig producers, their advisors, and other people associated with the pig industry with information about production performance, disease occurrence, and the impact of disease at the herd and national levels. Zovex (Enting, 2000) is another system that was developed to provide farm advisors with knowledge about the interaction between disease problems in a pig herd, and environmental and managerial factors on the farm influencing manifestation of disease. Several other questionnaires inquiring about health status and farm condition have been developed, tested and published. The Production Animal Disease Risk Assessment Program (PADRAP), owned by the American Association of Swine

Veterinarians (AASV) and designed and developed by Boehringer Ingelheim Vetmedica, assesses overall herd biosecurity and risk for introduction of Porcine reproductive and respiratory syndrome virus (PRRSV). The web-based tool is useful for assessing overall PRRS biosecurity at the herd level and can be used for estimating the success of a PRRSV elimination program (http://www.padrap.org). When applied at several time points, PADRAP can be used to measure changes in biosecurity over time (Rowland and Morrison, 2012).

Based on a literature study Hovi *et al.* (2003) suggested that, whilst organic standards offer a good framework for animal health and welfare management, there is a need to solve apparent conflicts between the organic farming objectives with regard to environment, public health, farmer income and animal health and welfare. This is supported by Kijlstra and van Eijk (2006) who conclude that important health problems in organic livestock farming are often related to the outdoor access area, exposing the animals to various viral, bacterial and parasitic infections some of which may only influence the animals' own welfare whereas others may also endanger the health of conventional livestock (e.g. Avian Influenza) or pose a food safety (Campylobacter, Toxoplasma) problem to the consumer. They propose various preventive measures, such as the use of better animal breeds, optimized rearing conditions, pre- and probiotics, and addition of acids to the drinking water. In case of infectious disease, tight vaccination schedules may prevent serious outbreaks.

Until now no tools have yet been applied to assess the health status among different contrasted, pig husbandry systems and this was the subject of the study presented here.

3.3 Material and methods

Because farm visits by veterinarians result in extra costs and additional risks, a questionnaire suitable for administration by non-specialist was developed. This questionnaire was tested in a variety of pig husbandry systems over Europe and the potential of further simplifications by elimination of redundancies were investigated.

3.3.1 The Questionnaire

Out of the known checklists, the elaborate questionnaire developed by Berns (1996), van der Wolf *et al.* (2004) and Mack (2007) was chosen, modified and translated. The questionnaire (table 3.1) was administered by experienced and trained animal scientists in each of the five countries, using data supplied by recent audits, data from of the management system and face-to-face interview with the farmer. The questionnaire consisted of a wide range of questions grouped into the following four dimensions:

1. Preventive health management;
2. Disease and vaccination status;
3. Parasites (endo and ecto parasites);
4. Health situation.

Each dimension consisted of one or more sub-dimensions, and sub-dimensions consisted of a coherent group of questions. The number of questions per sub-dimension and the number of sub-dimensions per dimension can differ. Not all answers are available on all questions. The dimension 4 ‘Health status’ consists of sub-dimensions sections with different stages of production, which are not always all present at all farms.

Table 3.1 Animal Health Questionnaire

1. Preventive Health Management (15) *All questions, except 1.1.d, to be answered by: (Yes / No)*

1.1 Herd health is monitored by:

- Observations
- Testing
- Production records
- Examining by Caretaker / Veterinarian / Bot

1.2 Following procedures are designed in consultation with a veterinarian

- Disease control/prevention programs
- De-worming
- Medication protocols
- Vaccination

1.3 Disease control/prevention

- Sick animals are immediately treated and/or removed from groups to treatment areas
- Any unusual illness is immediately brought to the attention of veterinarian
- All inn, all out
- Antibiosis as a routine (*Yes = negative*)

1.4 Pest control

- Professional pest control services are used to prevent rodent and insect infestations.
- Access to feed by rodents is minimized by storage in rodent-proof containers and the prompt clean-up of spills.
- Building design and maintenance discourage the entry and harbourage of pests.

2. Disease and Vaccination (14) *Status (Present / Absent / Don’t know)*
Vaccination (Yes / No)

- Parvovirus
- Erysipelothrix rhusiopathiae (Red murrain)
- Influenza
- Porcine Reproductive & Respiratory Syndrome (PRRS)
- Mycoplasma hyopneumoniae
- Escherichia coli (E. coli)
- Actinobacillus Pleuropneumonia (APP)
- Clostridium
- Atrophic Rhinitis (AR)
- Streptococcus
- Lawsonia intracellularis (Lawsonia, Ileïtis, PIA)
- Salmonella
- Staphylococcus hyicus
- Haemophilus Parssuis

3. Parasites (8) *Status (Present / Absent / Don’t know),*
Therapy (Yes / No)

Presence and therapy of endo-parasites and ecto-parasites in sections for young breeding sows, sows and boars, piglets and finishers are questioned separately.

4. Health Status *Status (Yes / No)*

4.1 Farrowing unit (7)

- Metritis (Sows)
- Uniformity litter
- Mastitis problem (Sows)
- Diarrhea problems (Piglets)
- Acute respiratory (Piglets)
- Locomotion problems (Piglets)
- Growth rate (Piglets

4.2 Mating unit (4)

- Defluxion/metritis
- Body condition
- Mastitis/ Udder inflammation
- Mating behaviour

4.3. Weaning unit (6)

- Diarrheal disease
- Acute respiratory
- Locomotion problems
- Skin disease
- Uniformity group
- Growth rate

4.4. Finishing unit (6)

- Diarrheal disease
- Acute respiratory
- Locomotion problems
- Skin disease
- Uniformity group
- Growth rate

At this instance no distinction between the value and consequences of different aspect has been made and all qualitative questions answered by ‘yes’, ‘no’ ‘don’t know’ were coded on a scale from 0 to 1, where the most favorable question always was rewarded highest by 1 and the unfavorable answer by 0. ‘Don’t know’ answers were coded differently depending on the probable consequences estimated by the authors. Table 3.2 presents the values used in different categories.

Dimension 1: Preventive Health management

Preventive health management consisted of sub-dimensions ‘Monitoring’, ‘Procedures designed with a veterinarian’, ‘Disease control and prevention’ and ‘Pest control’. Answers could be ‘Yes’ and ‘No’. For each stage of production (farrowing, mating, gestation, integration, rearing, finishing), the examination method, application of ‘all-in all-out’ and ‘routine antibiosis’ was asked. Examination method could be answered by ‘Veterinary investigations’, ‘Observation by caretakers’ and ‘Both’ and was included in the ‘Monitoring’. ‘Antibiosis as a routine’ was considered to be negative, an average value over different stages of production was calculated for each farm, and included in ‘Procedures with a veterinarian’.

Dimension 2: Disease and vaccination status

Disease and vaccination status was questioned on the presence and therapy for the following diseases: Parvovirus, Red murrain, Influenza, Porcine Reproductive & Respiratory Syndrome (PRRS), Mycoplasma, Escherichia coli (E. coli), Actinobacillus Pleuropneumonia (App), Clostridium, AR, Streptococcus, Lawsonia, Salmonella, Staphylococcus, Haemophilus Parssuis. Each disease was checked on Status (Present/Absent/Don’t know), Vaccinations (Yes/No) and clinical symptoms. Clinical symptoms were only reported in combination with a ‘Present” answer. This was not included in the further analyses. ‘Absent’ is rewarded with a value 1.0, Present with a value 0.0. ‘Don’t know’ was regarded to be close to ‘Absent’. When ‘Absent’ was inserted in combination with ‘Yes’ for vaccination also the highest value 1.0 and without vaccination, a value of 0.8 was given.

Dimension 3: Parasites

The occurrence of endo- and ecto-parasites was questioned separately for sections with different stages of production ‘young breeding sows’, ‘sows and boars’, piglets’’ and ‘finishers’. Each stage was analyzed as a sub dimension. Parasites: Answers ‘Don’t know’ in combination ‘Yes’ for therapy were coded 0.5 points. Answers ‘Don’t know’ in combination ‘No’ for therapy were coded 0.0 points.

Dimension 4: Health status

In this dimension the health status in the farrowing section, the mating section, the weaner section and the finishing section were questioned on specific diseases or disorders and were analyzed as sub dimensions. Answers were rated 0 for undesired and 1 for desired, and the average value was calculated for each stage of production. For each farm an average value was calculated as an average of all sections. In the farrowing section answers on litter uniformity, for sows metritis, and mastitis, and for piglets’ diarrhea, locomotion, acute respiration problems and growth were rated. In the mating unit body condition, mastitis, metritis and mating behavior were rated. Weaner and finishing sections were rated for diarrhea, locomotion, skin diseases, acute respiratory problems, and uniformity and growth rate.

Table 3.2 Validation of the questionnaire answers

Table 3.2.a. Presence of pathogens and associated vaccination

Answer	Presence of parasites Vaccination	Presence of diseases Vaccination
Absent / No	1	1
Don't Know / Yes	0.5	1
Don'tKnow / No	0	0.8
Absent / Yes	1	1
Present / No	0	0
Present / Yes	0	0.2

Table 3.2.b. Other questions

Answer	Way of examination	Antibiosis	Health disorders (1)	Health qualities (2)
By vet. and caretaker	1			
By veterinary only	0.6			
By caretaker only	0.6			
Don’t know		0.5	0.5	0.5
Yes		0	0	1
No		1	1	0

(1) Metritis/Deflux, Mastitis/Udder, Diarrhea, Respiratory, Locomotion, Feeding insufficient, Excessive feeding

(2) All in-all out, Growth Rate, Uniform, Mating behavior, Condition

3.3.2 Data acquisition

The health questionnaire was administered in five different countries. A total of 130 farms were entered into this study. The farms belonged to a total of 15 different husbandry systems, in four categories ‘Conventional’, ‘Adapted conventional systems’, ‘Organic’ and ‘Traditional’ (table 3.3). Details of the different husbandry systems are given in Edwards *et al.* (2012). In all countries one ‘conventional’ system, where the circumstances inside the building are generally standard, intensive and according to EU Directive 2008/120/EC, was included. Besides, two other systems have been selected by availability in each country.

Table 3.3 Categories, production systems and number of investigated farms

Category	Husbandry system	No. of farms
Conventional (C)	C-1	9
	C-2	10
	C-3	10
	C-4	12
	C-5	3
Adapted Conventional (AC)	AC-1	4
	AC-2	9
	AC-3	11
	AC-4	9
	AC-5	10
Organic (O)	O-1	5
	O-2	5
Traditional (T)	T-1	13
	T-2	11
	T-3	9

3.3.3 Statistical analysis

In all analyses, the farm was the statistical unit. The conformity with investigated meat safety aspects was quantitatively analyzed using a linear scoring model as described above. Boxplots were created to show the distribution of single farm scores for each dimension. Mann-Whitney-U-Test was applied to evaluate significant differences between the various categories of husbandry systems. Differences were considered as significant at $p \leq 0.05$. Each farm was considered as an individual within a category. To check for redundancies within the questionnaire, correlations between the different dimensions and the overall assessment were tested by the Kendall Tau method and a principal component analyses (PCA) was performed. The levels of conformity for the 6 individual dimensions were included in the PCA as active variables whereas the overall conformity was included as additional, inactive variable. A hierarchical cluster analysis (Ward’s method) was performed to search for farms grouping by the level of conformity with the requested meat safety aspects. All statistical analyses were performed using SPSS 19 (IBM, USA) and Excel 2007 (Microsoft, USA).

3.4 Results

3.4.1 Variability of investigated husbandry systems and categories

Results range from 0 and 1 in which 1 represents the most favorable case and 0 represents the least favorable grade for the relevant dimension. The dispersion and skewness among conformity of farms to desired values in different dimensions in the investigated husbandry systems is presented by boxplots in figures 1 to 5, where the dispersion is presented by the interquartile range (IQR) from 25 % to 75 % of the farms within a system, the range within 1,5 * IQR and the median, while outliers are presented by a dot outside this range.

Figure 3.1 shows the dispersion of the level conformity of farms within systems to the desired ‘Health management’ dimension. The average level of conformity was 0.82 (SD 0.19) and most medians were close to the mean. Eight systems had one or more farms reaching the maximum level. The ‘Traditional’ category had a lower ($p<0.05$) conformity than the other category’s and the dispersion of farms and systems was high, with no parallel interquartile ranges (IQR) and several outliers. The IQR were very low in 4 systems in the ‘Conventional’ category. Variability in the ‘Organic’ category was low within systems, but there was no overlap between systems and outliers. In 4 systems the IQR and median were zero, but they showed outliers.

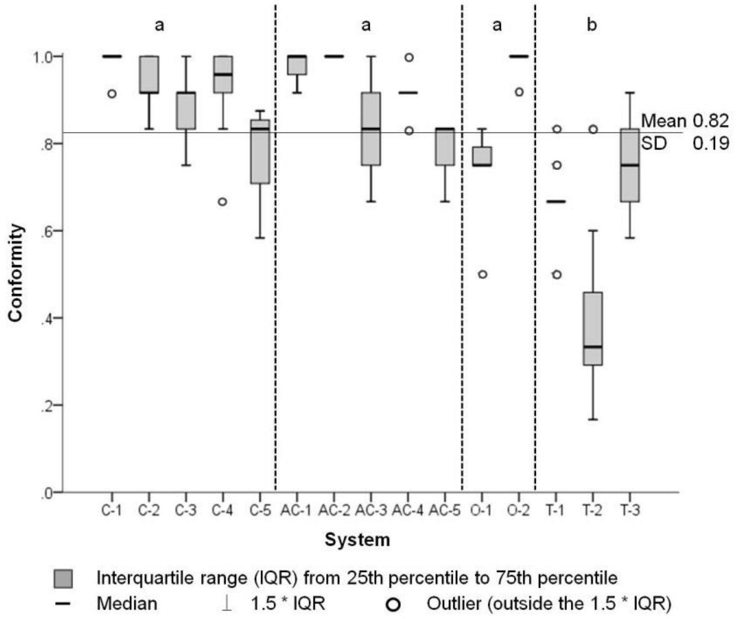

Figure 3.1 Conformity (0 – 1) to ‘Health management’ of farms within systems. Significant differences (p ≤ 0.05) between categories of systems are indicated by lower case letters. Mean and SD are for all 130 farms. C = conventional; AC = adapted conventional; O = organic; T = traditional

In the dimension ‘’Diseases and vaccination’ (figure 3.2), the variability and median of conformity to the desired status in all categories was rather close to the mean value of 0.63 (SD 0.23). In all categories IQR of one system did not overlap the IQR of the other systems in all categories some farms reached the maximum value. In T1 conformity was very low without variation between farms.

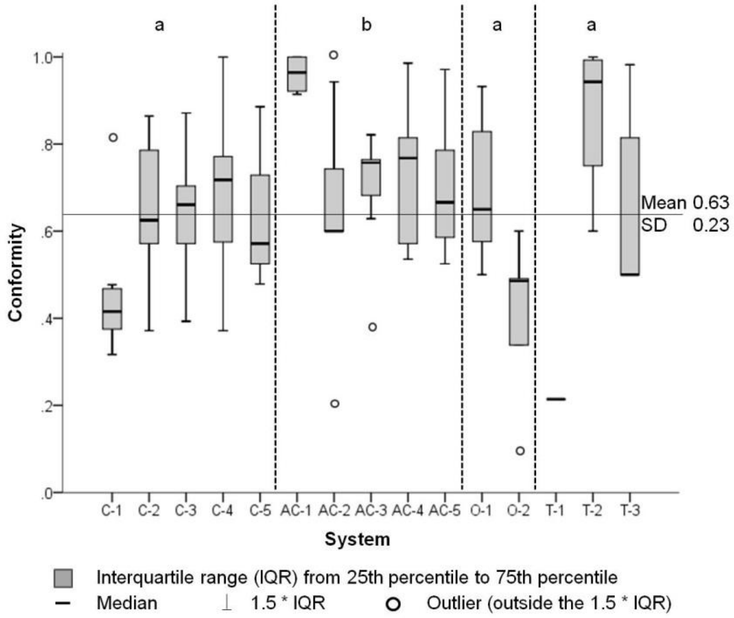

Figure 3.2 Conformity (0 – 1) to ‘Disease status’ of farms within systems. Significant differences ($p \leq 0.05$) between categories of systems are indicated by lower case letters. Mean and SD are for all 130 farms. C = conventional; AC = adapted conventional; O = organic; T = traditional

In the ‘Parasites’ dimension (figure 3.3) the average conformity is 0.44 (SD 0.44). There are very long IQR’s in all categories but also clear differences between systems within one category. Some medians are on the zero level, meaning that at most farms endo and ecto parasites are present. Maximum levels, without parasites, were reached in Conventional and in Adapted Conventional, but both had systems with a very low score, too. In this dimension was only one outlier. The Organic and Traditional categories had a lower conformity than the others

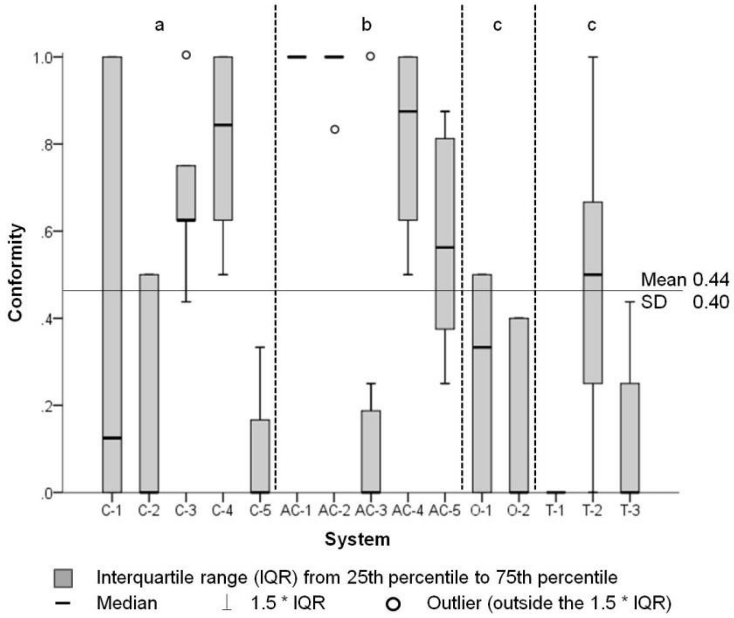

Figure 3.3 Conformity (0 – 1) to ‘Parasites’ of farms within systems. Significant differences ($p \leq 0.05$) between categories of systems are indicated by lower case letters. Mean and SD are for all 130 farms. C = conventional; AC = adapted conventional; O = organic; T = traditional

The dimension ‘Health status’ had an average conformity of 0.64 (SD 0.12) and all medians were very close to the mean. The performance of Conventional is better than the others ($p<0.05$), but we find the same IQR also in the Adapted Conventional category. In C and AC categories some farms reached the maximum level of conformity, while in O and T categories almost all farms performed on or below the general average (figure 3.4).

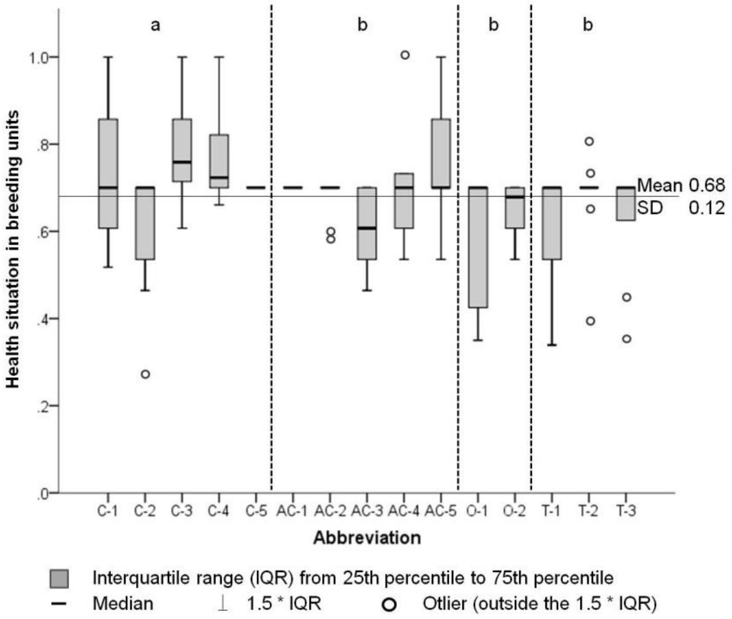

Figure 3.4 Conformity (0 – 1) to 'Health status' of farms within systems. Significant differences (p ≤ 0.05) between categories of systems are indicated by lower case letters. Mean and SD are for all 130 farms. C = conventional; AC = adapted conventional; O = organic; T = traditional

The mean overall average level of conformity (figure 3.5) to all dimensions 0.64 (SD 0.16) and performance of farms is very disperse in all systems and except the Traditional, no category shows a uniform picture. Most farms in C and AC categories perform better than this average, while most farms in the O and T categories are below this average.

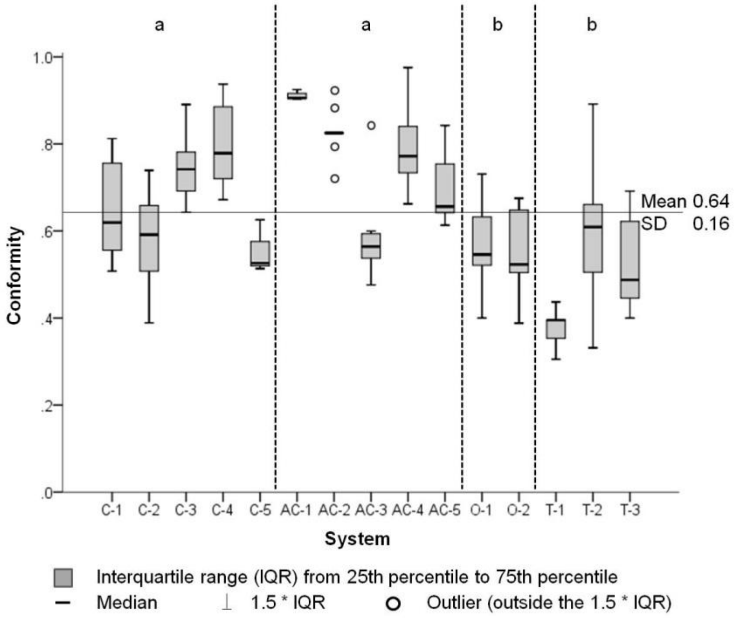

Figure 3.5 Overall level of conformity of farms in the systems. Significant differences between categories of systems (C = conventional; AC = adapted conventional; O = organic; T = traditional) are indicated by lower case letters (Mann-Whitney U-Test; $p \leq 0.05$); Mean and SD were presented for all 130 farms

3.4.2 Profiles of the various categories of husbandry systems

In figure 3.6 the total conformity of each system as a sum of the average level of farms within a system is presented with each dimension and the average of four at one point in the spider graph. We see that only Adapted Conventional, with one exception, is scoring well on the Parasites a few in the Conventional. Health management is a strong dimension in C and AC, but also one Organic system can reach a good level of conformity. We see most consistency in the AC and more different patterns in other categories. Two systems in C, and one in AC, O and T show the same pattern, with all a relative high conformity on health management and a low conformity on parasite control.

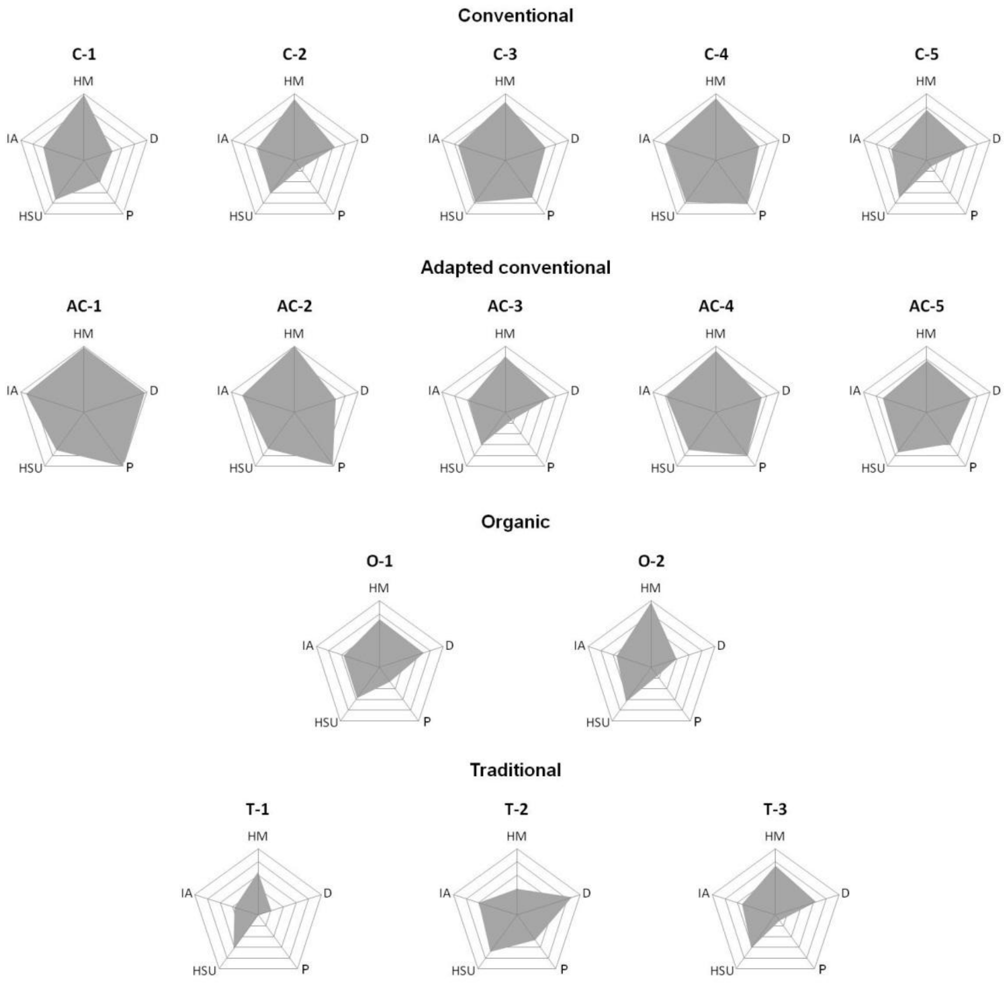

Figure 3.6 The average level of conformity (scale 0 – 1) for all dimensions and systems: HM = Health management, D = Disease, P = Parasites, HSU = Health situation, IA = Average of 4 dimensions

3.4.3 Correlations between dimensions

The correlation between the four dimensions was analyzed using the Spearman correlation test. The results of the overall analysis are shown in table 3.4. The highest correlation coefficient was identified between ‘Parasites’ and ‘Diseases’ (r = 0.44, $p < 0.01$) (Table 3). The second highest correlation was identified between ‘Parasites’ and ‘Health situation’ (r = 0.31, $p < 0.01$). ‘Parasites’ was highly correlated ($p < 0.01$) with dimension Health management’, too. Thus, the dimension ‘Parasites’ seemed to be substitutable by the other three dimensions or the evaluation of ‘Parasites’ could be seen as a good indicator for overall animal health situation. The only negative correlation was identified due to dimensions ‘Health management’ and ‘Diseases’. Though, this correlation was not significant ($p > 0.05$).

However, the result indicates that good health management leads to an improved disease status.

Table 3.4 Spearman correlation coefficients between dimensions

Spearman correlation, r	Diseases	Parasites	Health situation
Health management	-0.02	0.26**	0.08
Diseases		0.44**	0.22*
Parasites			0.31**

* = $p < 0.05$; ** = $p < 0.01$.

The information about the correlations did allowed the identification of one major link between dimensions, indicating that there was some redundancy in the information brought by the various dimensions. To obtain more detailed information a principal component analysis was performed.

3.4.4 Multivariate analysis (PCA)

Principal component analysis (PCA) is a mathematical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of uncorrelated variables called principal components. The graph 7 shows the results of the performed principal component analysis with all farms as active variables; different symbols indicate the categories of husbandry systems. Figures 3.7 and 3.8 show that the two principal components explained 68.5% of total variation. Bi-plots for PCA showed that dimensions ‘Health management’ and ‘Disease status’ had weight on component 2. On the other hand, dimensions ‘Parasites’ and ‘Health Situation’ had weight on component 1. The dimension ‘Parasites’ was very close to the results of the integrated analysis (IA) and showed the biggest distance from the centre of the PCA. Thus, the dimension ‘Parasites’ seemed to have a major impact on the overall analysis. Although traditional category was well discriminated, the other three husbandry categories revealed overlapping values (figure 3.7).

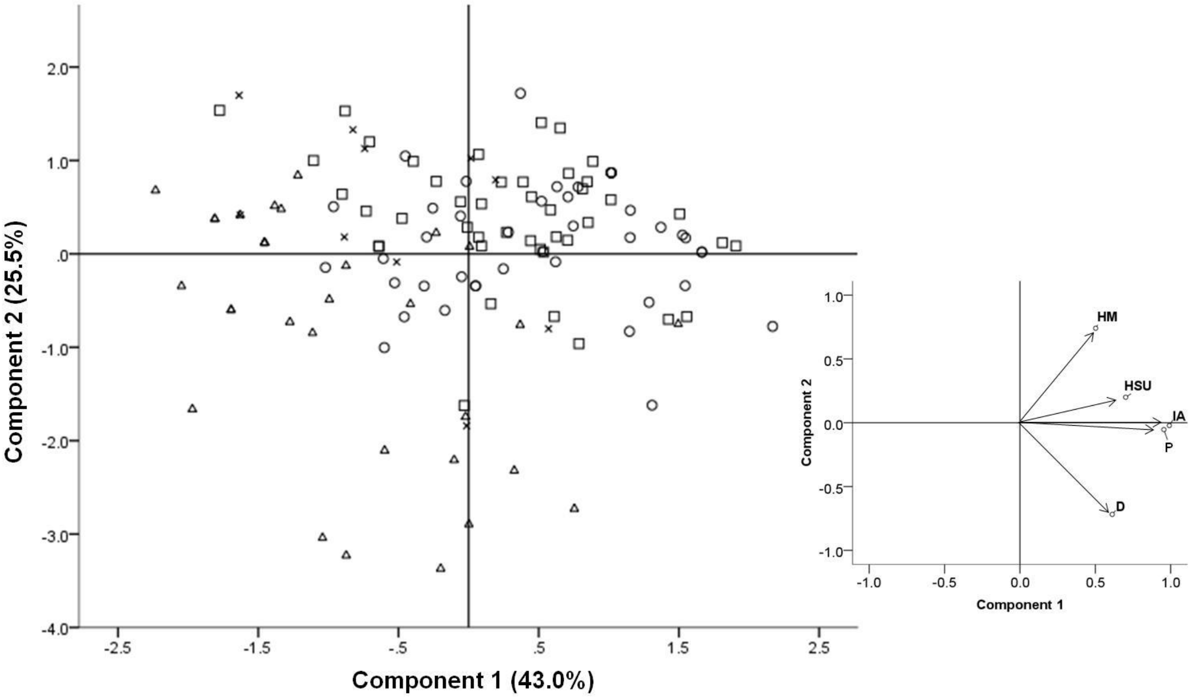

Figure 3.7 Principal component analysis with all farms; different symbols indicate the categories of husbandry systems: ○ Conventional, ☐ Adapted conventional, × Organic, Δ Traditional. Levels of conformity for the 4 individual dimensions were included as active variables: HM = Health management, D = Disease, P = Parasites, HSU = Health situation in breeding units. The Integrated analysis (IA) was included as additional, inactive variable.

The results of the hierarchical cluster analysis are tapestried in figure 8. The different symbols indicate the four distinguished clusters. It is obvious, that the clusters are dedicated to differences within both components. The farms are the active individuals within this analysis. The systems are displayed as inactive individuals. Component 1 subdivided to groups of clusters (group 1 = clusters 1 and 3; group 2 = clusters 2 and 4) and component 2 did the same (group 1 = clusters 1 and 2; group 2 = clusters 3 and 4). Cluster 1 represented three conventional and four adapted conventional systems. Cluster 2 comprised two conventional, one adapted conventional, all organic and two traditional systems. Cluster three mainly arised from farms of one traditional system and cluster four is build by three outstanding farms (one traditional and two conventional once).

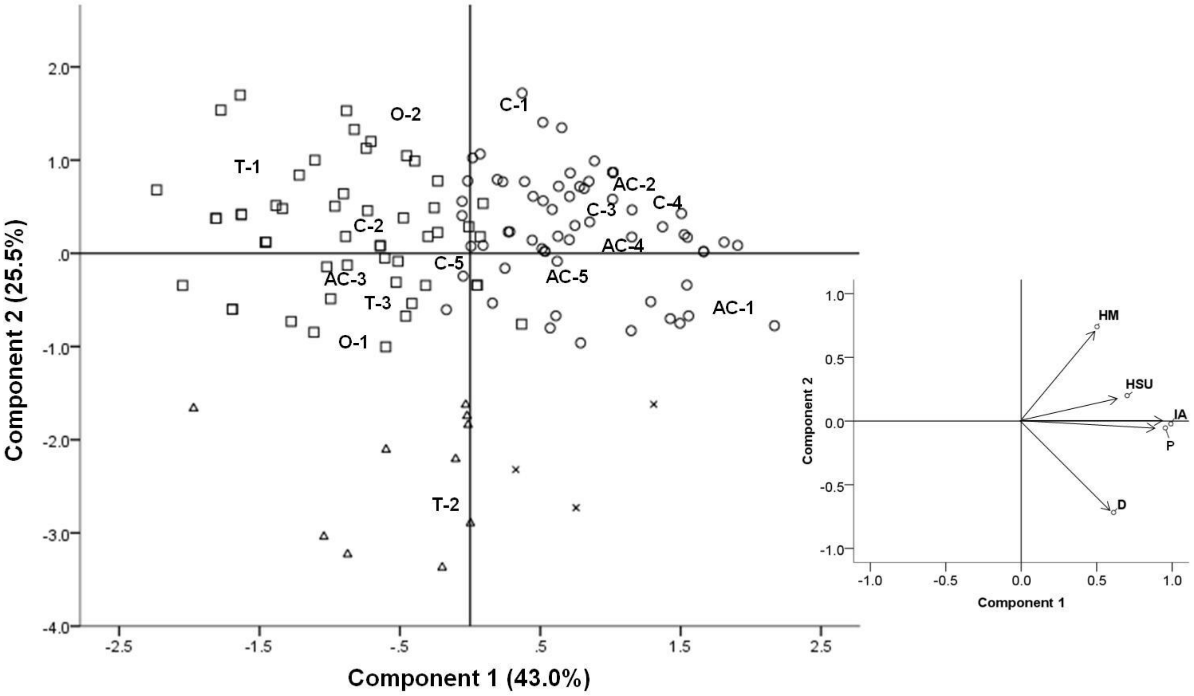

Figure 3.8 Principal component analysis with all farms; different symbols indicate the affiliation to clusters determined by hierarchical clustering: ○ Cluster 1, ☐ Cluster 2, × Cluster 3, Δ Cluster 4; systems were included as inactive individuals. Levels of conformity for the 4 individual dimensions were included as active variables: HM = Health management, D = Disease, P = Parasites, HSU = Health situation in breeding units. The Integrated analysis (IA) was included as additional, inactive variable.

3.5 Discussion

The main aims of this study were to develop a simple and comprehensive tool to assess and benchmark health status and potential of different pig husbandry systems, to identify structural differences in health status and potentials between systems and also to check the developed tool for possible simplifications. In this model for the health sustainability assessment average values are calculated per sub-dimension, per dimension and per system, based on available data. This means that averages and values are supported by different and often a small number of data.

The level of conformity of each aspect of each farm is calculated using the validation of answers in table 2, which at this instance was filled without weighting the different aspects and by authors ‘best guess’ of consequences of answers which are not clearly ‘yes’ or ‘no’. The power of the validation of table 2 can be improved by meta-analysis of literature and expert opinion.

Generally there is little uniformity of farms within systems, which is illustrated by many 1.5 * IQR's covering more than 50 % of the maximum range and outliers. Only 'Health management' and the overall average show shorter IQR's. A high level of conformity to the desired status on the dimension 'Health management' was observed in all categories and most systems. Variability between farms and between systems was rather low in the Conventional category and more disperse in other categories. In the dimension 'Disease and Vaccination' four of five systems of Conventional and Adapted Conventional did not differ very much in variability and medians were close to the mean.

Weak and strong points in health in the four dimensions can be made clear on farm level, but dispersion is too high to make a general qualification of systems in each dimension. Because the overall means, medians and IQR in categories are disperse, the category can only be used as a general indicator.

The developed tool performed reasonably well in terms of feasibility and practicability. However, during analyses and interpretation researchers met a dilemma how to value systematic treatments like de-worming and antibiosis. It was decided that when these measures are taken there will be signs that a threat is present. This can be disputed because on this aspects there can be a strong urge by veterinarians and farmers to be on the safe side. It is commonly accepted that a systematical use of antibiosis is highly undesired and depending on strong regulations. Because data generally are very often best guesses and have been gathered by different persons, systems are not randomly chosen, have unequal numbers, on three systems data are only based on information on 3 or 4 farms the quality of data permits cautious interpretation. The tool has been designed to evaluate current 'Health situations'. But the health situation can change within several days (Brown, 2000). This tool however includes other dimensions 'Health management', 'Disease and vaccination' and 'Parasites' control, that are rather affected on the long term. To assess animal health on the long term, a combination with regular monitoring measures is recommended (Petersen *et al.*, 2002a; Petersen *et al.*, 2002b, Piñeiro, 2009). However, farmers rank on-farm biosecurity measures as a more effective risk management strategy than animal health programs (Vaaleva et al., 2011). Therefore, a combination with biosecurity measures will increase the willingness of farmers to participate the evaluation and make health programs more effective.

The dimension 'Health status' showed a remarkable equality in medians which were all very close to the general mean, but the IQR's are at a different level. This suggests that the system is not a strong indicator yet to the health status. Systems and farms in the Organic and Traditional categories a higher vulnerability on the health status. For the dimension 'Health management' all systems except one of the traditional category ranked well. For 'Disease and vaccination' a high variability for organic and traditional farms was recorded.

The dimension ‘Parasites’ presented increased risks for the categories traditional and organic in general, likewise. Thus, the adapted conventional and conventional systems showed high variability of farms and systems, too. Other studies showed an increased risk for parasites in animals with outdoor access, likewise (Hovi et al., 2003). Contrary to these results, Cagienard *et al.* (2005) found no differences for intestinal parasite burden between different housing types. Results of the dimension wise analysis for ‘Health situation in husbandry units’ showed homogeneity for all investigated husbandry systems. All systems obtained a high health status. The integrated analyses indicate a tendency for increased risks due to traditional husbandry systems.

The dimension ‘Parasites’ was highly correlated with all other dimensions. This would indicate a possibility to reduce the number of questions within the tool. The results of the PCA proved the outstanding impact of ‘Parasites’ on the overall animal health compliance (IA), as both of them were located close to each other. The distance from the center of the PCA graph also indicated the high impact of this dimension. Furthermore, the negative coherence of heath management measures and disease status of the pigs ($r = -0.02$, $p > 0.05$). Although, results of the multivariate analyses indicated the major impact of the dimension ‘Parasites’ on the overall animal health situation an influence of all four dimensions to the explained variability was observed. The cluster analysis resulted in four clusters. Each of the clusters was affected by both principal components and thus all dimensions. This result underlines the importance of all four dimensions. A larger and evident database would be needed to decide about a simplification of the developed tool. Anyway, husbandry systems T-1, T-2 and O-2 were well discriminated among the fifteen investigated systems by PCA. The other thirteen husbandry systems revealed overlapping values in the multivariate statistical analysis.

The health assessment tool can be used to benchmark on farm diagnosis of health management. The selection of investigated systems can be used as a first sufficient database. An extension of the database will increase the reliability of drawn conclusions. All investigated systems had the potential to reach a required level of animal health. However, farms of the category ‘traditional’ ranked discernible lower than others. Except for one system, these low levels cannot be explained by systematically weaknesses of the systems, as variability is high.

3.6 Conclusions

The developed tool covered four dimensions of health in pig husbandry: preventive health management, diseases and vaccinations, parasites and current health status. The tool performed reasonably well in terms of feasibility and practicability. Because of the limited size of this survey, it is only possible to assess the health sustainability on farm level and no conclusions can be drawn about the conformity of systems to desired values. The achieved dataset however enables an overview of the current dispersion of conformity in different dimensions of health sustainability. Thus, the model is able to show strong and weak point in health dimensions in farms. High dispersion of conformity to desired levels of farms within systems in this survey does not permit definite conclusions on system level.

As the results of the tests for redundancies did not present consistent results, a larger dataset is needed to reduce the number of questions and simplify the tool. However, the current results indicate a major impact on the animal health assessment by the dimension ‘Parasites’. By assigning weights to sub dimensions and dimensions the model could be improved. The chosen dimensions ‘preventive health management’, ‘parasites’, ‘presence of diseases’ can be used to illustrate the sustainability and point health aspects which can be improved. However, a weighing of different aspects will determine the final interpretation. This can be determined by the importance of a question, a sub-dimension or a dimension to the sustainability of the pork chain. This can be answered by literature meta-analysis or expert opinions.

Acknowledgement

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Disclaimer

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4 Concept of a HACCP based quality management system towards more sustainability in finishing pig farms

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4.1 Abstract

Aim of this study was to develop an approach to extend and simplify the implementation of a hazard analysis and critical control points (HACCP) based system to control and improve sustainability of finishing pig farms. From nine defined sustainability themes three themes (animal health, animal welfare, meat safety) were chosen based on increased risks for overall sustainability. Failure mode and effects analysis was applied to identify the high-risk themes. The present study is mainly based on expert interviews to rank risks according to specific sustainability themes. Furthermore, a literature research was performed to find common, measureable parameters for a combined risk assessment. As they were already well investigated and known the sensitive and unspecific Acute Phase Proteins, haptoglobin (Hp) and pig major acute phase protein (Pig-MAP) have been identified as biological markers with a high potential for quality management applications in finishing pig herds. Critical limits were defined for both indicators and monitoring measures were described. The proposed inspection and auditing concept as a result of an international cooperation provides farms and advisors with the elements of a HACCP based approach towards more sustainability in fattening farms.

Keywords: HACCP, finishing pig herds, quality management, sustainability, Acute Phase Protein

4.2 Introduction

In the classical context, the quality concept addresses mainly the product. Quality as a subjective entity comprises both technical and technological characteristics, as well as emotional and ethical aspects. Many definitions of quality can be found in the literature, each trying to address quality from one or more of the forenamed points of view. Most important is that a product should fulfil the demands put forward by the consumers and is attractive enough to be bought under the aspect of sustainability of the value chain.

Meat industry faces many significant risks from public criticism of corporate social responsibility (CSR) issues in supply chains. Literature draws upon previous research and emerging industry trends to develop comprehensive farm work supply chain CSR in pork chains. Applications in meat supply chains include animal welfare, biotechnology, environment, fair trade, health and safety as well as labour and human rights.

Nowadays, meat production chains in Europe are more and more affected by changing consumer demands. However, approaches to implement CSR principles and thereby improve sustainability are limited for the level of animal husbandry. Thus, the aim of this study was to develop an approach of an HACCP based system to assess potential risk for the overall sustainability of fattening farms.

The objective of this substudy was to determine and test the organizational structure behind CSR and sustainability activities. Pork chains have not jet widely integrated CSR activities into business strategy. On farm level the cost and benefits of CSR is a critical issue to explore. Five categories are defined to explore the impact of best practice CSR and sustainability activities: Workforce (e.g. health, safety, and wellbeing…), Environmental (e.g. Resource and energy use, pollution and waste management, environmental product responsibility, transport planning…), Marketplace (responsible customer relations, product responsibility like animal health and welfare, product safety, materials origin and ethical competition like fair pricing), Supply chain (being a fair customer and driving standards), Stakeholder (mapping stakeholders, relation with enterprise , feedback communication, liaison and reporting, external validation).

The challenge behind the ongoing surveys in the meat sector is to find methods and indicators to measure conformity or nonconformity with CSR and sustainability criteria.

4.3 State of the art

4.3.1 Sustainability

The expected doubling in global food demand within the next 50 years will be the major challenge for sustainability both of food production and of terrestrial and aquatic ecosystems and the services they provide to society. Agriculturalists are the managers of useable lands and they will mainly shape the surface of the Earth in the coming decades. New incentives and policies for ensuring the sustainability of agriculture and ecosystem services will be necessary to meet the demands of improving yields without compromising environmental integrity or public health (Tilman *et al.*, 2002). Since the World Commission on Environment and Development published the Brundtland report “Our Common Future” (WCED, 1987), academic, scientific and policy-making communities were focused considerably on the concept of sustainable development. A general definition of sustainable agriculture was formulated by Harwood (1990):

> *“Sustainable agriculture is a system that can evolve indefinitely toward greater human utility, greater efficiency of resource use and a balance with the environment that is favorable to humans and most other species.”*

Brown *et al.* (1987) already named the three general definitions of sustainability ecological, social and economic. Bloksma and Struik (2007) supported farmers to redesign their farms in accordance with these three sustainability aims, now called “people, planet and profit“. In 2011 Bonneau *et al.* defined nine themes for an enquiry to evaluate the overall sustainability of fattening pig farms (figure 4.1). However, there is an absolute necessity of effective information systems along the whole production chain and a high potential of quality management methods to control and improve chain wide sustainability (Schmitz, 2006; Lehmann *et al.*, 2011; Wever *et al.*, 2011). Quality management systems are wide spread, well known and even required by law in food production chains (EC, 2002). This fact even enhances the recommendation of quality management methods for sustainability assessment.

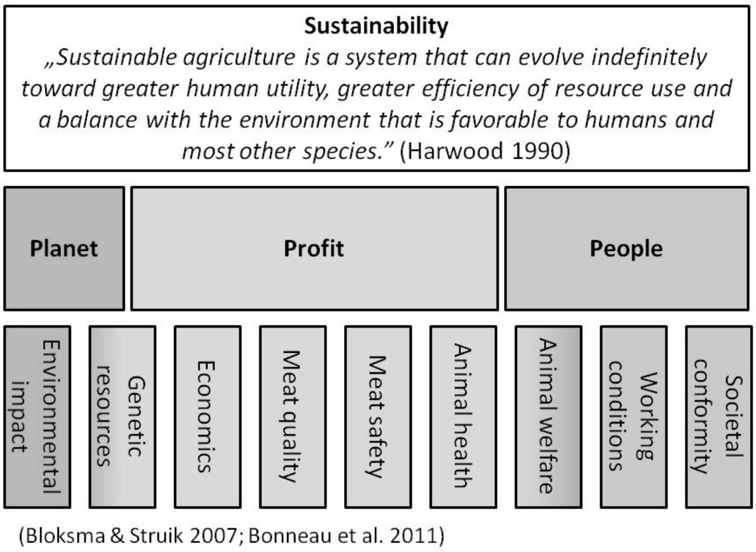

Figure 4.1 On-farm sustainability of pig production

4.3.2 Methodology for risk assessment

Risk management concepts consist of three parts. These are the risk assessment, the risk management and the risk communication. The first two parts can be met by an implementation of a quality assurance system, like HACCP (Mack, 2007). HACCP systems are often directly connected with a Failure Mode Effects Analysis (FMEA). This connection needs no further efforts, as the two tools match very good. Schmitz (2005) describes a possible combination of the two quality tools. He reduces the seven HACCP and the six FMEA principles to six “method modules” by combination of the quality strategies. The definition of hazards according to the HACCP concept means exactly the risks to consumers’ health, whereas the FMEA prevents every deviation from a target value. By the combination of both methods, the possibility to minimize the risks to consumers’ health and the deviations from targets of the sustainability tools can be assessed. The third part is an organizational challenge for the whole production chain needing effective information management systems (Brinkmann *et al.*, 2011, Lehmann *et al.,* 2011).

4.3.3 Hazard analysis critical control points (HACCP) approach

A very well known preventive quality management method heading for consumers protection is the Hazard Analyses and Critical Control Point system (HACCP). This concept originally focuses on hazards of food origin for human health (CAC, 1997). It consists of seven principles that allow to point out hazards to human health and to define critical control points in food production. But this established approach is not only suitable to cope with hazards, but also with other aspects of food production, like nonconformity with the defined sustainability criteria. HACCP is implemented in pork chains in all stages of the production, except the farm level. Thus, concepts to implement HACCP systems on farm-level have been developed (Horchner *et al.*, 2006). Based on prerequisites seven principles have to be followed to implement the concept in food production and to ensure food safety (CAC, 1997). In pork production chains the implementation of HACCP is advanced in large companies on feed production, slaughter and processing level according to European regulations (EC, 2002, EC, 2004). At these stages of a chain SMEs often have problems, because of a deficit of essential human resources. This is more distinctive on farm-level. Here a gap still exists because a practicable way of implementing HACCP has not been found, yet. Hence primary production was excluded from the legal requirement to implement HACCP in the self control of farms for now, but the member states shall motivate primary production to develop a concept for the HACCP implementation (EC, 2002). Crucial is that the EU aspires to have a coherent HACCP in the whole food chain (EC, 2002). Research has to be conducted to develop a concept that accounts for the specific circumstances on farms that have in most cases the organization of a family farm. For some pathogens the use of a HACCP system is well described in publications, especially *Salmonella* ssp. (Noordhuizen and Frankena, 1999; Borell *et al.*, 2001). During the life cycle of a pig that follows the product main stream vertically in the chain in addition several co-products accrue which leave the chain during the production process to the abattoir. This can e.g. be old sows or barrow etc. Such pigs have a very similar hazard potential like the main products (finisher) and because of this fact the same measures to control hazards should be conducted in the product main stream, likewise.

The HACCP-concept consists of seven principles that allow to point out hazards to human health and to define critical control points in food production (CAC, 1997). The principles are:

- Conduct a hazard analysis
- Determine the critical control points (CCPs)
- Specify and validate critical limits for each CCP
- Implement monitoring measures
- Define corrective actions
- Establish verification procedures
- Record keeping

The first principle might be subdivided into six steps according to Horchner *et al.* (2006). The authors stated that the hazard analysis has to start with the assembly of an HACCP team. This team should consist of professionals trained in HACCP methodology. In addition specialists for sustainability, the farmers, veterinarians and advisors could be part of the team. The team must develop a product description and define the intended use of the HACCP system (steps 2 and 3). Afterwards, a process flow chart must be set up (step 4) and verified (step 5). The final step (6) of principle 1 should be the hazard analysis. A useful tool for the hazard analysis is the Failure Mode and Effects Analysis. Due to the aims of this study, the hazard analysis will be central theme of it.

4.3.4 Failure Mode and Effects Analysis (FMEA)

The FMEA is a quality management tool. It is a standardized method to identify and assess possible weak points and their consequences in production processes, preventive (Mortimore and Wallace, 1998). The FMEA might be connected to the risk assessment of HACCP-systems (Schmitz, 2005; Ellebrecht, 2012). Therefore, a risk priority number (RPN) is calculated. It is the product of three factors. Each of these factors is given a value between 1 and 10 to weight their importance. The 1st factor is named “severity rating (S)”, the 2nd “occurrence rating (O)” and the 3rd “detection rating (D)” (Daily, 2004). The RPN is a measure of risk. It is used to rank different problems and the correcting actions (Mack, 2007). Welz (1994) makes a first approach to assess animal health by the use of a FMEA. He defines criteria for the three ratings. For the assessment of the occurrence rating the morbidity of illnesses is taken into account. To calculate the severity (S) mortality, the effect on the production, restrictions for trading and/ or processing and the duration of the illness are raised. The detection rating evaluated according to the used inspection methods. For a reduction of the RPN the occurrence rating and the detection rating can be affected by taking measures. However, for an adaption of this method to identify weaknesses in overall sustainability the detection rating was defined to be equal for all sustainability themes, as changes in such broad sectors will be recognized by farmers quite soon. Thus, the used formula to calculate the risk priority number was based on severity rating and occurrence rating.

Formula 1: Risk priority number (RPN)

$$RPN = S \times O$$

Severity rating (S), Occurrence rating (O)

Where, the severity rating is given by the impact of one theme on other themes and the occurrence rating by the possibility of rapid negative changes within each sustainability theme. Both ratings were evaluated in a small expert survey (5 persons).

4.3.5 Promising indicators for risk assessment

From literature Acute Phase Proteins (APPs) have been identified as biological markers with a high potential for quality management applications in finishing pig herds. It is possible to address several sustainability themes by the measurement of APPs. Table 4.1 gives an overview of the investigated coherences between animal health, animal welfare, meat safety and the concentration of different APPs. Besides these high risk themes also the correlations of meat quality traits and APPs were investigated.

Table 4.1 Literature on Acute Phase Proteins as indicators for choosen sustainability themes

Theme	Acute phase protein	Literature
Animal health	Hp; SAA; AGP	Loughmiller *et al.,* 1999
	Hp	Gymnich, 2001
	Hp	Dickhöfer, 2002
	Hp; CRP	Chen *et al.,* 2003
	general	Murata *et al.,* 2004
	Hp; CRP; SAA	Petersen *et al.,* 2004
	general	Gruys *et al.,* 2006
	Hp; Pig-MAP; SAA; CRP; Albumin	Parra *et al.,* 2006
	Hp	Quaye 2007
	General	Eckersall *et al.,* 2010
Animal welfare	Hp; AGP	Grellner *et al.,* 2002
	Hp	Petersen *et al.,* 2002b
	Hp	Geers *et al.,* 2003
	general	Murata, 2006
	Hp; CRP	Scott *et al.,* 2006
	Hp; Pig-MAP; SAA; CRP	Piñeiro *et al.,* 2007a
	Hp; Pig-MAP; SAA; CRPI	Piñeiro *et al.,* 2007b
	Hp	Van den Berg *et al.,* 2007
	Hp; SAA; CRP	Pallares *et al.,* 2008
	Hp; Pig-MAP; CRP	Salamano *et al.,* 2008
	Hp; SAA; CRP	Heinonen *et al.,* 2009
Meat safety	Hp	Jungersen *et al.,* 1999
	Hp	Knura-Deszczka, 2000
	Hp	Petersen *et al.,* 2002
	Hp	Witten, 2006
	Pig-MAP	Yamane, 2006
	Hp	Klauke *et al.,* 2011
Meat quality	Hp	Eurell *et al.,* 1992
	Hp; Pig-MAP; CRP	Saco *et al.,* 2011
	Hp	Blagojevic *et al.,* 2011
	Hp	Klauke *et al.,* 2012 (submitted)

Hp = haptoglobin, Pig-MAP = Pig major acute phase protein; CRP = C reactive protein; SAA = serum amyloid A; AGP = acid glycoprotein

Petersen *et al.* (2004) described Hp and Pig-MAP as the major APPs for pigs. Thus, during infection diseases the serum concentration of these APPs increased by more than the 10-fold. Gymnich *et al.* (2003) formulated a list called ”Do and Don’t” to interpret different APP values. These list will help to prevent misinterpretation of results. Table 4.2 displays the reference values for healthy fattening pigs presented by different studies.

Table 4.2 Acute Phase Protein reference values for healthy fattening pigs

Hp concentration [mg/ml]	Pig-MAP concentration [mg/ml]	Author
0.8 – 0.9	0.7 - 0.8	Piñeiro *et al.,* (2009a)
1.06 +/- 0.73		Segales *et al.,* (2004)
1.42 +/- 0.02		Chen *et al.,* (2003)
0,68 +/- 0,39		Hiss *et al.,* (2003)

Hp = haptoglobin, Pig-MAP = Pig major acute phase protein

For the measurement of APPs several methods have been described. The most common methods to measure APP concentrations are enzyme linked immunosorbent assays (ELISA). ELISAs were developed for both Hp (Hiss, 2003) and Pig-MAP (Piñeiro *et al.,* 2009b). But also new and rapid detection methods were described. For the qualitative assessment of Pig-MAP an immunochromatographic method was developed by Piñeiro *et al*. (2010) and in chapter 6 an application of modern surface acoustic wave biosensor technology for the measurement of Hp is presented. This technology enables a combined measurement of several parameters. Thus, the measurement of Hp could be combined with specific antibody measurements of production diseases. Klauke *et al.* (2011) reported an improvement of the accuracy of testings to assess animal health and meat safety by combinations of specific and unspecific indicators. The authors described a method to compare the accuracy of different testing methods based on the information theory by Shannon (1949).

4.4 Material and methods

The presented tools to assess risks to sustainability are combined to an overall model. The basic approach is illustrated in figure 4.2. It shows steps of the design for an effective development and implementation of inspection methods.

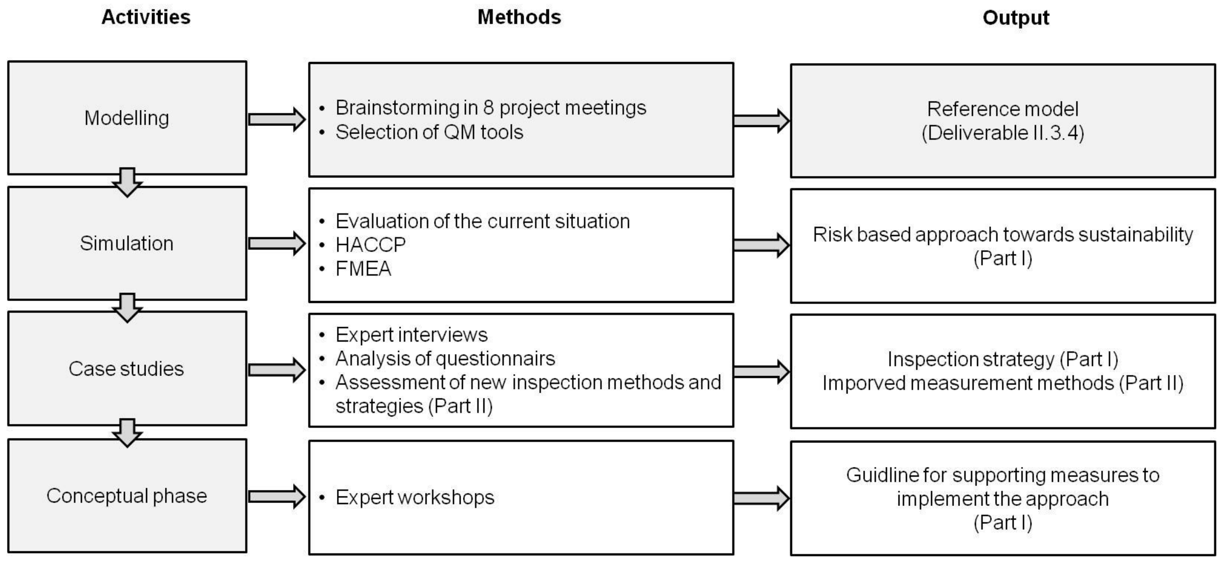

Figure 4.2 Steps for the development and implementation of an inspection strategy towards more sustainability

4.5 Results

4.5.1 Identified sustainability themes with increased risks for overall farm sustainability (principle 1)

From discussions with farming and quality management experts the possibility of rapid changes with hazardous effects for sustainability of farms were evaluated as high for the themes animal health, animal welfare, meat safety and economics. Thus, changes effecting sustainability of the other four themes, genetic resources, meat quality, environmental impact, human working conditions and social acceptability occur only on the long term. This information was used for the calculation of the RPN. For occurrence rating a 2 was given when rapid changes have to be expected, whereas a 1 was given when not.

Table 4.3 shows the results of the determination of the severity ratings. The severity rating was assessed by the number of sustainability themes which might be affected by a negative change of one specific theme. A negative change of animal health shows the most crucial effect on the overall sustainability, as seven of the eight other themes were affected. Thus, to control farm sustainability major ambitions should focus on the control of animal health. Half of the possible eight other themes were affected by negative development of animal welfare. Negative changes of meat safety and economics had a negative effect on three other themes. Changes of meat quality showed an effect on two other themes. Developments to the worse affected only one other theme for all four remaining themes.

Table 4.3 Mutual impact of negative developments of sustainability themes genetic resources (G), animal health (AH), animal welfare (AW), meat safety (MS), meat quality (MQ), economics (E), environmental impact (EI), human working conditions (HWC) and social acceptability (SA), average results from an expert survey

		Impact on …									
		G	AH	AW	MS	MQ	E	EI	HWC	SA	**sum**
Negative development of…	G		(-)	(-)	(-)	-	(-)	(-)	(-)	(-)	**1**
	AH	(+)		-	-	-	-	-	-	-	**7**
	AW	(+)	-		-	-	ni.	ni.	-	-	**4**
	MS	ni.	ni.	ni.		-	-	ni.	ni.	-	**3**
	MQ	(+)	ni.	ni.	ni.		-	ni.	ni.	-	**2**
	E	(-)	-	-	(-)	(-)		(-)	-	(-)	**3**
	EI	(+)	ni.	ni.	ni.	ni.	ni.		ni.	-	**1**
	HWC	ni.	ni.	ni.	ni.	ni.	ni.	ni.		-	**1**
	SA	ni.	ni.	ni.	ni.	ni.	-	ni.	ni.		**1**

ni. = no direct impact; - = negative impact; (-) = possible negative impact; (+) = possible positive impact

Themes with a potential for rapid changes were the same with impact on many other themes. Thus, the calculation of the RPN enhanced the differences between the themes. Animal health (RPN 14) presented crucial risks for farm sustainability. With some distance animal welfare (RPN 8) ranked second. Meat safety and economics (RPN 6) were important risk factors for the overall farm sustainability, too. But genetic resources, environmental impact, human working conditions and social acceptability (RPN 1) as well as meat quality (RPN 2) presented low risks for overall farm sustainability. This ranking does not express the general importance of single themes, but the potential risks sourcing from each theme. However, a HACCP based approach towards more sustainability must focus on animal health and welfare, followed by meat safety and economics assessment.

4.5.2 Identified indicators for risk control (principle 2)

The identified themes with increased risks for sustainability of farms can be clustered in two groups. Group one consists of the biological themes animal health, animal welfare and meat safety. The second group is about business and represented by economics. For economics several possibilities to detect and manage changes and risks have been developed by business economists. The monthly income is an indicator easy to control by each farmer himself and changes in prices of feedstuffs, additives and energy are presented in specialized literature every week as well as the possible income from slaughter. Thus, for this theme preventive control mechanisms do already exist and can be applied easily. For the biological themes risk assessment is more complex. An unspecific but sensitive indicator is needed to assess general herd health, animal welfare and meat safety with health being the core theme. Table 1 shows haptoglobin (Hp) and pig major acute phase protein (Pig-MAP) being the best investigated APPs. They have been investigated in correlation with all biological sustainability themes presenting increased risks to overall sustainability.

4.5.3 Definition of critical limits for the indicators (principle 3)

As APPs haptoglobin and pig major acute phase protein were identified as indicators for the sustainability assessment of farms, critical limits for both indicators must be defined. Due to the reported reference values for Hp and Pig-MAP (table 4.2), a critical limit of 1.5 mg/ml should be preferred.

4.5.4 Monitoring measures (principle 4)

To apply monitoring measures two things are needed, first a measurement method and second a strategy for the screening. The strategy must contain a sampling time schedule and a tool to interpret the results. In 2001 Gymnich proposed a strategy for an APP-screening at farm level to assess the herd health situation. The stated sampling schedule is given in table 4.4. The author requires 5 samplings during the lifespan of a pig. This sampling is not required for each pig, but for the assessment of the situation 10 % of the herd must be tested.

Table 4.4 Sampling time schedule

Age (d)	28	60	80	116	Slaughter (-3 days)
Weight (kg)	7	20	35	60	100+

However, two of the five sampling terms have to be performed at the weaning section and the remaining three tests during fattening. The described sampling schedule can be used for the sustainability assessment as it is.

4.5.5 Corrective actions (principle 5)

Following the systematic approach to identify weak points (figure 4.3) positive and negative sustainability situations can be identified. In cases of negative sustainability situation further steps and corrective actions must be applied. Thus, in depth studies of each of the high-risk themes will help to identify the real source of an upcoming problem. For animal health this might be a check of the serological profile of a finishing herd or the evaluation of production data e.g. the growth performance or feed conversion of the pigs (Blackshaw *et al.,* 1980). Iceberg indicators for animal welfare are the occurrence of cannibalism and abnormal behavior (Edwards, 2006, Smulders *et al.,* 2006). Changes in *Salmonella* seroprevalence might indicate hygiene problems affecting meat safety (van der Wolf *et al.,* 2001). Based on the results of the in depth studies specific measures must be applied to improve sustainability. For animal health the development of vaccination programs or the dispensing of medical drugs might be required. To improve animal welfare the husbandry systems must be adapted to the needs of pigs and for meat safety preventive measures like cleaning and disinfection might be applied. For a detailed weak point analysis the tools developed within Module II of the Q PorkChains project to measure overall farm sustainability can be applied (Bonneau *et al.* 2012).

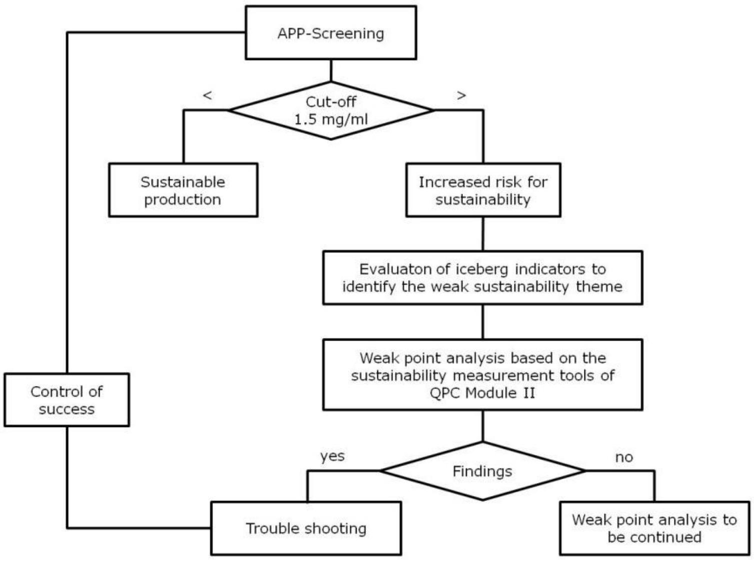

Figure 4.3 Systematic approach to identify weak points and apply corrective actions (modified according to Gymnich, 2001)

4.5.6 Remaining HACCP principles (principles 6 and 7)

The establishment of verification procedures (principle 6) is described in figure 4.3 by the continuous restart of the approach. The control of success can be assured by auditing procedures. However the verification procedures and the frequency of verification should be enough to ensure that the HACCP based system towards more sustainability is working effectively. The final principle 7, the documentation, is essential for the application of a HACCP based system. Documentation should include the laboratory results of the screening and all reference documents used in the risk assessment. In case of identified deviations, all further assessment steps, the results of the in depth studies like the evaluation of iceberg indicators and results of the applied tools must be recorded.

4.6 Discussion

Within this study a practical approach was developed to apply preventive quality management methods on the farm level to control and improve sustainability. Three themes of on-farm sustainability were identified as high-risk themes to overall farm sustainability. These themes were:

- animal health
- animal welfare
- meat safety

The outstanding importance of these themes is also reported by others (Blaha and Köfer, 2009). To improve the sustainability situation, reduce and control the risks of sustainability and thereby satisfy consumer and social demands. A combination of methods HACCP and FMEA was used to enable continuous control following the ideas of Schmitz (2005) and Ellebercht (2012). Practical applications could be presented for all seven HACCP principles to implement a risk based management system towards more sustainability. The practicability of HACCP based systems to control and manage specific sustainability themes (e.g. animal health) or subdimensions (e.g. Salmonella infections in fattening pigs) were already reported (Borell *et al.*, 2001, Doyle and Erickson, 2011, Horchner *et al.*, 2011). Already implemented quality management and information management systems might support the necessary monitoring actions and lower the costs of an implementation (Brinkmann *et al.*, 2011, Lehmann *et al.*, 2011). Acute phase proteins were identified as measureable indicators for monitoring measures to identify upcoming risks for sustainability. Pig-MAP and Hp were identified as proteins with major potential for the purpose. These proteins were defined as major APPs from other authors, too (Petersen *et al.*, 2004, Gruys *et* *al.*, 2006). Based on literature specific critical limits for the risk assessment were defined. A critical limit of 1.5 mg/ml is beyond normal biological concentrations of healthy animals but will be exceeded in case of hazardous situations. This limit is also reported by Piñeiro *et al.* (2012). The sampling strategy developed by Gymnich (2001) was adapted for the risk assessment of sustainability themes. Corrective actions were recommended and useful tool for in depth studies were provided. Thus, a whole HACCP system based on the seven principles of the HACCP method was developed to ensure sustainable pig production on the farm-level.

Supporting measures for an implementation in practice

Within current literature a high interest of farmers to participate in monitoring programs for pig health is reported (Schütz, 2010, Ellebrecht, 2012). But the willingness of farmers to attend such monitoring activities seems to be lower than the willingness to improve their biosecurity measures (Vaaleva *et al.*, 2011). A combination of both, improved biosecurity measures and monitoring programs to control the effectiveness of taken actions would be advantageous from the point of quality and animal health management (Petersen *et al.*, 2005) and thereby important for the improvement of farm sustainability. Therefore, Brinkmann *et al.* (2011) defined a chain coordination model to encourage quality management strategies of pork supply chains. The model highlights the importance of service organizations (Network coordinators) supporting all enterprises along the production chain sharing their data (collection, analyses, communication) and by this enables a joint decision making. These Network coordinators can support farmers during implementation and performance of the developed HACCP based approach. The communication with laboratories and the analysis of the results might be supported but also the verification procedures and documentation (Schütz 2010). Besides these supporting organizations, the use of incentive mechanisms for food safety and animal health control is recommended (van Wagenberg, 2010). This might also apply for the improvement of farm sustainability.

In herd health and production management programmes it is common practice to make an inventory of the herd performance status (Petersen *et al.*, 2002a; Petersen *et. al.*, 2005). The activities comprised under “inventory” are often called “monitoring”. Monitoring is an important component of quality risk management programmes following the rules of a HACCP concept as shown by this study. Monitoring is an act of conducting a planned sequence of observations or measurements of certain control parameters to assess whether a certain point in the production process is under control or functioning correctly or shows conformity with market or society demands. It is highly indicated to conduct also an inventory (i.e. monitoring) regarding the prevailing risk conditions on the farm in animals, their surroundings, the management and the farm records (Berns, 1996; Mack, 2007). Such risk conditions can be found by the application of strengths- and weaknesses assessment on the farm as presented in this study. Preventive quality management methods proposed in ISO 9000 and 22000 have been introduced to support management in decision making, to reduce failure and cost, to assure conformity to demands and to increase income and thereby the sustainability of fattening farms. Thus, many steps towards the implementation of the described HACCP based approach have already been taken. A collaboration of stakeholders from several steps of pork production chains like piglet production, rearing, fattening and slaughter could lower the costs and make the system more effective.

4.7 Conclusions

This study shows a possibility to implement a HACCP based management system on the farm-level aiming for an improvement and assurance of sustainability. Sustainability themes with major effects on the overall sustainability are animal health and welfare as well as meat safety. Acute phase proteins Pig-MAP and Hp are indicators with high potential to identify risks at an early stage. Practical recommendations for an implementation to practice were given. Thus, a complete management concept towards more sustainability was developed.

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Disclaimer

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5 Coherence of animal health, welfare and carcass quality in pork production chains - Correlations of acute phase proteins and quality traits

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5.1 Abstract

Aim of the study was to measure the coherence of animal health and welfare on the one hand and carcass quality on the other hand. The study has been performed at the experimental farm of the University of Bonn. 99 pigs under equal housing and feeding conditions have been involved in the study. The pigs have been divided into three subgroups, for which the intervals of blood sampling differed. Effects of the immune system on carcass composition, meat quality and performance data of slaughter pigs became measureable by quantification of acute phase proteins, haptoglobin and pig major acute phase protein. The results were not significantly affected by the sampling term, gender or breed. The calculated correlations between animal health and carcass quality parameters prove an influence of health and welfare. Time points of measurement and the measured acute phase protein affect the resulting correlations. Significant correlations of acute phase protein concentrations were calculated for many of the value determining factors of carcass quality. The acute phase proteins could also be valuable as a predictive indicator for risk assessment in meat inspection, as increased haptoglobin concentrations in slaughter blood indicate a 16 times higher risk for organ abnormalities.

Keywords: Carcass quality, acute phase protein, haptoglobin, pig major acute phase protein, meat quality, meat safety

5.2 Introduction

Health of an animal has been defined as the absence of disease, the normal functioning of an organism and as normal behavior by Baker and Greer (1980). In production animals, health might 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 of 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). Consequently, the strong linkage between animal health and welfare becomes more and more important. For both animal health and welfare, acute phase proteins are known to be well-investigated, unspecific indicators (Eckersall and Bell, 2010, Murata *et al.*, 2004, Petersen *et al.*, 2004, Geers *et al.*, 2003). Besides the increasing aspects of veterinary medicine, the demands of consumers are changing nowadays. Branscheid *et al.* (1998) defined carcass quality as the combination of carcass composition and meat quality. Carcass composition includes factors like percentage of valuable cuts, lean meat content, fat content and the percentage of saleable meat. Meat quality comprises technological, hygienic, sensory and nutritionally attributes of meat (Hoffmann, 1987). However, this definition has to be adapted to the new challenges of pork production. Consumers are more and more interested in how their food is produced, due to some outbreaks of disease that affected food safety within the last decades (Ahola, 2008). High animal welfare standards at the production stage are demanded as this is seen to be an indicator for safe, healthy and high quality food (Fallon and Earley, 2008, Verbeck, 2001).

This study investigates the coherence of pig health and welfare, measured by acute phase protein concentrations in serum, with pig performance data, carcass quality attributes as well as organ findings.

5.3 Material and methods

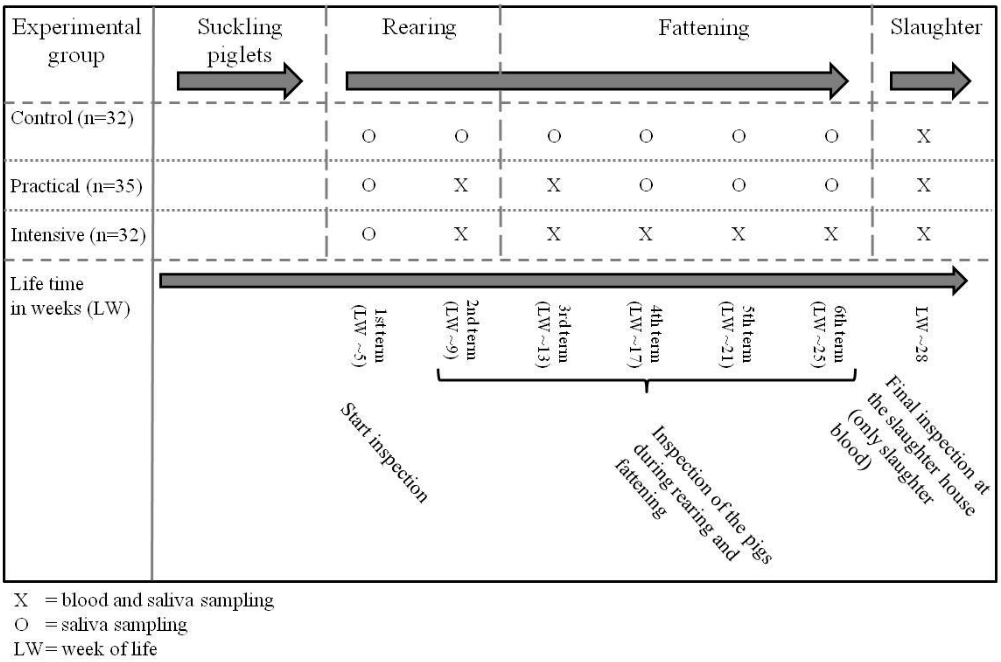
5.3.1 Experimental animals

99 pigs were housed under the same conditions in the experimental farm of the University of Bonn. Twelve litters were included in the study. The pigs stayed for 30 days (± 4 d) with the sows. Average weight at weaning was 8.86 kg (± 2.02 kg). A maximum of 2 litters were mixed into one batch at the rearing station. Fattening started at an average age of 70 d (± 4 d) and at a weight of 26.78 kg (± 4.85 kg). The amount and composition of the rations were the same for all animals. The pigs were fed unrestricted with a standardized diet (13.0 MJ

ME, 16.0 % crude protein) during fattening. Two animals were housed in one batch together. The pigs were divided into three subgroups, for which the intervals of blood sampling differed. From the pigs of the intensive group up to seven blood samples, from the practical group three and from the control group only the slaughter blood samples were taken. The experimental animals originated from two different breeds. The breeds of the mother sows were Large White (DE; n=4) or German Landrace (DL; n=12), all boars were Pietrain (Pi; n=5) breed. The different breeds were almost equally distributed over the three experimental groups, where litters were held together within experimental groups. There was nearly a balanced ratio of the gender in the experimental groups, except in the control group, which showed a predominance of female pigs (71.9 %).

5.3.2 Sampling intervals (program)

Saliva samples were taken starting at an average age of five weeks in regular intervals of four weeks. From the ninth week on also blood samples were collected (figure 1). For the control group blood samples were taken only at the time point of slaughter. The blood sampling points of the practical group have been adapted to the very important time points: at the end of rearing and beginning of fattening as well as slaughter. Only the intensive group had to undergo regular blood sampling once a month. Figure 5.1 shows all sampling intervals and collected matrices.



Experimental group	Suckling piglets	Rearing		Fattening				Slaughter
Control (n=32)		O	O	O	O	O	O	X
Practical (n=35)		O	X	X	O	O	O	X
Intensive (n=32)		O	X	X	X	X	X	X
Life time in weeks (LW)		1st term (LW ~5)	2nd term (LW ~9)	3rd term (LW ~13)	4th term (LW ~17)	5th term (LW ~21)	6th term (LW ~25)	LW ~28

X = blood and saliva sampling
O = saliva sampling
LW= week of life

Figure 5.1 Sampling intervals for the measurement of acute phase proteins

The time points of sampling had to be adapted to the practical procedures at the experimental farm. In the intensive group the number of blood samples collected differed depending on the slaughter age. The first sampling was performed at an average age of 36.5 days (± 2.75 d). From that day onwards a regular testing every forth week was performed. The pigs were sorted for slaughter due to their weights. All pigs were slaughtered at a weight of 108.6 kg (± 3.53 kg). Thus, one pig was tested just three times whereas the others up to seven times. The average age at slaughter was 174 days (± 13 d).

5.3.3 Collection of samples

The saliva samples were taken using a Foerster-Ballenger sponge forceps (Instruments4you, Wurmlingen, Germany) and a Salivette® (Sarstedt AG & Co., Nümbrecht, Germany). The Salivette® contains a cotton swab which was introduced into the mouth of the pigs fixed by the forceps. The pigs chew on the swabs for about one minute. After taking the samples, the Salivettes® were closed and stored for a maximum of three hours at room temperature. The samples were centrifuged at 2000 U/min (950 g) for 10 minutes at room temperature using a Cryofuge 6-6 Heraeus (DJB Labcare Ltd., Newport Pagnell, England). The obtained saliva was stored at -20 °C till laboratory analysis. The results of the saliva haptoglobin analysis were used to identify systematical differences between the three groups of experimental pigs caused by the sampling procedures. Blood samples were obtained following standard procedure of good veterinary practice. The obtained serum was stored at -20 °C till laboratory analysis.

Meat samples were taken 24 h post slaughter. The loin from the 14th rib cranial was collected and brought to the laboratory for further analyses.

5.3.4 Analytical methods

The concentration of haptoglobin (Hp) was measured by a competitive ELISA developed by Hiss *et al.* (2003). Pig Major Acute phase Protein (Pig-MAP) was measured using a commercial sandwich ELISA (Pig-MAP®, PigCHAMP Pro Europa S.L., Segovia, Spain) based on the method developed by Piñeiro *et al.* (2009a).

5.3.5 Performance parameters

The assessed performance parameters were the average daily gain of the pigs and the feed conversion. Both parameters were presented for a weight range from 30-105 kg life weight. Thus, for the calculation of the average daily gain the whole weight gain (75 kg) was divided by the days the growth needs. Feed conversion was the quotient of used feed quantity in kg and the realized gain of the pig (kg). For the calculation of average weight gain during fattening, the pigs were weight at the start of fattening and at the end. The difference was divided by the number of days.

5.3.6 Meat quality parameters

The content of intra muscular fat, water, protein and collagen in the meat samples from musculus longissimus dorsi (m. long. dorsi) was measured using near-infrared spectroscopy (NIRS). The meat samples were chopped up using a Tefal La Mulinette 1000 (Groupe SEB Deutschland GmbH, Offenbach am Main, Germany). After chopping, samples were placed in a spectrometer (NIRS™ DS2500, Foss, Rellingen, Germany) and fully automated analyzed. Electrical conductivity, pH value and the color were measured at the slaughterhouse. Devices developed by the Ingenieurbüro R. Matthäus (Nobitz, Germany) used for these measurements, the pH-Star, LF-Star and the OPTO-Star. Drip loss measurements were performed according to the Bag-method (Kauffman *et al.,* 1992), using a two rib peace of the m. long. dorsi stored at 4 ºC for 48 h. The same meat samples were frozen at -20 ºC after drip loss measurement. Afterwards, samples were thawed at room temperature for 12 – 16 h. Differences of weights gave the thawing loss of the samples. The thawed samples were vacuum packed and cocked for 50 min at 75 °C. After cooking, the samples were cooled in a water-bath at 15-20 °C for 40 min, and weight to obtaining the cooking loss. Again, the weight was taken and the difference presented the cooking loss of the sample. Drip, thawing and cooking loss are given as percentages of weight.

5.3.7 Parameters of carcass composition

Carcass composition was measured due to the routine procedures of the slaughter house. Weights of the value-determining parts of carcasses were detected using the autoFOM device. Lean meat content has been determined following the “Bonner Formular” (Schmitten *et al.*, 1986) and the lean meat content of the belly following the formula of Tholen *et al.* (1998). All analyses of meat quality parameters and carcass composition were performed in accordance with the German regulation for station testing of fattening performance, carcass yield and meat characteristics (ALZ, 2007).

5.3.8 Statistical analyses

For the statistics SPSS 19 (IBM, Armonk, USA) was used. Descriptive statistics give the mean values, minima (min) and maxima (max) as well as standard deviations (SD) and the coefficients of variation (CV). T-tests were used to analyze significant differences between the mean values due to factors like gender and breed. Correlations were calculated using Pearson’s product-moment correlation coefficient. Correlations were considered as significant from the 0.05 niveau and as highly significant from the 0.01 niveau. The Kolmogorov-Smirnov test showed normal distribution for all carcass quality and performance data. As the acute phase proteins showed no normal distribution, these parameters were transformed by logarithmic function. After transformation these parameters showed normal distribution as well. For the calculation of correlations between acute phase proteins (untransformed values) and the number of organ findings, Kendall-tau-b coefficients were given. Odds ratios were calculated and the results proved by Fisher’s exact test.

5.4 Results

5.4.1 Distribution and correlation of the analyzed acute phase proteins

The numbers of tested pigs, mean values with standard deviations as well as minimum and maximum values are given in table 5.1 for each sampling term. Though, no significant differences between the terms were observed, haptoglobin mean concentration at the 2nd term and Pig-MAP mean concentration at the 3rd term showed a tendency to be lower. Haptoglobin concentration in saliva showed a higher dispersion of values in the first sampling term, compared to the other terms

Table 5.1 Descriptive statistics of different terms of APP testing

Term	Number of animals (n)	Min	Max	Mean	SD
		Hp serum concentration [mg/ml]			
2	67	0.012	2.460	0.679	0.606
3	67	0.160	3.950	1.448	0.679
4	32	0.680	2.580	1.296	0.448
5	30	0.530	2.770	1.211	0.505
6	13	0.500	2.270	1.352	0.565
Slaughter	99	0.210	3.200	1.162	0.699
		Pig-MAP serum concentration [mg/ml]			
2	67	0.462	1.922	0.852	0.342
3	67	0.316	3.296	0.664	0.430
4	32	0.404	2.290	0.880	0.407
5	30	0.440	2.214	0.884	0.344
6	13	0.627	3.380	1.102	0.790
Slaughter	99	0.273	4.812	0.986	0.750
		Hp-concentration in saliva [ng/ml]			
1	99	0.900	111.500	16.881	20.871
2	99	0.054	15.800	2.266	3.090
3	98	0.194	65.000	4.487	9.229
4	99	0.114	34.075	2.992	5.222
5	97	0.050	25.500	1.528	2.917
6	42	0.110	3.700	0.959	0.872
7	1	0.900	0.900	0.900	.

min = minimum; max = maximum; SD = standard deviation

Extreme APP serum values (outside the 1.5 interquartile range) were detected from each sampling term and analyte, creating boxplots (not shown). Pigs with extreme APP values had shown clinical signs of respiratory diseases in the controls performed by a veterinarian before or after the blood sampling.

The factors breed and gender had no significant effect on mean Pig-MAP and haptoglobin concentrations. Furthermore, the measurement of saliva Hp was not affected by the blood sampling procedure, as t-test showed no significant differences between the experimental groups at each sampling term ($p<0.05$). The two measured acute phase proteins were significantly correlated (figure 5.2). The correlation coefficient was $R=0.473$ ($p<0.001$).

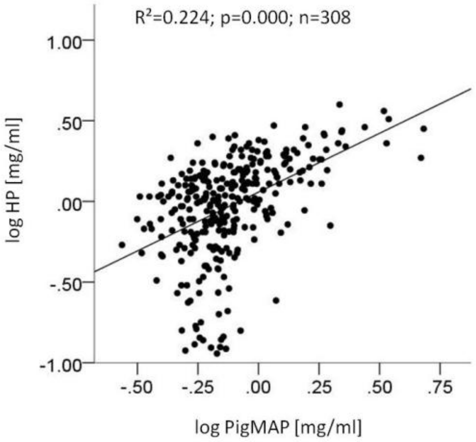


Figure 5.2 Correlation of Hp and Pig-MAP concentrations in serum samples

The concentrations of Hp in serum and saliva were also correlated (R=0.296, p<0.001). However, Pig-MAP concentrations measured in serum samples and Hp concentrations in saliva showed no statistical significant correlation (R=0.125, p=0.072).

5.4.2 Acute phase proteins and performance parameters of fattening pigs

Descriptive statistics on the performance parameters during the fattening period are outlined in table 5.2.

Table 5.2 Descriptive statistics on performance data

Parameter	n	min	Max	mean	SD	CV
average daily gain during fattening [g]	99	551.18	1000.00	792.09	88.49	0.11
average daily gain from 30 - 105 kg live weight [g]	99	591.00	1087.00	816.57	98.19	0.12
feed conversion ratio from 30 - 105 kg live weight [kg]	99	2.26	3.36	2.61	0.18	0.07

min = minimum; max = maximum; SD = standard deviation; n = number; CV = coefficient of variation

The performance parameters average daily gain and feed conversion showed slight but significant correlations to the acute phase protein concentrations in pigs. The positive correlations between animal health and feed conversion (30- 105 kg) were mainly found for Pig-MAP. The statistical coherence was higher for measurements closer to the time point of slaughter (term 4 and 5). Haptoglobin concentration measured at term 5 was correlated to the average daily gain of the animals (30-105 kg) with a correlation coefficient of up to R=0.503 (figure 5.3).

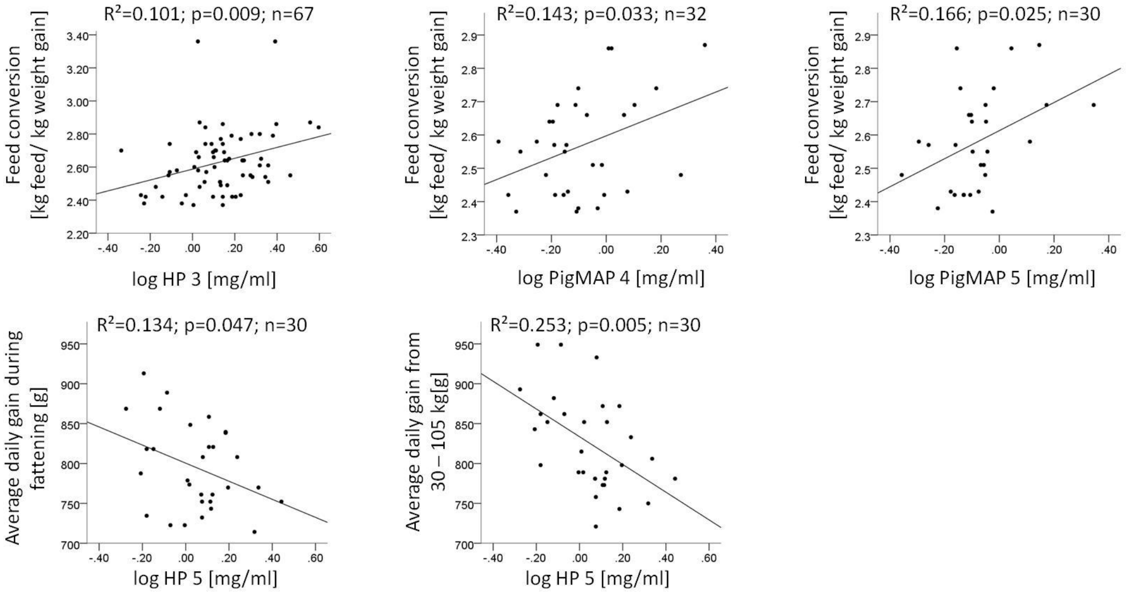


Figure 5.3 Correlations of acute phase proteins and performance data of finishing pigs

5.4.3 Acute phase proteins and meat quality traits of pigs

The mean intra muscular fat (IMF) content was 1.66 %. It ranged from 0.84 % up to 5.58 % with a standard deviation of 0.58 % and a coefficient of variation of 0.35 %. The average water content in m. long. dorsi was 73.77 %, with a minimum content of 70.91 % and a maximum of 74.80 % (SD 0.56, CV 0.01). Descriptive statistics on all investigated meat quality parameters were presented in the Annex (table 5.A1).

Pig-MAP concentrations at term three and at the time point of slaughter showed positive significant correlations with the IMF content of the pigs and negative correlations with the water content in m. long. dorsi (figure 5.4). Other meat quality traits also showed significant correlations to the concentration of APPs. All observed significant correlations were presented in the annex (table 5.A2 and 5.A3).

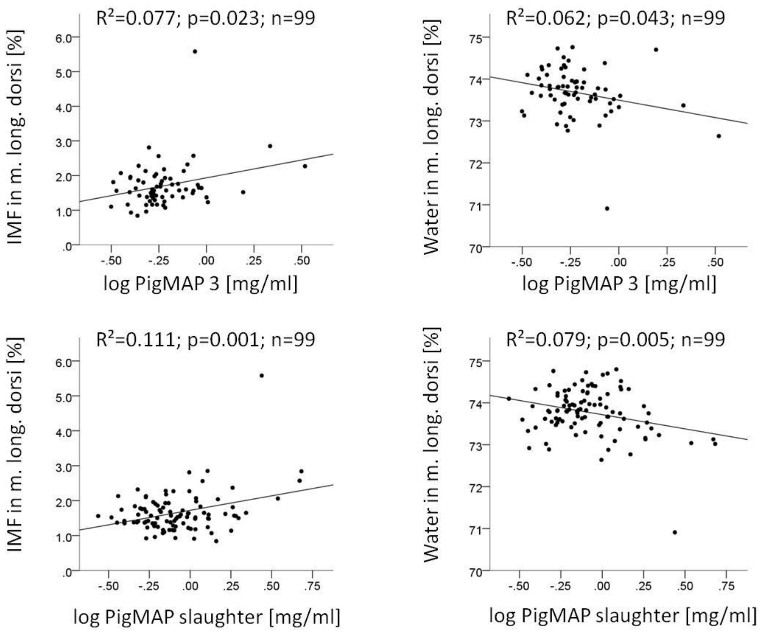


Figure 5.4 Correlations of APPs in slaughter blood and chosen meat quality parameters

5.4.4 Acute phase proteins and carcass composition of slaughter pigs

Descriptive statistics on all investigated parameters of carcass composition are outlined in table 5.3.

Table 5.3 Descriptive statistics on carcass composition

Parameter	n	min	Max	mean	SD	CV
autoFOM Index	99	56.20	100.26	83.71	7.68	0.09
back fat [cm]	99	1.33	2.70	1.94	0.29	0.15
belly percentage	99	46.50	62.00	54.28	3.02	0.05
dressing percentage	99	72.96	82.73	79.19	1.59	0.02
fat-meat ratio	99	0.16	0.49	0.30	0.07	0.23
lean meat content [%]	99	53.41	66.25	59.83	2.67	0.04
lean meat content of belly [%]	99	51.25	65.58	58.99	3.23	0.05
weight of belly [kg]	99	11.90	15.06	13.47	0.63	0.05
weight of ham [kg]	99	13.36	19.44	17.04	1.12	0.07
weight of loin [kg]	99	4.86	8.12	6.51	0.52	0.08
weight of shoulder [kg]	99	6.28	8.50	7.53	0.40	0.05
slaughter weight [kg]	99	73.20	96.40	86.00	3.40	0.04

min = minimum; max = maximum; SD = standard deviation; n = number; CV = coefficient of variation

The Hp concentration at term 3 showed significant negative correlations to the dressing percentage as well as the weights of hams recorded during slaughter. On term 4 the measurements of Pig-MAP were negatively correlated to dressing percentage, again. Furthermore, an increase in Pig-MAP concentrations at this time point resulted in lower weight of loin and a reduced proportion of belly in the carcass. The negative correlation of Pig-MAP to the dressing yield could also be detected at sampling term 5 (figure 5.5).

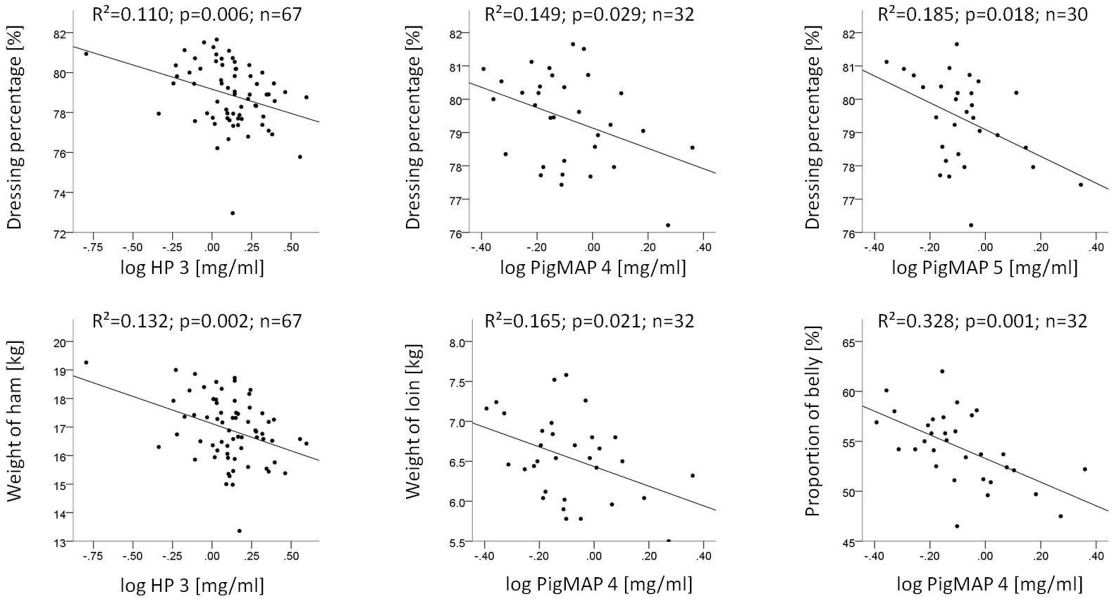


Figure 5.5 Correlations of APPs with weights of dressing percentage and important cuts

Hp and Pig-MAP concentrations measured from serum samples taken at term 3 both were negatively correlated with the lean meat content of the whole carcasses and also with the lean meat content of the belly. Thus, both were positively correlated with the fat-meat ratio of the carcasses. This was not only related to a decrease of lean meat content but to an increase of subcutaneous back fat (table 5.A3). The correlations to lean meat content and fat-meat-ratio were found for Pig-MAP measurement at sampling term 5 (figure 5.6).

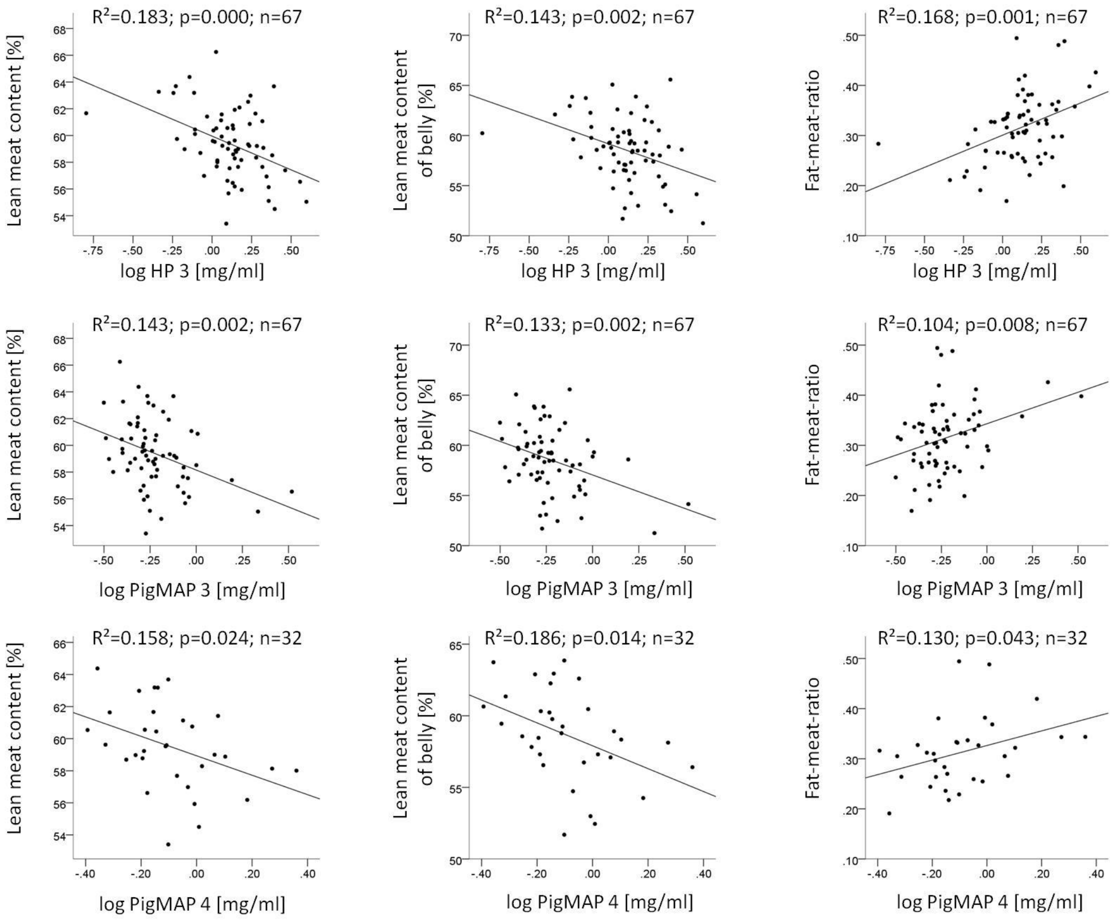


Figure 5.6 Correlations of APPs and parameters of meat and fat content

All in all, 51 parameters of performance (n=10), meat quality (n=19) and carcass composition (n=22) were investigated and 94 significant correlations with Hp (n=37) or Pig-MAP (n=57) concentrations were identified. Not all of the correlations were shown within this paper. Figure 5.7 presents the number of correlations for both APPs due to the time points of sampling.

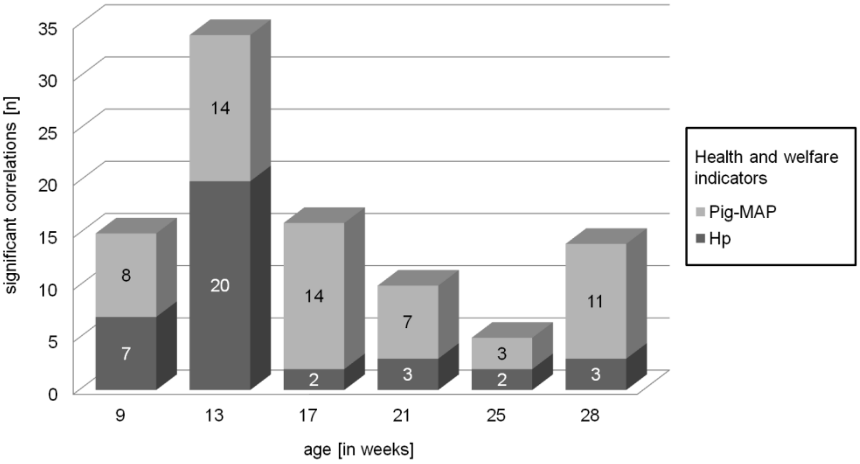


Figure 5.7 Number of identified significant correlations of APPs and 51 investigated parameters of carcass quality and performance due to the age of the pigs

5.4.5 Acute phase proteins as predictors of increased risks for organ findings

Although no clinical symptoms of disease were detected by adspections before slaughter, eighteen of the 99 slaughter pigs showed organ findings during the meat inspection at the abattoir. In nine cases pneumonia has been diagnosed, two pigs suffered from pericarditis, two other pigs showed milk spots on their livers and five had combinations of more than one organ finding. Analysis of correlations between acute phase protein levels and the number of organ findings by Kendall-tau-b showed a significant, positive coherence of the two parameters (table 5.4).

Table 5.4 Correlation between APPs and the number of organ findings

		Hp slaughter [mg/ml]	Pig-MAP slaughter [mg/ml]
number of organ findings	Correlation coefficient (Kendall-tau-b)	0.180	0.194
	Significance (bilateral)	0.027	0.017
	N	99	99

To allow the calculation of odds ratios for acute phase proteins and organ findings, optimal cut off values for Hp and Pig-MAP concentrations were selected via try and error. Pigs with Hp concentrations above 0.8 mg/ml and/or 0.7 mg/ml of Pig-MAP in slaughter blood were defined as pigs with increased risks for organ abnormalities. Pigs with increased Hp levels showed a 16 times higher risk to be found with organ findings, and animals with increased Pig-MAP a 10.58 times higher risk. Both odds ratios were proved by Fisher’s exact test ($p<0.001$).

5.5 Discussion

The correlation of Pig-MAP and Hp concentrations in blood were close to the known results from other studies (Clapperton *et al.*, 2007, Pineiro *et al.*, 2009b). The correlation of Hp concentrations in serum and saliva indicated the possibility to use saliva as an alternative matrix for the health assessment of pigs. This was also reported by others (Gutierrez *et al.*, 2009, Gómez-Laguna *et al.*, 2010). However, the correlations of Hp concentrations in saliva with the investigated performance and quality traits (not shown) were much lower and in parts inconsistent with the correlations based on serum samples. As no significant differences between the saliva sampling terms were found, it was assumed that the sampling procedures (blood collection) did not affect the APP concentrations of the pigs. The Hp concentrations at the first term (5th week of life) tended to be higher than these measured in the later terms. This effect might be caused by gum bleeding, as several blood-stained swabs were identified by visual inspection. During the later sampling terms none blood-stained swabs were found.

The average daily weight gain (mean 0.8 kg/day) during fattening as well as feed conversion (mean 2.6 kg feed/kg weight gain) showed the good performance of the investigated pigs. However, the coefficients of variation show differences between individual experimental animals of up to 12 %for the performance parameters (table 5.2). All investigated parameters of performance, meat quality and carcass composition were in a normal range (Losinger, 1998; Laube *et al.*, 2000; Möhrlein *et al.*, 2005; Correa *et al.*, 2006, Liao *et al.*, 2010, Stege *et al.*, 2011). The coefficients of variation of meat quality and carcass composition parameters ranked between 0 and 30 %. This range was not fixed by the genetic potential of the investigated breeds.

All extreme values identified by the creation of boxplots could be attributed to clinical signs of respiratory diseases in the controls performed by a veterinarian before or after the blood sampling. In only one case a treatment with antibiotics was performed. Some of the presented correlations were related to these extreme values. Due to the fact that only rare presence of disease was shown by the animals and even the extreme values were in the physiological range shown during acute phase reaction (Petersen *et al.*, 2004), the extreme values were not excluded from the analysis. However, a detailed analysis including more sick animals could lead to more conclusive results.

The decrease in performance, slaughter weight, dressing percentage, the weights of valuable cuts, lean meat content and the autoFOM index of pigs showing increased APP concentrations might be caused by sickness behavior and the biological cost of the acute phase reaction (APR). Behavior of sick pigs during the APR with decreased appetite or anorexia is mediated by pro-inflammatory cytokines. The cytokines induce the formation of prostaglandins causing fever and the secretion of APPs. Additionally, the immunological stress induces adrenal gland medullary hormone release with catecholamines causing redistribution of the blood flow to brain and muscles instead of to the splanchnic system. Intestinal villus atrophy and reduced enteric absorption might result in diarrhoea. The changed metabolism results in negative energy balance (Gruys *et al.*, 2005). Thus, the effects lead to a catabolic metabolism of pigs with increased APP concentrations and growth retardation is ameliorated (Knura-Deszczka, 2000). Eurell *et al.* (1992) and Gymnich *et al.* (2004) proved the negative linkage of haptoglobin concentrations and weight gain of fattening pigs.

The positive coherence of APPs and proportional as well as absolute fat of the investigated pigs has to be studied in depth. 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 intermuscular fat development and composition in pigs. Maybe the APR leads to an increased storage of rapid mobilizable energy in adipose tissue. However, the results of this study contradict the positive coherence of slaughter weight and fat contend reported by Kouba and Bonneau (2009).

The investigated reduction of thawing and cocking loss might be related to the decrease of water content in m. long. dorsi in case of increased APP concentrations. But the coefficient of variation (CV 0.01) for this parameter was rather low. The water content ranged between 70.9 and 74.8 %.

The results of this experimental study must be proved under commercial conditions. However, the results are promising for the development of a testing strategy to predict carcass quality traits. Furthermore, the measurement of APPs might be a useful indicator in terms of risked based meat inspection. The increased risk for organ abnormalities in animals with elevated APP concentrations at the time point of slaughter is also reported by others (Witten, 2006, Blagojevic *et al.*, 2011). A combination of APP concentrations and specific serological analysis even improved the validity of risk assessment in case of risk based meat inspection (Klauke *et al.*, 2011).

5.6 Conclusions

The study showed a lot of significant correlations between Hp and Pig-MAP and carcass quality traits and performance parameters. In terms of increased APP concentrations the daily weight gain, slaughter weight, dressing yield, weights of valuable cuts, lean meat content autoFOM index, water content as well as thawing and cocking loss decreased. Feed conversion ratio, subcutaneous and intramuscular fat as well as the risk of organ findings increased with the concentration of the investigated APPs. Anyway, the time point of measurement and the measured APP were important factors for the significance of the correlation. The most significant correlations resulted from serum samples taken at an age of 13 weeks. Pig-Map concentrations presented more significant correlations to carcass quality and performance traits, but the Hp concentrations proved the results and showed correlations to additional parameters. Hp values present higher odds ratios regarding the occurrence of abnormalities of organs and indicated a high potential for the risk assessment during meat inspection.

However, health and welfare of the pigs measured by APPs were proved to have a measurable impact on carcass quality and thereby added value.

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5.8 Annex

Table 5.A1. Descriptive statistics on meat quality traits

Parameter	n	Min	max	mean	SD	CV
cocking loss [%]	99	19.70	28.10	23.62	1.79	0.07
conductivity of loin 24h post slaughter [mS/cm]	99	2.20	4.10	2.88	0.44	0.15
conductivity of loin 45min post slaughter [mS/cm]	99	2.80	5.70	4.00	0.58	0.15
conductivity of ham 24h post slaughter [mS/cm]	99	2.10	5.70	3.13	0.72	0.23
drip loss [%]	99	0.40	4.70	2.05	0.98	0.48
intra muscular fat [%]	99	0.84	5.58	1.66	0.58	0.35
pH of loin 24h post slaughter	99	5.28	5.55	5.40	0.06	0.01
pH of loin 45min post slaughter	99	5.99	6.88	6.49	0.14	0.02
pH of ham 24h post slaughter	99	5.33	5.68	5.50	0.08	0.01
protein [%]	99	23.51	25.53	24.34	0.43	0.02
thawing loss [%]	99	4.60	13.10	8.38	1.71	0.20
water [%]	99	70.91	74.80	73.77	0.56	0.01

min = minimum; max = maximum; SD = standard deviation; n = number; CV = coefficient of variation

Table 5.A2. Pearson correlations of APPs and meat quality traits

Quality attribute	Acute phase protein	R	p-value
cocking loss [%]	PigMAP slaughter [mg/ml]	-0.213	0.034
color [OPTO-Star]	PigMAP term 2 [mg/ml]	-0.272	0.026
conductivity of loin 24h post slaughter [mS/cm]	PigMAP term 2 [mg/ml]	-0.244	0.046
conductivity of loin 45min post slaughter [mS/cm]	HP term 2 [mg/ml]	0.304	0.012
conductivity of loin 45min post slaughter [mS/cm]	HP term 5 [mg/ml]	-0.522	0.003
conductivity of loin 45min post slaughter [mS/cm]	PigMAP term 2 [mg/ml]	0.252	0.040
conductivity of ham 24h post slaughter [mS/cm]	HP term 3 [mg/ml]	0.254	0.038
drip loss [%]	PigMAP term 2 [mg/ml]	-0.271	0.027
intra muscular fat [%]	PigMAP term 3 [mg/ml]	0.278	0.023
intra muscular fat [%]	PigMAP slaughter [mg/ml]	0.333	0.001
pH in loin 24h post slaughter	PigMAP term 4 [mg/ml]	0.351	0.049
pH in loin 45min post slaughter	HP term 5 [mg/ml]	0.274	0.006
pH in ham 24h post slaughter	PigMAP term 4 [mg/ml]	0.491	0.004
thawing loss [%]	HP term 2 [mg/ml]	-0.299	0.014
water [%]	PigMAP term 3 [mg/ml]	-0.248	0.043
water [%]	PigMAP slaughter [mg/ml]	-0.282	0.005

R = correlation coefficient

Table 5.A3. Pearson correlations of APPs and carcass composition

Quality attribute	Acute phase protein	R	p-value
autoFOM Index	PigMAP term 5 [mg/ml]	-0.389	0.034
autoFOM Index	PigMAP term 6 [mg/ml]	-0.613	0.026
back fat [cm]	HP term 3 [mg/ml]	0.303	0.013
belly percentage	HP term 3 [mg/ml]	-0.345	0.004
belly percentage	PigMAP term 4 [mg/ml]	-0.573	0.001
dressing percentage	HP term 3 [mg/ml]	-0.331	0.006
dressing percentage	PigMAP term 3 [mg/ml]	-0.367	0.002
dressing percentage	PigMAP term 4 [mg/ml]	-0.386	0.029
dressing percentage	PigMAP term 5 [mg/ml]	-0.430	0.018
fat-meat ratio	HP term 3 [mg/ml]	0.410	0.001
fat-meat ratio	PigMAP term 3 [mg/ml]	0.323	0.008
fat-meat ratio	PigMAP term 4 [mg/ml]	0.360	0.043
fat-meat ratio	PigMAP slaughter [mg/ml]	0.230	0.022
lean meat content [%]	HP term 3 [mg/ml]	-0.428	0.000
lean meat content [%]	PigMAP term 3 [mg/ml]	-0.378	0.002
lean meat content [%]	PigMAP term 4 [mg/ml]	-0.398	0.024
lean meat content [%]	PigMAP slaughter [mg/ml]	-0.218	0.030
lean meat content of belly [%]	HP term 3 [mg/ml]	-0.378	0.002
lean meat content of belly [%]	PigMAP term 3 [mg/ml]	-0.365	0.002
lean meat content of belly [%]	PigMAP term 4 [mg/ml]	-0.431	0.014
weight of belly [kg]	PigMAP term 3 [mg/ml]	-0.250	0.041
weight of belly [kg]	PigMAP term 6 [mg/ml]	-0.610	0.027
weight of ham [kg]	HP term 3 [mg/ml]	-0.363	0.002
weight of loin [kg]	PigMAP term 4 [mg/ml]	-0.406	0.021
weight of loin [kg]	PigMAP term 5 [mg/ml]	-0.416	0.022
weight of shoulder [kg]	HP term 6 [mg/ml]	-0.650	0.016

R = correlation coefficient

6 Measurement of porcine haptoglobin in meat juice using surface acoustic wave biosensor technology

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6.1 Abstract

Aim of the study was the application of biosensor technique to measure the concentration of an acute phase protein (APP) within complex matrices from animal origin. For the first time, acute phase protein haptoglobin (Hp) was detected from unpurified meat juice of slaughter pigs by a label-free biosensor-system, the SAW-based sam®5 system. The system uses a sensor chip with specific antibodies to catch Hp while the mass-related phase shift is measured. The concentration is calculated as a function of these measured phase shifts. The results correlate very well with regular enzyme-linked immunosorbent assay (ELISA), R = 0.98. The robust setup of the surface acoustic wave (SAW)-based system and its possibility to measure within very short time periods qualifies it for large-scale analyses and is apt to identify rapidly pigs in the meat production process whose consumption would have an increased risk for consumers.

Keywords: Surface-acoustic wave sensor, Love waves, porcine haptoglobin, meat juice

6.2 Introduction

Whenever the tissue of a pig is damaged, e.g. due to inflammation or bacterial infection, the animal shows an unspecific immune response, the acute phase reaction (Gruys *et al.* 2005). Within 6 to 48 hours, the production of more than 30 different acute phase proteins (APP) increases (Miller *et al.* 2009). One of these acute phase proteins is haptoglobin (Hp) (Baumann and Gauldie, 1994). A role of the blood plasma protein Hp is to bind and transport hemoglobin (Murata *et al.,* 2003). Porcine Hp has a molecular weight of about 120 kDa. The α2-globulin consists of two light α-chains (9.1 kDa) and two heavy β- chains (40 kDa) (Petersen *et al.,* 2004). It is not exclusively present at inflammation, but it increases significantly from a base level by more than ten times (Piñeiro *et al.,* 2009a)**.** Since half-life of Hp is about four days, an increase is still detectable after several days (Hall *et al.,* 1992). Hp can be used as a parameter of infection and inflammation (Eckersall, 2000). Thus, Hp is an excellent not disease-specific marker for health assessment, e.g. of pigs in a screening test (Gymnich, 2001, Knura-Deszczka, 2000, Murata *et al.,* 2003, Petersen *et al.,* 2002, Petersen *et al.,* 2004, Piñeiro *et al.,* 2009a, Piñeiro *et al.,* 2009b). The evaluation of animal health and welfare has also been investigated based on Hp concentrations in meat juice (Hiss *et al.,* 2003, Piñeiro *et al.,* 2009c, Witten, 2006). The described temporary rise of Hp only for a number of days back to base level, shows the importance of a rapid on-site method taking Hp measurements.

Many methods have been developed to determine Hp from various origins. Manual methods detecting Hp content are EIA/ELISA outlined by Lequin (2005). Hp from porcine origin has also been detected by enzyme-linked immunosorbent assay (ELISA) (Hiss *et al.,* 2003). Another assay measuring Hp in samples of various body fluids of swine is based on time-resolved immunofluorometry (TR-IFM) (Gutierrez *et al.,* 2009). Lately, (semi-) automated methods have been developed, i.e. immunoturbidimetric assays (Saco *et al.,* 2010) and automated spectrophotometric methods (Martinez-Subliela *et al.,* 2007). At present, acute phase proteins mostly are quantified by ELISA.

Concentrations of porcine Hp from ELISA are generally well accepted and trusted. Empirical values of Hp concentrations of healthy animals measured by ELISA maximally are stated as 1100 µg/ml in full blood and 70-330 µg/ml in meat juice (Petersen *et al.,* 2002, Piñeiro *et al.,* 2009b, Witten, 2006). Comparable reference values for Hp of pigs from commercial farms were presented by Piñeiro *et al.* (2009a).

The EU project ‘Coordination, harmonization and standardization of measurement of bovine and porcine acute phase protein in blood; reference preparations for animal protein assay’ focused on round robin tests to assess and reduce inter-laboratory effects. The measurement of haptoglobin was considered to be standardized if the variation between the laboratories could be reduced to a maximum of 30 %. Three rounds of tests in ten laboratories from different European countries have been performed, but variations were still higher than 30 %. The intra-laboratory repeatability for the same sample was ascertained with a maximum of 16 % (Eckersall, 2002, Skinner, 2001). Therefore, a comparison of ELISA results from different laboratories is critical. Tecles *et al.* (2007) presented a comparison of different commercial APP tests. Intra and inter assay coefficients of variation (CV) of the Hp assay were lower than 5.7 % (see table 1) at any tested APP concentration. Dilutions of samples with high concentrations resulted in linear regression equations with coefficients of correlation (R) ranging from 0.98 to 0.99. The detection limit (0.02 g/L) of the Hp assay was low enough to detect Hp levels even in healthy animals (Tecles *et al.,* 2007). Table 6.1 gives examples of intra and inter assay CV for different methods that are used to measure acute phase protein in serum of pigs.

Table 6.1 Intra- and inter-assay coefficients of variation (CV) due to different measurement methods of porcine haptoglobin

Method	Protein	Mean (SD) [g/L]	CV [%]		Author
ELISA	Hp	0.86 (0,03)	4.00	Intra-assay	Tecles *et al.* (2007)
		0.66 (0.08)	5.70	Inter-assay	
		4.82 (0.08)	1.70	Intra-assay	
		4.85 (0.25)	5.10	Inter-assay	
		u.	3.31	Intra-assay	Hiss (2001)
		u.	10.27	Inter-assay	
TR-IFMA	Hp	0.36 (0.01)	4.81	Intra-assay	Gutierrez *et al.* (2009)
		0.32 (0.01)	5.97	Inter-assay	

Hp = haptoglobin; SD = standard deviation; CV = coefficients of variation; u. = unknown

Although the results of ELISAs within one laboratory are trustable, these measurements still are tedious and time consuming. Specialized laboratory equipment is necessary as well as technical training of the laboratory personal. Therefore, the pig is already traded, processed and consumed before it is tested on conspicuities. Hence, the goal of this study was to simplify and speed up the measurements of Hp and to lower the cost. A well suited approach to automate and simplify the analyses is the measurement by chip-based biosensors. The tubing, detection and the sensitive surface of such sensors might be affected by the conglomerate of proteins, fat, cells, salts and many other contents of the Hp sources blood, serum, saliva and especially meat juice.

A Surface Plasmon Resonance (SPR)-based biosensor was used to detect Hp in milk (Akerstedt *et al*., 2006). The flexible sam®5 system (Perpeet *et al.,* 2006, Schlensog *et al.,* 2004), measuring mass related phase shifts of Love waves, has already been used to detect small molecules, proteins and antibodies, membrane vesicles and complete cells from various matrices. Thus, it is ideally suited to test the unpurified, Hp-containing meat juice, saliva, serum and also whole blood samples. In this article, the usability of this chip-technology to perform Hp tests and to standardize the course of action, the data flow and the outcome of the resulting data is proved. Tested samples were validated by standard ELISA (Hiss, 2001). Previously, it was shown that the sam®5 system can be more sensitive than ELISA tests and that its unique approach to surface acoustic wave (SAW) technology is little affected by pH changes, salts or other matrix contents (Perpeet *et al.,* 2006). Problems arising from a background of accompanying contents in the samples masking the wanted results are targeted by surfaces reducing unspecific binding. Advantages of such a biosensor approach are short detection times, low detection limits, high rates of automation and low costs for large numbers of samples (Cho and Park, 2006, Lan *et al.,* 2008).

6.3 Materials and methods

The used biosensor was the chip-based sam®5 system (SAW instruments, Germany). A binding surface specific for porcine Hp has been developed on a standard gold chip. The biosensor measures phase shifts φ and amplitude alterations of longitudinal Love waves within a piezoelectric crystal. The analyte was bound to the surface by a specific ligand. The concentration of the analyte was calculated from the measured phase shift using the sensor sensitivity of $515° \text{ cm}^2 \text{ µg}^{-1}$. In a first test, porcine Hp was measured from unpurified meat juice, which is a crude mixture of water, proteins, fat and other undefined contents. Thus, the measurement of one specific protein is a big challenge. The results were compared to reference measurements by using a commercial Hp assay (RAIDASCREEN®Haptoglobin, R-Biopharm, Germany).

6.3.1 Collection and preparation of the samples

The meat juice was obtained by thawing frozen muscle samples. Within this study, muscle samples were taken from boars which have been investigated and watched during their growth as a first trial. All pigs used for these measurements had the same origin and genetic setup to minimize possible side effects. After electrical stunning, bleeding, scalding and splitting of the carcasses at a commercial slaughterhouse, the muscle samples were dissected from the diaphragmatic pillars and frozen in meat juice containers described by Christensen (2003) at -20 °C. By freezing the muscle samples, cell membranes were damaged and meat juice was obtained after thawing.

6.3.2 The ELISA reference of Hp concentrations

Hp contents were detected with a commercial ELISA, the RAIDASCREEN®Haptoglobin developed by Hiss (2001). It is based on the concept of a competitive ELISA with a second antibody. The CV of this assay is 5.7 % at a concentration of 0.9 g/L, and the limit of detection is given with 0.033 g/L (product information).

6.3.3 The sam®5 system

The chip-based sam®5 system (SAW instruments, Germany) uses proprietary surface acoustic wave technology. The sam®5 allows to record directly the phase shift φ and an amplitude signal A of an applied longitudinal wave as a function of the quantity of bound molecules on the chips surface. A rigid mass load to the surface leads to a pure phase shift that is proportional to the mass density, whereas a fluid loading leads to a phase shift companied by an increased damping of the acoustic wave, amplitude attenuation (Perpeet *et al.,* 2006). By this, it enables the analyst to separately interpret the mass loadings by changes of φ and viscoelastic effect alterations by changes of A. Concentration of the analyte was calculated from the measured phase shift using the sensor sensitivity of 515 °cm² μg^{-1}. The measurements were performed at room temperature at a continuous buffer flow of 40 µL/min.

6.3.4 Preparation of the chip surface

The measurement is based on the mass-related phase shift (φ) as a function of mobile Hp diluted in PBS running buffer, which binds to antibodies immobilized on the surface of a sam®5 chip with a carboxymethyl dextran (CMD) surface. The chip contains five sensor elements (Perpeet *et al.,* 2006). At each injection, phase shifts at defined time points are taken and compared. Antibodies were (1) directly immobilized via carbodiimide chemistry

(Schlensog *et al.,* 2004), or (2) indirectly bound via their Fc region to a recombinant protein A/G surface. Rabbit antibody solutions enriched with highly selective antibody against porcine Hp, or containing crudely mixed antibodies (reference) were applied to the sensor elements at 1:500 dilution. After preparation, the chip was placed into the sam®5 biosensor at 40µL/min in phosphate buffered saline (PBS) pH7.4. Hp samples were diluted in running buffer and injected into the buffer stream. Between injections, the antibody surface was regenerated to baseline level using 10mM Glycine pH2.2 (surface 1), or with 10mM Acetate buffer pH4.5 (surface 2). The complete antibody layer was stripped off the protein A/G surface at pH2, and fresh antibodies were applied for another set of experiments. By using an identical surface, the Hp responses could be compared directly. The antibody surface used was relatively stable for several days of continuous usage at conditions applied. This was tested by repeated injections at identical conditions.

6.3.5 Performed statistical analysis

To prove the accuracy of the new developed measurement method, coefficients of correlation (R) between concentrations measured by ELISA and detected phase shifts have been calculated by linear regression. All statistical analyses were performed by using the standard analytical software of the sam®5 system. Kinetic data were evaluated with the Origin 8.1 (Origin Lab, Northhampton, MA, USA)- based FitMaster. The FitMaster is a routine developed by SAW instruments to automate the kinetic analyses. Selected consecutive injections were cut out and integrated fits were applied. The resulting overlay plot and the individual fits following an 1:1 interaction of ligand and ligate are displayed in figure 3A. The pseudo-first order kinetic constants (k_{obs}) as determined by the FitMaster were plotted versus calculated haptoglobin concentrations in figure 3B. A linear best fit was applied using the equation shown with k_{on} = association rate constant (on-rate) and k_{off} = dissociation rate constant (off-rate). The average k_{off} [Unit in sec^{-1}] equals the intersection with the y-axis. The slope of the fitted straight line is a measure of the k_{on} rate [Unit in $conc^{-1}$ sec^{-1}]. The dissociation constant (K_D) is calculated with $K_D = k_{off}/ k_{on}$, as described by Gronewold *et al.* (2006). CV and detection limit was compared with the reliable values ascertained by Tecles *et al.* (2007).

6.4 Results and discussions

6.4.1 Reference concentrations of Hp in samples measured by using ELISA

Haptoglobin samples from porcine meat were tested by ELISA as previously described. In table 6.2 the Hp concentrations of 13 samples measured by ELISA and the coefficients of variation are given. For sample 1 (357E), the high CV of 18.5 % indicates an over- or underestimation.

Table 6.2 Haptoglobin concentrations measured by ELISA

Number	Sample ID	Hp [µg/ml]	CV [%]
1*	357E*	39	18.5
2*	359E*	71	10.0
3	371E	75	7.2
4	340E	82	2.3
5	373E	99	0.6
6	367E	103	3.2
7	337E	122	7.6
8*	360E*	131	3.3
9	375E	151	3.9
10	344E	163	1.2
11	362E	167	1.6
12	376E	199	6.3
13*	358E*	213	7.5

Hp = haptoglobin; CV = coefficients of variation; * = samples showed in figure 1

6.4.2 Immobilization of the antibody

Unpurified mixtures of rabbit serum were used in the ELISA experiments and were as well applied to the sensor chips, either from animals immunized with haptoglobin, or from control animals. The coating was performed externally. On recombinant protein A/G surfaces, the IgG fraction of the antibodies bound randomly based on their content in the serum, but directed via their Fc region. The antigen binding sites are presented to the mobile porcine meat juice contents. Antibodies coupled using carbodiimide chemistry were randomly bound to their exterior primary amines.

6.4.3 Proof of principles for the detection of Hp in meat juice by using biosensor technology

After preparation, the chip was placed into the sam®5 biosensor at 40µl/min PBS running buffer on all five sensor elements. The PBS buffer was also used for dilution of the subsequently injected meat juice samples. In the following graph (figure 6.1), the ELISA results are faced with the maximal phase shifts of the injections. Meat juice samples were used in dilution of 1:5,000. The displayed samples were chosen, as they covered the whole range of Hp concentrations presented in the analyzed samples.

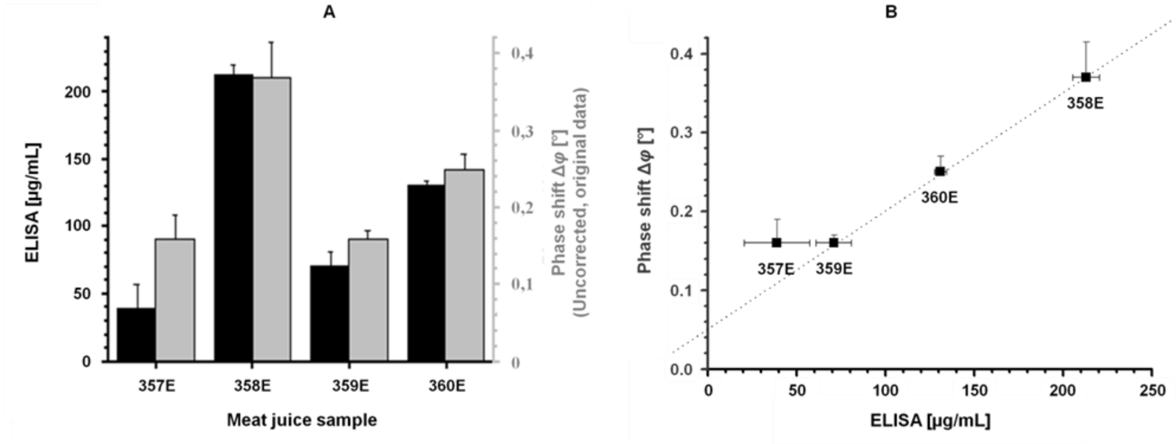

Figure 6.1 Comparison of Hp concentrations and phase shifts of selected meat juice samples (375E, 358E, 359E and 360E); grey barns = phase shifts; black barns = ELISA results

Within the standard deviations, the ELISA results were highly corresponding with the phase shifts with one exception, 357E (figure 6.1 A). This significantly differed by about 50 %. The resulting phase shifts were the uncorrected values. Unpurified antibody-enriched serum has been used and, as the following experiments showed, concentrations required for preparation of the reference from the sensor surfaces differ significantly. It has to be taken into account that some unspecific reactions are included from the serum contents of the immobilized ligand or the meat juice contents of the analytes. The high differences in the ELISA and biosensor test of sample 357E might be based on an especially crude, undefined mixture of this meat juice sample. Figure 6.1 B also shows the phase shifts plotted against the ELISA results, independent of the individual samples.

As has been indicated in the previous graph, the phase shifts were corresponding extremely well with the ELISA results with one exception. This could be attributed to sample 357E. The dotted linear connection indicates a very low base of unspecific binding to the sensor surface at about 0.05 ° (see figure 6.1 B). This can be corrected by a well prepared reference. In all measurements, the native rabbit serum was not suited to be useful as a reference in the current setup. The progress of the linear graph indicates that based on a number of reference injections, the Hp content of meat juice samples could be calculated. The linear regression of the measured phase shifts [°] and the Hp concentrations measured by ELISA for all tested meat juice samples are shown in figure 6.2. The presented phase shifts display the average of three measurements. The standard deviation (SD) was 0.17 and the coefficient of variation (CV) has been calculated with 5.5 %. Therefore, the results are comparable with the values measured by Tecles *et al.* (2007) for the Hp ELISA (CV 5.1% – 5.7 %). Again, sample 357E showed an aberrant behavior from all other samples. It can only be assumed, which test method (if any) failed. (1) The repeat of the sam$^{®}$5 result on completely different surfaces showed identical results, (2) The ELISA result has a very high CV of 18.5 % and (3) the ELISA result of sample 357E is out of the linear range of the ELISA method.

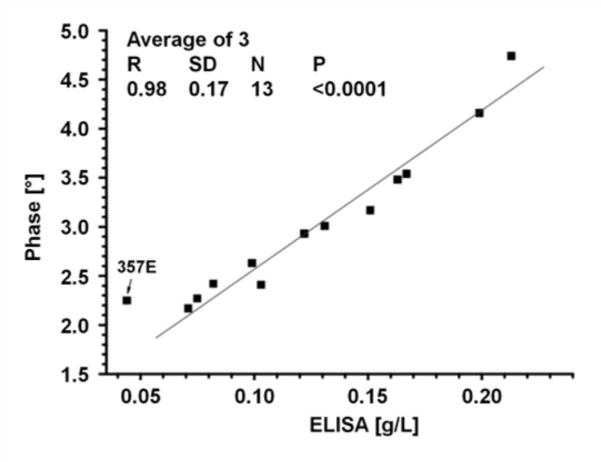

Figure 6.2 Linear regression of ELISA results and phase shifts

The coefficient of correlation (R) is 0.98. This indicates the high quality of the measured phase signals, which correspond highly with the measured ELISA results as well as the linearity and accuracy of both methods.

In the following graph an overlay plot of increasing concentrations of meat juice sample is shown. The concentrations have been calculated as follows:

Example 1:1000 dilution (highest concentration) of 362E (see table 2) with undiluted about 214 mg/L. A 1:1000 dilution would contain 0.214 mg/L = 214 µg/L. Based on an assumed molecular weight of 120000 g/L, a Molar content of $1.43 \cdot 10^{-9}$ M = 1.43 nM was calculated. Accordingly, the Molar content for further dilutions 1:2000, 1:3000, 1:4000, 1:5000, 1:7500 and 1:10000 were calculated.

For kinetic evaluation, the resulting curves were automatically exported into Origin 8.1 (Origin Lab), using the integrated FitMaster (SAW instruments), injections were cut and fits based on a 1:1 binding model were applied. The resulting overlay plot and the individual fits are displayed in figure 6.3.

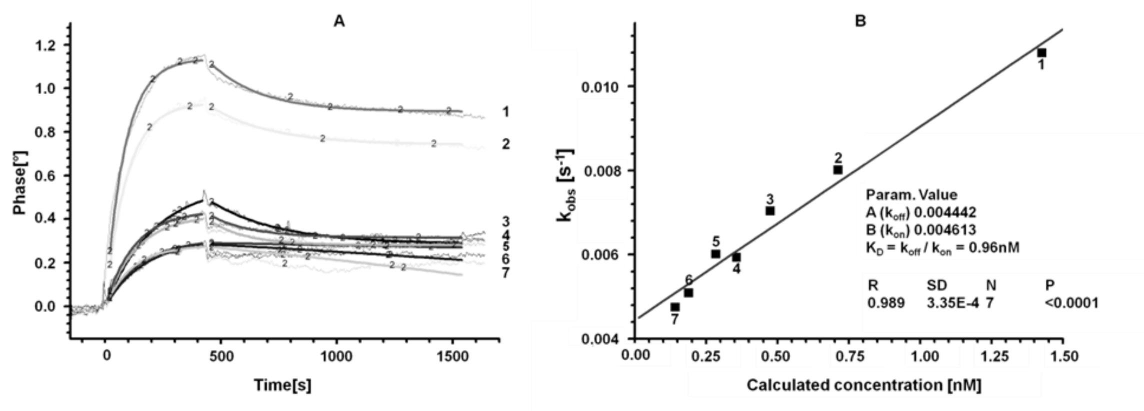

Figure 6.3 Overlay plot of phase shifts (A) and linear regression (B) of sample 362E diluted to calculated Hp concentrations in dilutions of 1:1 000 (1); 1:2 000 (2); 1:3 000 (3); 1:4 000 (4); 1:5 000 (5); 1:7 500 (6) and 1:10 000 (7)

The phase shifts display the dilutions of the sample. The coefficient of correlation (R) 0.989 was calculated by linear regression. Based on the pseudo-first order kinetic constants (k_{obs}) determined by the FitMaster and the calculated concentrations, a $K_D=k_{off}/k_{on}=0.96$ nM was determined, assuming the concentrations of injected meat juice were calculated correctly.

The sam®5 measurement results are linear to size and amount, while the ELISA results show a slightly S-shaped curve response to applied concentrations. In a central interval, which is roughly linear, measurements are performed. At higher and lower concentrations, the results are leaving the quasi-linear regime. This sets the lower end of the limit of detection for the

ELISA method to 20 mg/L. Contrarily - due to the linearity of the sam®5 signal - concentrations beyond the upper and lower limit of the ELISA method can easily be handled. As an example equals the lowest concentration applied in figure 6.3 0.0214 mg/L. Thus, in this experiment, 100- to 1000-fold lower concentrations were applied. This also depends on the affinity of the ligands on the surface. In figure 6.2, ELISA signals face the corresponding sam®5 signals. A slight S-shape becomes visible with a turning point at phase differences of 3.0 ° (sam®5) or 120µg/ml (ELISA). Lower concentrations show a phase signal slightly above the linear regression and higher concentrations slightly below. This can be attributed to the non-linearity of the ELISA method, introducing a small, but significant methodological error into almost all results.

6.5 Conclusions

Porcine Hp has been successfully identified and quantified by using the sam®5 system. The biosensor is easy to clean even with harsh methods and is unaffected by many impurities and debris. The SAW technology can be easily minimized due to specific measurements. Due to the robust setup of the sam®5 system, with a few alterations on-site measurements are envisioned. A regeneration of the chip surface enables fast testing of multiple samples, which lowers costs as well as comparative measurements on identical surfaces. First tests to regenerate the surface by a simple pH shift and usage for several days showed very promising results. The chip containing five sensor elements, allows testing of four markers simultaneously, since the reference could be used on the remaining element for all markers. The calculated coefficients of correlation (R) and variability (CV) indicate that the sam®5 system has the same or better potential to measure Hp from unpurified meat juice within a shorter period of time than ELISA. The nearly complete automated setup of the biosensor improves the comparability of measurements in different laboratories.

The results shown can simply be improved by (1) enhancement of both the sensor and the reference surface. (2) In the experiments, dilutions of complete serum enriched with specific antibodies were used. The use of purified antibody will surely improve accuracy of the measurement and further lower detection limits. (3) Those improved solutions enable the standardization of the experimental setup.

The quasi-linear graph of the ELISA-vs-phase shift plot shows the possibility to standardize the system with injections of a number of reference samples. This would enable the calculation of the original concentration of unknown samples. The linear concentration range has to be defined. Since concentration of Hp is within a relatively small range for samples originated from blood, meat juice, or saliva, the necessary dilution can be standardized and even prepared in a sampling device destined for specific origins of samples.

The next step is a routine application of the technology for measurement of porcine Hp also from blood, serum and saliva of pigs. First tests on those body fluids have successfully been performed. Additionally, the Hp tests can be combined with other markers for animal health and meat safety to improve reliability and trustworthiness in respect to conditional diseases of the pigs and differentiation from stress and inflammation symptoms. This will support animal health management as well as quality management in pork production to prevent consumers from communicable diseases possibly leaping the animal-human barrier. The results of this study hold out the prospect of this detection method to measure Hp from complex matrices on site.

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Disclaimer

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7 General conclusions

7.1 Introduction

Over the last years biosecurity at farm level as well as preventive animal health and hygiene menagement became key factors to improve and obtain meat safety in primary pork production (Food and Agriculture Organization, 2010; European Food Safety Authority, 2011; Wilke *et al.* 2012). Animal health is one of the most important dimensions in this context, as without a desired animal health status regional and international trade is restricted and production at pig farms is reduced (Sofos, 2008). As animal health and food safety are vulnarable to biological and abiological factors within short periods of time, rapid data acquisition and timely communication along the whole chain are important to apply measures to eliminate possible hazards on the following stage of pork production chains (Petersen *et al.*, 2002; Lehmann *et al.*, 2011). In addition to that, the pork chain has a high commitment in reducing the use of antibiotics and transmittance of zoonosis (Coenraadts and Cornellissen, 2011). Quality management systems are well suited to guide and support food production and to limit risks for the health of animals and consumers. Many of the successful quality management concepts need measurable indicators to identify and control risks along food production chains. This challenge has also been identified earlier:

"If you can't measure it, you can't manage it" (Kaplan and Norton, 1996)

In the classical context, the quality concept addresses mainly the product. Quality as a subjective entity comprises both technical and technological characteristics, as well as emotional and ethical aspects. Many definitions of quality can be found in the literature, each trying to address quality from one or more of the forenamed points of view. Most important is that a product should fulfil the demands put forward by the consumers and is attractive enough to be bought under the aspect of sustainability of the value chain. Meat industry faces many significant risks from public criticism of corporate social responsibility (CSR) issues in supply chains. Applications in meat supply chains include animal welfare, biotechnology, environment, fair trade, health and safety as well as labour and human rights. Nowadays, meat production chains in Europe are more and more affected by changing consumer demands. However, approaches to implement CSR principles and thereby improve sustainability are limited for the level of animal husbandry.

7.2 Answers to the research questions

The major aim of this thesis was to design a conceptual approach for a risk based quality management system towards more sustainability in pig production. The performed evaluations of the meat safety and animal health situation in fife European countries showed a high variability within and between different farming systems and showed the need for continuous assessments of farm sustainability aspects. An assessment should be performed on the farm level, as inter-farming system variation was quite high. Thus, the farming system does not present reliable information for a risk assessment. Based on the results of the surveies on farm level, the following hypotheses were tested:

1. It is possible to design a risk based approach towards more sustainability.
2. Parameters with high potential for on-farm risk assessment can be identified.
3. A rapid method for the measurement of these risk indicators can be developed.

The possibility to implement a HACCP based management system on the farm-level aiming for an improvement and assurance of sustainability was presented. Sustainability themes with major effects on the overall sustainability are animal health and welfare as well as meat safety.

Acute phase proteins Pig-MAP and Hp are indicators with high potential to identify risks at an early stage. This potential was proved by literature and an additional experiment with 99 pigs at the experimental farm of the University of Bonn. The results showed a lot of significant correlations between Hp and Pig-MAP and carcass quality traits and performance parameters. The most significant correlations resulted from serum samples taken at an age of 13 weeks.

By the use of a mass related biosensor a rapid detection method for the measurement of porcine Hp was developed. Thus, porcine Hp was identified and quantified by using the sam®5 system from unpurified meat juice. The biosensor is easy to clean even with harsh methods and is unaffected by many impurities and debris. The calculated coefficients of correlation (R) and variability (CV) indicate that the sam®5 system has the same or better potential to measure Hp within a shorter period of time than ELISA. The automated setup of the biosensor improves the comparability of measurements in different laboratories.

7.3 General conclusions

Within chapters two and three different tools based on questionnaires were presented to evaluate and assess the current animal health and meat safety status of farms and partly farming systems. For a clear assessment of the given answeres, regarding the compliance with a desired situation, scoring models were developed. The given scores enable a clear differentiation of desired and un-desired situations. The tools performed reasonably well in terms of objectivity, practical feasibility and applicability to individual farms. Anyway, the limited number of investigated farms per farming system disabled an assessment on the systems scale. The achieved datasets enable an overview of the current dispersions of conformity in different dimensions of animal health and meat safety. Thus, the models are able to show strong and weak points regarding the evaluated dimensions of sustainability per farm. Audits on side were proved to be very successful, to evaluate the real, current situation on the farms. High dispersion of conformity to desired levels of farms within systems in this survey does not permit definite conclusions on system level. Thus, the sustainability assessment should be performed on the single farm scale.

A combination of the presented tools and the risk based approach towards more sustainability, presented in chapter four, will enable a more precise assessment of on farm sustainability. A combination of on side audits with measurements during the production process is highly recommended. Anyway, the developed approach shows that the sustainability themes animal health, welfare and meat safety have major effects on the overall sustainability of farms and acute phase proteins are indicators with high potential to identify risks at an early stage. Practical recommendations for an implementation to practice were given. Thus, a complete management concept towards more sustainability was developed. However, a combination with data from systems aiming at improved piglet health like the German TiGA (TierGesundheitsAgentur) or the Danish SPF-system (Specific Pathogen Free), information given by the responsible veterinarian or systems monitoring specific pathogens or their antibodies, could complete and improve the assessment of risks along the whole added value chain.

The study presented in chapter five was performed to solve some potential problems for an implementation of the developed concept to practice. Again, the potential of acute phase proteins as indicators was presented by a lot of significant correlations between Hp and Pig-MAP and carcass quality traits and performance parameters. In terms of increased APP concentrations the daily weight gain, slaughter weight, dressing yield, weights of valuable cuts, lean meat content autoFOM index, water content as well as thawing and cocking loss decreased. Feed conversion ratio, subcutaneous and intramuscular fat as well as the risk of organ findings increased with the concentration of the investigated APPs. Anyway, the time point of measurement and the measured APP were important factors for the significance of the correlation. The most significant correlations resulted from serum samples taken at an age of 13 weeks. Pig-Map concentrations presented more significant correlations to carcass quality and performance traits than Hp concentrations, but the Hp concentrations proved the results and showed correlations to some additional parameters. Hp values present higher odds ratios regarding the occurrence of abnormalities of organs and indicated a high potential for the risk assessment during meat inspection. Thus, the measurement of both indicators is favourable. Health and welfare of the pigs measured by APPs were proved to have a measurable impact on carcass quality and thereby added value. A combination of these unspecific indicators with specific antibody or pathogen monitoring measures will enable faster and more effective reactions to increased risks.

To allow such complex measurements of different parameters and limit the costs to an acceptable level, modern measurement technology is needed. Within chapter six a mass based biosensor was used to measure Hp from unpurified meat juice. This investigations should be understood as a prove of principles. The measurement of this exemplary parameter could be expanded to other additional parameters. Anyway, the performed study was successful, as the Hp concentrations measured via biosensor technology showed very strong correlations with the results obtained from ELISA analysis.

All in all the performed work showed one possibility to measure, control and manage risks to overall farm sustainability. The system is prepared for an expansion to other sustainability themes like environmental impact or economy. Anyway, a implication of the designed approach will have a positive impact to all sustainability themes and can be used for an continuous improvement process.

7.4 Managerial implications

A combination of both improved biosecurity measures (presented in chapters two and three) and monitoring programs to control the effectiveness of taken actions (chapter four) would be advantageous from the point of quality and animal health management (Petersen *et al.*, 2005) and thereby important for the improvement of farm sustainability. However, the amount of data and the conversation of informations along the chains show the necessity of suporting organisations. Brinkmann and coauthors (2011) defined a chain coordination model to encourage quality management strategies of pork supply chains. The model highlights the importance of service organizations (network coordinators) supporting all enterprises along the production chain sharing their data (collection, analyses, communication) and by this enables a joint decision making. These network coordinators can support farmers during implementation and performance of the developed HACCP based approach. Communication with laboratories and the analysis of the results might be supported but also the verification procedures and documentation (Schütz, 2010). Besides these supporting organizations, the use of incentive mechanisms for food safety and animal health control is recommended (van Wagenberg, 2010). This might also apply for the improvement of farm sustainability. Public Private Partnership (PPP) organizations such as GIQS (Grenzüberschreitende Integrierte QualitätsSicherung GIQS e.V.) have a high potential to be drivers for an implementation and persistence of such innovations in food production chains.

7.5 Technical implications

In herd health and production management programmes it is common use to make inventories of the herd performance status (Petersen *et al.*, 2002; Petersen *et al.*, 2005). Such monitoring measures are important components of quality risk management programmes following the rules of a HACCP concept as shown by this study. Monitoring is an act of conducting a planned sequence of observations or measurements of certain control parameters to assess whether a certain point in the production process is under control and in conformity to market and society demands or not. It is highly indicated to conduct inventories (i.e. monitoring) regarding the prevailing risk conditions on farm for animals, their surroundings, the management and the farm records, too (Berns, 1996; Mack, 2007). Such risk conditions can be found by the application of strengths- and weaknesses assessment on the farm as presented in this study. The use of sensors to identify risks at an early stage of production is summarized under the term “prescision livestock farming”. Prescision livestock farming has a big potential to improve farming systems by efficient utilisation of nutrients, early warning of ill health, reduction in pollutant emissions and provision of useful information to skilled stockmen (Wathes *et al.*, 2008). Many steps towards the implementation of the described HACCP based approach have already been taken. Modern sensor technology to measure indicators for a risk assessment was proved to be possible also for farm sustainability evaluation. Anyway, the technique must be continuously improved and adapted to changing situations. Biosensor or lab-on-a-chip developments can be used to keep the costs for measurements on an acceptable level. In addition to that, a collaboration of stakeholders from several steps of pork production chains like piglet production, rearing, fattening and slaughter could also lower the costs and make the systems more effective.

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8 List of tables

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