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Effects of Environmental Stress Induced by Modified Stable Routine or Feed-Borne Mycotoxin Exposure on Feed Intake Patterns of Pigs under Practical Housing Conditions

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Zusammenfassung

In diesem Promotionsprojekt wurde untersucht, ob die Erfassung tierindividueller Futteraufnahmedaten von Absetzferkeln und Mastschweinen eine sinnvolle Ergänzung zu den in routinemäßig erfassten Fütterungsdaten darstellt. In der allgemeinen Praxis werden Tierernährungsdaten für ganze Tiergruppen, d. h. für alle Tiere, die von der gleichen Futterstation gefüttert werden, erfasst. Vor dem Hintergrund aktueller Herausforderungen an die Agrarwissenschaften und Tierernährung, wie Ressourceneffizienz und vor allem Tierwohl, könnten zusätzliche hochpräzise einzeltierbezogene Messungen der Futteraufnahme in praktischer Haltungsumgebung eine nötige Innovation sein.

Zur objektiven Beurteilung von Tierwohl ist eine Beobachtung des Tierverhaltens nötig, da nur diese direkt Aufschluss über das Befinden des Tieres gibt. Die gängige Praxis, die Haltungsumwelt als Indikator zu verwenden, ist daher hauptsächlich ein Hilfsparameter, da die Erfassung des Verhaltens der Tiere zeitaufwendig ist und häufig von der Messperson abhängt. Aus diesem Grunde könnte die Ergänzung der bereits in der alltäglichen Routine erfassten Futterdaten um eine Einzeltier-Komponente sinnvoll sein. Dadurch könnten in der Praxis das Fressverhalten verfolgt und potentielle Störungen frühzeitig erkannt werden. Auch eine Anpassung der Fütterung an den Bedarf des Einzeltieres wäre denkbar.

In dieser Doktorarbeit werden drei Versuche behandelt. In allen Versuchen wurden die Tiere mit Abrufstationen gefüttert, was die Erfassung sehr detaillierter Fressdaten ermöglichte. Diese setzten sich aus der täglichen Futteraufnahme pro Tier, der Anzahl der täglichen Besuche an der Station, der verzehrten Futtermenge pro Besuch, und der höchsten verzehrten Futtermenge in einem Besuch pro Tier und Tag zusammen. Zusätzlich wurden die klassischen zootechnischen Leistungsparameter Gewichtsentwicklung, tägliche Zunahmen und Futterverwertung erfasst. Alle Mastschweine wurden nach den Vorgaben der Leistungsprüfung geschlachtet und untersucht. Zur statistischen Auswertung wurde ein hierarchisches Varianzanalysenmodell etabliert, anhand dessen die Hauptstreuungsquellen festgestellt wurden. Dadurch konnten die wichtigsten Parameter identifiziert werden, die den beobachteten Zusammenhängen zu Grunde lagen.

In einem publizierten ersten Experiment beim Mastschwein wurde in vier Behandlungsgruppen der Einfluss von kurzfristigen Störungen der Stallroutine auf zootechnische Leistungen und Futteraufnahmeparameter untersucht. Die Behandlungen setzten sich aus einer Kontrollgruppe ohne Störung, einer Gruppe mit Problemen der Fütterung (24-stündiger Fütterungsausfall, bzw. restriktive Fütterung für 48 Stunden), einer Gruppe mit kurzfristigen Änderungen der Futterzusammensetzung (nur Getreide in der Vormast und Vormastfutter in der Mittelmastmast für jeweils 48 Stunden) und einer Gruppe mit (sozialem) Stress (Reduktion der verfügbaren Wassermenge an den Tränken und Tierwechsel zwischen den Buchten) zusammen. Die Störungen wurden in der Vor- und Mittelmast eingebracht und waren so konzipiert, dass kein Effekt auf die zootechnischen Leistungen zu erwarten war, was auch eintrat. Die Abänderungen beeinflussten die Futteraufnahmeparameter nicht. Die Fressverhaltensmuster waren nur vom Einzeltier beeinflusst, was auch an der starken Tag-zu-Tag-Variation der Parameter lag. Ein Effekt der Tiergruppe konnte nicht festgestellt werden. Da es sich bei Schweinen aber um hochsoziale Tiere handelt wurde schlussgefolgert, dass sich die sehr individuellen Verhaltensmuster nur im Kontext der Gruppe, d. h. durch Faktoren wie der Rangordnung, etablieren konnten. Ein Einfluss der sozialen Hierarchie auf das Futteraufnahmeverhalten ist in der Literatur beschrieben konnte in dieser Arbeit aber nicht untersucht werden.

In einer zweiten publizierten Studie wurden Mastschweine dauerhaft hohen Mykotoxingehalten, in diesem Fall Deoxynivalenol (DON), ausgesetzt. Einer Kontrollgruppe mit niedrigen DON-Gehalten im Futter wurden drei Gruppen mit hochkontaminiertem Futter (fünffache Überschreitung des Richtwertes der EU-Kommission) gegenübergestellt. Um Praxisnähe herzustellen, wurden in zwei dieser Gruppen unterschiedliche Mykotoxinbinderprodukte zugesetzt. DON verringerte die Futteraufnahme erwartungsgemäß um 30 %, was alle zootechnischen Daten negativ beeinflusste und eine um ca. 30 Tage längere Mastdauer in allen drei DON Gruppen zur Folge hatte. Die Mykotoxinbinder zeigten teils zusätzliche adverse Effekte. Alle anderen Parameter des Futteraufnahmeverhaltens waren jedoch nicht durch DON beeinflusst. Wieder war hier der Hauptfaktor das Einzeltier, häufig aber auch die Tiergruppe. Das Verhalten der DON-ausgesetzten Schweine wurde außerdem erratischer, was sich in einer signifikanten Erhöhung der Variationskoeffizienten zeigte. Wieder wurde schlussgefolgert, dass das Verhaltensmuster von Schweinen hochgradig individuell ist

und vom sozialen Status des Tieres innerhalb der Gruppe abhängt. Dieses „Gruppenverhalten“ schien vor dem Hintergrund der andauernden Konfrontation mit DON verstärkt zu werden. Das Mykotoxin hatte hingegen keinen Einfluss auf das Verhaltensmuster.

Zur Verifizierung der Ergebnisse wurde zusätzlich eine Ferkelstudie durchgeführt. Hier wurden ebenfalls dem Lebensalter der Ferkel angepasste kurzfristige Störungen der Stallroutine simuliert (Fütterungsausfall für 12 h und Reduktion der Wassermenge). Zusätzlich wurden zwei Vaterlinien getestet, wodurch eine Piétrain sowie eine Duroc Kontrollgruppe einer jeweiligen Behandlungsgruppe gegenübergestellt wurde. Die Zootechnik war nicht beeinflusst. Auch beim Ferkel war der Haupteffekt auf das Futteraufnahmemuster wiederum das Einzeltier. In der Phase nach dem Absetzen zeichneten sich aber auch wieder Buchteneffekte ab. Vor allem in dieser Phase war bei den Ferkeln die Variation der Futteraufnahmeparameter bedeutend höher als bei den Mastschweinen der ersten Studie. Die Variationskoeffizienten lagen hier in etwa auf dem Niveau der hohen DON-Belastungen ausgesetzten Mastschweine der zweiten Studie. Nach dem Absetzen scheinen die Tiere also eine Anpassungsperiode zu benötigen, bis sich ein konsistentes Verhaltensmuster etablierte.

Aufgrund der hohen Streuungen der Verhaltensparameter und der sehr tierindividuellen Natur des Futteraufnahmeverhaltens muss somit geschlussfolgert werden, dass eine Erfassung derartiger Daten zum aktuellen Zeitpunkt keinen bedeutenden Mehrwert für die praktische Tierernährung bieten. Ethologische Methoden zur Erkennung von kurzfristigen Abweichungen im Zeitverlauf lägen vor, konnten aber aufgrund fehlender Datenpunkte nicht angewandt werden. Zukünftige Studien sollten zum einen die Wiederholungszahl erhöhen, um potentielle gleichsinnige Reaktionsmuster der Tiere oder auch Tiergruppen abzuleiten. Zum anderen sollte eine Implementierung ethologischer zeitbasierter Methoden in die Futteraufnahmemessung untersucht werden.

Summary

The aim of this doctoral project was to examine whether an additional measurement of animal-individual feeding data can deliver added-value to the nutritional measurement routine. Feed intake data are routinely generated for animal groups i.e., all animals feeding from the same feeder. Recent challenges for agriculture and especially animal nutrition, namely resource efficiency and especially animal welfare, require innovations in pig feeding. The implementation of high precision animal individual feed intake data could represent such an innovation.

Objective evaluation of animal welfare requires the examination of behaviour as only the behaviour provides direct insight into an animal's wellbeing. However, behaviour observation is time consuming and sensitive for cognitive bias. Therefore, it is common practice to evaluate animal welfare via examination of the environment as an auxiliary parameter. Extending the routinely assessed feeding data with measurements of the individual animal could hence provide an added benefit. Hereby the feeding behaviour could be observed and potential disruptions in the daily routine could be identified at an early stage in practical conditions. In addition, an adjustment of the feed according to the requirement of the individual animal could be a further application of such measurements.

This doctoral thesis covers three experiments in swine nutrition. All pigs were fed with automatic single space feeders, recording feed intake data in utmost detail. The measurements comprised daily feed intake, number of daily visits to the feeder, feed intake per visit, and highest amount of feed consumed in one visit per animal and day. Additionally, the classic zootechnical performance parameters, weight development, daily weight gain, and feed conversion ratio were measured. All finishing pigs were slaughtered and analysed according to the German guidelines of progeny testing. A hierarchical ANOVA model was established to identify all important sources of deviations. This allowed for the determination of the main factors influencing the observed relations.

The first published study in fattening pigs consisted of four different treatment groups. During starter and grower phases, the animals were confronted with several short-term disturbances of the stable routine. Effects on zootechnical performance and feeding patterns were assessed. The treatments comprised an undisturbed control group, a starving group (24 h feed deprivation, restrictive feeding for 48 h), a feed change group

(only cereals during starter phase and starter feed during grower phase for 48 h each), and a social stress group (reduced water efflux at drinkers and animal exchange between pens). These disturbances were simulated during starter and grower phases to allow recovery, as they were designed to have no influence on zootechnical performance. As expected, no effect on performance was measured. The treatments did not affect the behavioural parameters either. Feed intake measures were only influenced by the individual animal, partly due to the high day-to-day variation of the behavioural parameters. The group, i.e., the pen, did not influence the behaviour. As pigs are highly social animals, we concluded that the individual patterns had to develop in the social context of the group (e.g., the pig's rank in the hierarchy). Influence of the social status of a pig is described in literature but was not part of the examination in this project.

Fattening pigs were permanently exposed to mycotoxins, namely Deoxynivalenol (DON) during the second published study. A control group with minimum DON contents in feed was compared to three groups with DON-levels exceeding the EFSA guidance values fivefold. To maintain practical relevance, two different mycotoxin binder products were tested in two of these DON groups. As expected, DON exposure reduced daily feed intake by 30% depressing zootechnical performance and prolonging the fattening period by about 30 days. The mycotoxin binders showed partially additional adverse effects. However, the treatments did not influence any further feed intake behavioural parameters. Again, the main factor was the individual animal as well as the group (pen) in several cases. When exposed to DON the animals showed more erratic behaviour as the Coefficients of Variation were significantly increased. Pigs developed highly individual feeding patterns depending on the social status within the group. This "group behaviour" seemed to gain importance under extended DON exposure. However, the mycotoxin itself did not influence any behavioural trait.

To verify these findings, a piglet study was conducted. Piglets originating from two different boar lines were confronted with short-term disturbances adjusted to the younger age (feed deprivation for 12 h and reduction in water efflux at the drinkers). A control group for each boar line (Piétrain or Duroc) was tested against a respective treatment group. As anticipated, the treatments did not affect zootechnical performance. Piglets also developed highly individual feeding patterns. During the phase directly after weaning, once again pen effects were recognisable. During this same phase, piglets showed elevated CVs, similar to the ones found in fatteners exposed to DON and higher

than the values of the pigs of the first study. Piglets seemed to require additional time to establish consistent feeding patterns after weaning.

In total, due to the high variation in feeding behavioural parameters and the individual nature of feed intake behaviour, measurement of the animal’s feeding data does not offer added value to current feeding practice at this point. Ethological methods to identify short-term aberrations of the feeding routine are available but could not be applied due to missing data points. Future studies should increase the number of replicates in both animals as well as groups in order to identify possible concordant reaction patterns. Also, the possibility to implement time-dependent ethological methodology into practical feeding systems should be examined.

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List of Abbreviations

ANOVA	Analysis of Variance
cm	centimetre
CV	Coefficient of Variation
d	day
DFI	Daily Feed Intake
DM	Dry Matter
DON	Deoxynivalenol
DWG	Daily Weight Gain
e. g.	for example
FCR	Feed Conversion Ratio
g	gram
h	hour
i. e.	that is
kg	kilogram
L	litre
µg	microgram
m	metre
min	minute
ME	Metabolisable Energy
MJ	Megajoule

List of Publications arising from this thesis

Articles in peer reviewed journals

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Loibl, P., Windisch, W., Preißinger, W.: Examination of high-resolution feed intake data of growing-finishing pigs confronted with high deoxynivalenol contents present in their feed. Czech Journal of Animal Science, 2020 65:411-423.

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Loibl, P.: The influence of stress factors typical to the specific environment of rearing piglets and finisher pigs on their feed intake behaviour and other welfare associated measure. In: Luksch, C. (edt.): Proceedings of the1st HEZagrar PhD Symposium, April 21st, 2015, p 71-72.

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Loibl, P. Spiekers, H., Preißinger, W., Windisch, W.: Feed intake patterns of fattening pigs exposed to short term disturbances in stable routine. In: Society of Nutrition Physiology (edt.): Proceedings of the Society of Nutrition Physiology (27). March 13th-15th 2018 p 173

Chapter I. General Introduction and General Overview of Methodology and Materials

1. *Introduction*

The progress of digital technology and its influence on all parts of life was tremendous in past decades. Thanks to the dramatic advances in computational technology, high performing computers became broadly available and are used in areas nobody thought possible (Campbell-Kelly et al. 2018). This led to a present focus on big data assessment, evaluation, and management in combination with the development of artificial intelligence and machine learning in nearly all industries and areas of life (Hwang 2018).

Agriculture is continuously implementing these smart technologies too. Applications in all areas are explored and will become a standard in developed countries soon, since highly qualified workers are scarce (Eurostat 2019) and need to be subsidised. Also, big data management will find many implementations in food production to optimise efficiency or increase transparency. This is necessary since the world's population is growing rapidly and with it the demand for food as well as food safety (Gerland et al. 2014; Garcia-Llorente et al. 2018).

In agriculture, one of the pioneers in implementing modern technology was crop production. Many innovations have taken place, from implementation of satellite data for autonomously working tractors or detailed assessment of the nutrient supply of crops for aimed fertilisation to using robots for automatic extinction of weeds without herbicides (Gerland et al. 2014; Ivanov et al. 2015; Finger et al. 2019). During this development, the dictum of "precision farming" was established and could roughly be summarised as precisely steering the input for maximum output through the combination of recent knowledge and technology.

"Precision livestock farming" is on the rise as well. Probably the most obvious technological advance in livestock husbandry was the establishment of milking robots. These systems increased their market share in Europe from 4% to 10% from 2006 to 2011 and were expected to have a share of 19% in 2016 (Lassen 2011). Such systems optimise the workload of the farmer while increasing animal welfare. Additionally, they generate an enormous amount of data with possible implications in areas like animal health, e.g. by measuring somatic cell-counts in real-time (Nørstebø et al. 2019).

Other techniques allow the near-real-time observation of physiology, e.g. when using rumen boli to observe animal health (Dye et al. 2007; Timsit et al. 2011).

The advances in feeding technology allowed the implementation of phase feeding systems to maximise the feed efficiency of animals (Schuster et al. 2020). German pig growers continuously increased the number of feeding phases from one universal feed to at least two or more to supply the animals more precisely with the nutrients needed in their respective growing phase (Schneider 2020). Other parts of the world implemented entirely flexible systems with near-daily adjustments to the growth status of the animals (Fernández et al. 2019). These advances in nutrition mainly transferred knowledge on nutrient requirements into praxis. The feeding technology evolved predominantly towards more automation, however, accurate assessment of the animal's status to disperse feed directly according to the growth state is still under development (Pomar and Remus 2019).

Many consumers as well as other stakeholders of the food chain start to demand utmost transparency of production. Here, modern technology is key to allow the traceability of the product back to its origin, e.g. the stable where the animal lived (Kassahun et al. 2014).

On the other hand, the demands of society and consumers regarding food switches from pure delivery of nutrients towards functional food, production based on ethical and ecological standards, and increased animal welfare (EMA 2017; Garcia-Llorente et al. 2018). At least in the percipience of the stakeholders, a lot of parameters regarding animal welfare are causally linked to the respective environment, like space allowance or window area (Schukat et al. 2020; Von Plettenberg and Heise 2020). From an objective point of view, however, animal welfare is not purely influenced by the stable environment. The most evident parameter to truly evaluate animal welfare remains behaviour as well as health, namely factors to be measured on each animal individually (Broom 1988; Broom 1991; Bracke 2001; Veissier and Forkman 2008).

The measurement and evaluation of both animal welfare and animal wellbeing itself can definitely be supported by modern technology. However, the animals' nature complicates the implementation of "precision livestock farming" techniques compared to e. g. wheat plants in a wheat field. The ability of animals to move around interferes with measurement of any parameter. Also, recording of physiological or other internal parameters often would require manipulation of the animal itself (Patel et al. 2016) by for instance, implantation of bio-sensors. This seems counter intuitive under the context of increasing animal welfare and economical value. Therefore, the implementation of smart technology in livestock husbandry is not as progressed as it is in plant production.

Of course, there were innovations such as the above-mentioned milking robots to assess animal health while milking. Many other areas in animal production lack such systems that are causally linked to an individual animal. However, novel technology is being developed, e.g. the observation of the locomotion of an individual pig or discerning the sound pigs make (Kashiha et al. 2014; Vandermeulen et al. 2015). These are often still too expensive and their added-value too limited to find reminiscence on the practical farms. Therefore, the observation and evaluation of animal welfare, especially from independent monitoring entities, mostly remains pure examination of the stable environment at a specific moment (Hinrichs 2016). Real-time or rather long-term aspects of animal welfare are still mostly examined in scientific approaches.

2. Ethological methods for behaviour examination

Behaviour is the best indicator of animal welfare, therefore a short overview of methods for behaviour observation will be given in the following. As this doctoral project examined feeding data of pigs, the focus will primarily be on feeding and swine.

There are several methods described in literature to assess, examine and evaluate animal behaviour (Commins 2018). The examination of the ethogram, i.e. the entity of all behavioural complexes like locomotion, defaecation, reproduction, etc., mostly comprises scanning the exhibited behaviour of all animals within a group at defined consecutive time points or observation of an individual animal (focal animal) at random time points (Signoret et al. 1975; Stolba and Wood-Gush 1989; Commins 2018). These behaviour complexes can be assessed simply by visual judgement and recording (Stolba and Wood-Gush 1989) up to automated recording e.g. by locating animals within a pen (Kashiha et al. 2014). Recently, using these automatic video methods, not only resting behaviour could be distinguished from locomotion but also more complex behaviour like tail-biting became recognisable (Liu et al. 2020).

Many behavioural complexes naturally follow rhythms. In most non-domesticated animals, reproduction-related behaviour follows a circa-annual rhythm. Red deer for instance seek to calve in early summer and therefore all reproduction-related behaviours follow this time point, or “Zeitgeber” (Hamilton and Blaxter 1980).

In wild boar the seasonal effects rather depend on the habitat, namely the climate, than on genetic predisposition (Macchi et al. 2010). During domestication, the relevance of such seasonal zeitgebers was reduced. The controlled environment in the production systems further reduces seasonal dependency. This can be observed best looking at the reproduction cycle of sows where the only zeitgeber is the duration of lactation and the weaning to oestrus interval. Due to lactation-anoestrus this time period is physiologically predefined and takes around five days in practice (Panisson et al. 2019). In practice hormones are used only to reduce the variation of this interval for optimisation of work-loads (Kraeling and Webel 2015). Hence, this has no influence on the zeitgeber itself. Looking at feed intake behaviour of wild boar living in the mid-latitudes shows a clear seasonal rhythm as feed is scarce during winter (Ballari and Barrios-García 2014).

Thanks to advances in sensory and video technology, feed intake of free roaming wild animals as well as group housed domesticated animals can be recorded with reasonable effort. Purely measuring the feeding times of an animal can be achieved by videotaping, motion sensors recognising the movement of the jaw, or recognising the animals close to a feeder (Berger et al. 2003; Maselyne et al. 2014; Matthews et al. 2017). Measuring the amount of feed intake of wild animals is difficult and often requires capturing and sacrificing of animals (Ballari and Barrios-García 2014). In livestock, however, measuring the daily feed intake is far easier, due to the controllable environment. In pigs at least, the feed intake of a group of animals fed by the same feeder is readily accessible, and routinely practiced for decades.

Combining the measurement of daily feed intake (DFI) with any kind of assessment of the individual animal's feeding behaviour, like duration or frequency of feeding acts has been studied in the past to some extent to better understand the behavioural complexity of the animal (Nielsen et al. 1995b; Morgan et al. 1998; Musial et al. 1999; Pichler et al. 2020). To do so, plenty of options are available even under practical conditions in pig stables. In most studies, the animals are videotaped to correlate the standard feed intake parameters with behavioural measurements (Matthews et al. 2017). In the past years, applications using RFID (Radio Frequency Identification) ear tags to identify animals feeding at multi-space troughs have been developed (Maselyne et al. 2014; Maselyne et al. 2015). Although these methods allow the examination of time and duration of feeding actions in utmost detail, the feed intake is still measured as the average over the whole group fed by one feeder. The use of automatic single space feeders optimises this situation in a way that the animals can still live out most of their social behaviours within the group while the individual feed intake behaviour is measured in utmost detail with high precision and next to no risk of misreading (Nielsen and Lawrence 1993; Labroue et al. 1994; Nielsen et al. 1995a; Kallabis and Kaufmann 2012; Pichler et al. 2020).

Behavioural examinations need to focus on the individual animal as each animal behaves somewhat differently. This, however, makes it necessary to take the hierarchy into account. All social animals such as pigs exhibit their behaviours individually in dependency of rank and status within its group, that combined results a "group behaviour" (Gonyou 2001). Therefore, examination or at the least inclusion of the individual animal's rank should be an important aspect in ethological studies as the place in the hierarchy influences the behavioural patterns of the animal (Nielsen 1999).

In examinations of feeding behaviour, the methods have been adjusted over time. The simplest approach was to measure how often an individual animal or a group feeds within a defined period. This was practiced for instance by Nielsen et al. (1995b). Such approaches can struggle with the time-dependency of any behaviour. However, time can be relevant as swine generally feed during the day with focus on evening's twilight hours (Ingram and Dauncey 1985). This is a crucial part of the feeding behaviour of pigs and should be included into the assessment of their behaviour.

Novel methods make better use of the refined and highly detailed data to deliver more sufficient statistical performance as well as predictability (Howie et al. 2009). Objective condensation of data can be useful especially when examining time-dependency. To do so, the concept of a "meal" was established (Slater and Lester 1982; Tolkamp et al. 1998; Tolkamp and Kyriazakis 1999; Yeates et al. 2001; Yeates et al. 2003; Howie et al. 2009). A meal consists of several feeding actions that can be separated by pauses of a defined length. These pauses are identified by so-called meal criteria that can be calculated based on fitting curves, like Gaussian curves, to the histograms of the pause-length (Tolkamp et al. 1998; Tolkamp and Kyriazakis 1999; Yeates et al. 2001). In this way, the data is condensed in a reproducible and objective manner maintaining transferability to any kind of similar study. However, there still is no consensus on the best method to calculate meals (Nielsen 1999).

Strubbe and Woods (2004) postulated that the timing of meals is generally determined by the following three interacting factors: firstly, the genetically programmed circadian feeding pattern; secondly, the light cycle during time, and thirdly the size of the preceding meal. Considering these factors, feeding behaviour develops a rhythmicity that can be identified by testing the time series of the data for rhythms. This is as well established methodology within time series analysis and makes use of the fact that a time series develops a wave-like pattern which can then be analysed on the basis of spectral-physical methods (Siegel 1978). Theory goes that as long as an animal or a group of animals is not disturbed, it soon reaches a normal behaviour keeping up constant harmonic rhythms within the behavioural complex. These rhythms are linked to the circadian rhythm by resulting in an even quotient (i.e., feeding could oscillate at 12 h and would result in 12 h/24 h = 2). These harmonic rhythms can then be identified calculating the "degree of functional coupling" (Scheibe et al. 1999). Here, a quotient between harmonic and all significant rhythms connected to the most important circadian period is calculated. This can then be used to evaluate the quality of the behaviour of animals. Berger et al. (2003) summarised in a review that this method proved that feeding behaviour of several different species changed drastically after disturbance.

These state-of-the-art ethological methods to examine feeding behaviour obviously need datasets with utmost detail regarding feeding data. Ideally, next to frequency and feed intake, all time-related parameters (point in time as well as duration of feed intake) are assessed. Condensed data could be more feasible to use for time-dependent analyses.

3. *Pig behaviour – normal behaviour and influence of husbandry*

Generally, the observation and examination of all aspects of the behaviour of pigs housed in practical condition are still advancing. With increasing political and consumer focus on animal welfare, the animal's possibility to exert its "normal behaviour", i.e. behaviour that it would show under nature-like conditions without restrictions of any kind (Gonyou 2001), becomes more and more important. However, the focus of welfare evaluation systems is primarily on examination of the environment (Veissier and Forkman 2008). The following will shortly summarise some behavioural possibilities in nature and possible implications on swine husbandry systems.

Firstly, wild boar develop groups of up to four sows with their youngest offspring depending on the availability of resources with a clear and persistent hierarchy amongst animals of the respective class (sows and juveniles) (Stolba and Wood-Gush 1984). In practical husbandry, pig groups are designed based on age and available space. This can lead to bigger groups, however, after only a few days a hierarchy is established as well (Ewbank 1976). The hierarchical structure of a pig group makes sufficient space necessary to allow a low-rank pig to evade a high-rank pen-mate (Matthews et al. 2017). Being highly social animals, pigs will synchronise a lot of their behaviours including feeding in the daily routine both, in nature, as well as in the stables (Gonyou 2001). Several studies show that the synchronisation can even facilitate inclusion of new animals into groups (Hsia and Wood-Gush 1983; Gonyou et al. 1999). This is one of the reasons why individually housed sows need to have at least visual contact to other sows (TierSchNutztV 2006).

When kept under near-nature like conditions or at least with sufficient space, a group of pigs separates the given space in different functional areas, e. g. for defaecating, feeding, drinking, etc., similar to what they would do in nature (Nannoni et al. 2020). A lack of space, therefore, can be a stress-factor since animals are inhibited to exert innate behavioural complexes such as defaecation behaviour.

Secondly, regarding feeding, evolutionarily wild boar are omnivores that spend most of the wake time searching for food that consists of roots, herbs, as well as insects, worms or small mammals (Ballari and Barrios-García 2014). In contrast, pigs under husbandry conditions usually are fed purely vegetarian feedstuff with high nutrient density, often at defined feeding times. This results in far shorter feeding duration than evolutionarily defined. In wild boar, rooting and grazing can represent up to 52% of the observable behaviour types (Stolba and Wood-Gush 1989), whereas feeding takes only about 70 min to 100 min per day in practically housed fattening pigs (Nielsen et al. 1995a; Pichler et al. 2020). Additionally, pigs show jealousy about food. In a husbandry situation, this could be used to improve performance. Preißinger et al. (2016) comparing different trough lengths (1.25 m, 1.50 m and 4.25 m) with different stocking densities (12 or 15 animals per pen, resulting in an animal-to-feeder ratio of 3.2 and 4.0, 2.6 and 3.3 and 0.9 and 1.1, respectively) found a reduction in FCR ($p < 0.10$) in the pens with longer troughs. Increasing the number of animals numerically increased the DWG by 4% – 7% indicating an effect of jealousy. However, other studies indicated that the positive effect of increased stocking density and thereby reduced feeder-space allowance led to more aggressive contacts between the animals (Preißinger et al. 2015). Comparing trough length, these authors also regularly observed higher feed wastage when using shorter troughs. They hypothesised that more feed was wasted due to aggressive fighting behaviour at feeding times.

When using a single-space feeding system the animals cannot synchronise their feed intake and the jealousy for feed is reduced. Therefore, the social stress could be decreased compared to other systems as the animals adjust their feeding behaviour according to their rank (Nielsen 1999). In restrictive animal individual feeding systems, often used for gestating sows, recent research showed that sows are able to understand and distinguish one “name” from the other. This way the sows could be conditioned to trisyllabic names and were called to the feeder individually. Thereby, stress due to feed jealousy and lack of behavioural synchronisation was reduced (Manteuffel et al. 2010). Since most individual feeding systems for piglets and fattening pigs are distributing feed *ad libitum*, however, the animals simply cope with the situation.

Thirdly, in contrast to wild boar, domesticated pigs are kept in artificial environments. Apart from mostly artificial lighting and ventilation, of course, one of the main differences in comparison to nature is generally the floor. Pigs are usually housed on fully slatted concrete floors. Since pigs are programmed to search for feed for the biggest part of the light-day, they are programmed to root in the earth (Stolba and Wood-Gush 1989). The lack of possibilities to exert rooting behaviour, e. g. by adding straw or other manipulatable and edible substrate to a pen, is assumed to be one of the many causes for behavioural aberrations like tail biting (Day et al. 1996; Moinard et al. 2003).

4. Current challenges in animal nutrition

Adjusting to market demands, animal nutrition constantly progressed in the past decades. In the following, the development of animal nutrition and current challenges with possible reactions will be depicted.

In the early stages of animal nutrition sciences, the main focus was getting the animals to feed as much as possible to maximise their performance without measuring feed intake at all (Wishart 1938). After that, identifying the requirements of the animals under the respective stage of performance became important in order to increase efficiency (Morgan et al. 1975b; Morgan et al. 1975a). This still is, of course, a crucial part of animal nutritional sciences as we observe rapid genetic progression in nearly all livestock (GfE 2006; Rostagno et al. 2011; NRC 2012).

However, the current challenges posed by society, industry and consumers also demand new approaches in animal nutrition. The update in the fertiliser ordinance 2020 in Germany (DüV 2020) made a new way of thinking necessary in some areas in Germany. Intensive husbandry with high counts of animals led to threatening levels of nitrate in groundwater (Wriedt et al. 2019). The updated legislation aims to reduce these levels that primarily originate from manure and other fertilisation measures by implementing a nutrient flow balancing system (StoffBilV 2017). Therefore feeding switches from maximising performance to minimising nitrogen (and phosphorus) emissions (Meyer and Vogt 2020; Stalljohann et al. 2020). Also, antibiotic reduction and animal health maintenance are constant problems animal nutritionists try to find solutions for (EMA 2017). The use of essential oils often in combination with efficacious organic acids can improve the gut health of the animals due to an effect on hazardous microbes in the intestines (Roth and Kirchgessner 1998; Bakkali et al. 2008). Feed-borne hygienical challenges, such as *Salmonella*- or mycotoxin-contamination also need to be managed. Hygienical measures in such cases comprise heat treatment (Jones 2011) or use of additives (Boudergue et al. 2009).

The main focus of western customers seems to be animal welfare (Garcia-Llorente et al. 2018). Like all of the above mentioned challenges, welfare is influenced by many factors, not only nutrition (Veissier and Forkman 2008), however animal nutrition can have an impact. It is possible to optimise formulas in a way that does not only meet requirements, but also manipulates pigs' behaviour positively. Doing so, e. g. Stewart et al. (2010) were able to show that a herd of gestating sows showed less aggressions when reducing the nutrient concentration and increasing the fibre contents in the feed.

There are two control points which are not exposed to cognitive bias of the personnel (Harding et al. 2004) to capture the efficacy of above mentioned animal nutritional measures: The performance of the animal (in swine, mostly weight gain or number of weaned piglets per sow) and feed intake. The feed intake is usually measured per pen, namely for a group of animals. This, of course, was sufficient when just optimising performance, however, under the recent challenges, innovations in practical animal nutrition are necessary to cope with these changes.

Focussing more on feeding the individual animal could be a next innovation toward "precision animal nutrition". Although feeding systems that measure the individual pig's feed intake are available, they are only common practice in stables for gestating sows (Hinrichs and Hoy 2010; DLG 2018). These animals are restrictively fed, as gestating sow nutrition aims primarily to optimise body condition for farrowing (Lindermayer et al. 2012). Therefore, the use of the individual feeding data in this case is rather limited, although, it could be shown that sick sows enter feed later than healthy ones (Hinrichs and Hoy 2010). For weaned piglets and fattening pigs, individual feeding systems would be available, but are mostly used in scientific experiments and for progeny testing (de Haer and Merks 1992; Zentral Verband der Deutschen Schweineproduktion 2007). Thus, the capable technique would be available; however, a rationale needs evaluation as group feeding systems already measure feed intake rather precisely.

5. Goal of this doctoral project

This doctoral project aimed to evaluate if the addition of the individual animal's feeding behavioural data to routinely assessed group measurements could be a valuable tool to improve precision swine farming and react to the current challenges of animal nutrition. Hence, several trials with weaned piglets and fattening pigs were performed. The animals were fed with automatic single space feeders that allowed the measurement of the feeding pattern of each individual pig while maintaining a common, practical stable environment. Typical threats of animal welfare and feed hygiene were simulated to examine the possible added-value in depth.

Welfare challenges comprised short-term disturbances of the stable routine, such as interchanging of animal or deprivation of feed. These disturbances were designed to have only limited effects on zootechnical performance. This way, a possible implementation of the obtained individual feeding data as a sensitive indicator for short-term distress was evaluated. If this was achieved, an additional easily accessible welfare indicator via the feeding system might be established.

Deoxynivalenol (DON)-contamination was selected as a feed-borne hygienical challenge to animal health. The levels exceeded the European guidance-values (The Commission of the European Communities 2006) by up to five-times. To maintain practical relevance, two different mycotoxin-binders were tested to assess their possible effect.

6. Material and methods

A series of studies was conducted at the Swine Research Centre Schwarzenau of the Bavarian Research Centre for Agriculture. Weaned piglets and growing/finishing pigs were chosen as experimental animals.

Stable and animals

The swine research centre Schwarzenau is a near-practical production stable that comprises a sow herd of ca. 250 sows and facilities to grow and fatten the produced piglets. The used genetics is (German Large White × German Landrace) × Piétrain.

The animal nutrition research stable contains two compartments fitted with automatic feeders ("Schauer Compident Station CID 2006 – MLP Ferkel" for piglets and "Schauer Compident Station CID 98 – MLP Mast" for fatteners, Schauer Agrotronic GmbH, Prambachkirchen, Austria). These allow the examination of newly weaned piglets (4 weeks until 10 weeks of age) as well as grower-finisher pigs (10 weeks of age until slaughter). Both compartments offer enough space for 96 piglets and fatteners in 8 pens. The piglet barn is usually only stocked with 80 animals due to technical reasons. Every pen is fitted with fully slatted floors (plastic for piglets and concrete for fatteners), containing one feeder and a minimum of two drinking nipples. Pictures of housing and feeders with and without animals are shown in Figure I-1 to Figure I-6.

Figure I-1: Empty fattener stable (source: Simone Scherb)

Figure I-2: Fattener feeding station (source: Simone Scherb)

Figure I-3: Fattener compartment with pigs (source: Simone Scherb)

Figure I-4: Fattener feeding stations with starter pigs (source: Simone Scherb)

Figure I-5: Piglet pen with piglet feeding station and piglets (source: Simone Scherb)

Figure I-6: Piglet feeding station with feeding piglets

Data Generation and Processing

The animals were individually weighed either weekly (Chapter IV and Loibl et al. (2020a)) or every two weeks (Loibl et al. 2020b) to obtain their weight development. Scales connected to the network uploaded the data directly to an off-site server. Based on these data, daily weight gain (DWG) was calculated. Additionally, for the studies in fattening pigs all animals were slaughtered at the institute's own slaughterhouse and meat quality parameters were assessed according to the guidelines defined by the Zentral Verband der Deutschen Schweineproduktion (2007).

The single space automatic feeders gave access to several feeding parameters of each animal. Daily feed intake (DFI) was measured as well as the feed intake per feeding act. Next to the time when an animal entered the station the feed intake of each visit was assessed. The data was uploaded to the server daily. FCR was calculated as the quotient of DFI and DWG. First data evaluation was done in Microsoft Excel 2010 (Microsoft, Seattle, United States of America), and all calculations, graphs, and statistical analysis used in the following were performed with SAS 9.4 (SAS Institute, Cary, United States of America).

Group housed pigs were fed concentrated, purely vegetarian practical feed based on barley, wheat, and soybean meal, or barley, maize and soybean meal respectively (details in Loibl et al. (2020a), Loibl et al. (2020b), and Chapter IV). Feeding actions with less than 5 g were excluded from the analysis due to restrictions of the weighing technique. The number of each animal's daily visits to the feeder, the average feed intake per visit to the feeder, and the feeding action with highest feed intake per animal and day was also calculated based on these data. For the unpublished weaner study (Chapter IV), all feeding actions were considered as a lot of data points would be lost because of the reduced feed intake capacity of piglets (e. g. the count of daily feeder-visits would have been reduced by 30%).

Development of analytical model for feed intake behaviour

The generated feeding data clearly had a hierarchic structure (i.e., the factor treatment comprised pens, which comprised the sexes which in turn comprised the individual animals). It was therefore logical to establish a hierarchical ANOVA model that specifically takes the inert hierarchy of the data into account (Munzert 2015). Such a model allows the examination of the sources of deviation. Therefore, in all analyses, the deviation of the highest resolving independent variable was tested against the residual deviation. Then, systematically, the lower resolving variates were tested against the next higher resolving one. In the case of zootechnical performance, the variable with highest resolution was sex, as all parameters were summarised per animal before analysis. For feeding behavioural variates, all values were summarised daily, thereby resulting in the animal as parameter with highest resolution. The detailed models are described in Loibl et al. (2020a) and Loibl et al. (2020b).

7. *Outline of this thesis*

This doctoral project aimed to evaluate the feasibility of detailed feed intake data to deliver an added value on group feeding data. These could result in possible, easily accessible parameters to quickly evaluate animal welfare of piglets as well as growing finishing pigs under practical conditions. Due to digitalisation, modern husbandry systems routinely generate an increasing volume of detailed data. These data could be an integral part in future practical precision feeding of the animals and might even allow for real-time observation through automatic systems.

Therefore, stables fitted with automatic single space feeders were used to produce feeding data for each animal in the stable in utmost detail. Next to a general assessment and examination of feeding behavioural patterns, several different harmful situations causing either short-term distress within the pen or leading to a sustained alteration of performance were designed. The possibility to use feeding behavioural measurements as an indicator system for animal distress was then evaluated. These disturbances comprised husbandry problems like interchanging of animals, technical problems in feed or drinking technique, or mycotoxin contamination of feed.

Based on these experiments two manuscripts were published by the time of the submission of this thesis (Loibl et al. (2020a), Loibl et al. (2020b), links to the respective publisher's websites are given on the title pages below, pages 28 and 49). An unpublished piglet study is described in Chapter IV. Several contributions to scientific conferences were also published and are mentioned in the publication list above.

Loibl et al. (2020a) presents the first study in growing finishing pigs conducted in this doctoral project. During the starter and grower period, several short-term disturbances were simulated to assess possible effects on the feeding behavioural patterns of the animals without altering the zoo-technical performance of the pigs. The animals were allowed sufficient recovery time between the challenges. The four different treatments comprised an undisturbed control group, a "starving" group with feed deprivation or restriction, a "feed change" group with changes in feed composition for 48 hours and a "social stress" group with exchanging animals between two pens and reduced water efflux at the nipple drinkers. As expected, these distress situations did not have a sustained effect on zootechnical performance. Interestingly, using the hierarchical ANOVA we found no effects on the feeding behaviour either. The animals developed rather consistent group-specific behaviour that remained constant even after facing short-term disturbances.

Loibl et al. (2020b) presents the second study in fattening pigs. The animals were chronically exposed with deoxynivalenol (DON). A low contaminated control group (~900 µg/kg DON, equal to roughly the guidance-value defined by The Commission of the European Communities (2006) was compared to three highly contaminated groups (>4500 µg/kg DON in all groups) with two of them supplemented with different mycotoxin binders. The animals clearly showed the expected depression in zootechnical performance mostly caused by feed refusal. However, the feeding behavioural structure again was not influenced by the treatments. The main source of deviation was the animal within the respective group. The DON-exposure led to a more erratic behaviour as shown in higher variation in the feeding traits.

Chapter IV presents the results of an unpublished study in weaned piglets. The reaction of progeny of either Duroc or Piétrain boar lines to short-term disturbances was tested. The 12h feed deprivation as well as a reduction of water efflux for 48h did not affect zootechnical performance of the animals. Feed intake behaviour was mainly specific for each individual animal. In piglets, the group was not as important of a factor as in fattening pigs.

The general discussion puts all studies into context and is followed by the general conclusion of this doctoral project.

Contribution statement

The doctoral candidate contributed with experimental conception and design, supervision of the animal experiment in the stable (except for study of chapter 3), collection of zoo-technical and feeding behavioural data, data analysis and interpretation and authoring of all manuscripts.

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Chapter II. Examination of High-Resolution Feed Intake Data of Grower Finisher Pigs Confronted with Typical Short-Term Disturbances in Stable Routine

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Authors' Contributions

Peter Loibl contributed with experimental design, conduction and supervision of the practical feeding trial, data generation and processing, development of the statistical model and conduction of statistical analyses and wrote the publication. Wilhelm Windisch delivered general support and supervision, ideas for statistical evaluation, and input on authoring the manuscript. Wolfgang Preißinger contributed with funding, experimental facility, input on design and supervision of the practical feeding trial.

Summary

This publication represents the results of the first feeding study in fattening pigs, comprising 96 animals in eight pens of 12 animals, being part of this doctoral project. The goals of this study were to examine a possible feasibility of feed intake data generated by automatic single space feeders to assess and examine behaviour of the individual animal. This way, an easily measurable welfare indicator might be established.

Assuming, that the animals quickly established a consistent feeding behaviour four treatment groups were designed to simulate different, rather typical disturbances of stable routine. These disturbances were designed with adequate recovery time in-between, therefore, only two such situations could be established during the whole fattening period. An undisturbed control group was compared to a starving group (no feed for 24 h, restrictive feeding), a feed change group (changes in feed composition,

either only grains or starter feed in grower phase) and a social stress group (exchanging of animals between the pens and short-term reduction of accessible water). Next to classical zootechnical performance parameters, daily weight gain, feed conversion ratio, daily feed intake and different meat quality measures, the single space feeders allowed the assessment of feeding behavioural traits, feed intake per feeder visit, number of daily feeder visits, and highest feed consumption in one visit per animal and day. Using a hierarchical ANOVA, we could identify the main causes of variation in our data, thereby identifying the factors influencing the measured parameters.

Since the short-term disturbances lasted at most 48 hours, as expected, no effect on zootechnical performance was observed, however, typical factors, like sex dimorphism could be identified statistically. The feeding behavioural traits were not affected by the treatments too. The most prominent parameters influencing feeding behaviour was the individual animal and partly the pen, namely the group of animals feeding at the same feeder. This led to the conclusion, that individual animals established a persistent, however highly varying feeding pattern that was not altered sustainably by any of the simulated disturbances. Our results suggested that group dynamics also influenced the individual animals' behavioural patterns.

The format of text, tables, figures, and references in the following will differ from the original publication to maintain uniformity in this thesis. Some of the cited references might have been updated since the publication of the paper. The original publication is Open Access and can be downloaded under https://doi.org/10.17221/25/2020-CJAS. A copy of the publisher's permission to include the publication in this thesis can be found in the appendix.

Abstract: Modern pig feeding systems allow the collection of highly detailed feeding data for each animal. These data enable the examination of individual feeding behaviours to assess an animal's wellbeing. As such, four different treatments – undisturbed control, starving (no feed for 24 h, restrictive feeding), feed change (changes in feed composition) and social stress (exchanging of animals between the pens and short-term reduction of accessible water) – were designed to simulate typical short-term disturbances in a practical stable routine. Each treatment was conducted over 2 pens with 12 animals each. Zootechnical performance and feed intake behaviour measures were assessed for each animal. Treatments did not affect zootechnical performance. Results showed that short-term disturbances did not influence feed intake behaviours, such as daily feed intake, amount of intake per feeder visit, number of daily feeder visits and daily feeding action with highest feed intake. Animals developed individual feeding patterns that persisted through artificial short-term disturbances. However, data suggested that an individual animal's behavioural pattern was strongly influenced by the group (pen) due to group dynamics among animals.

Keywords: fattening pigs; feed intake behaviour; single space feeder; feeder visit

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Improving animal welfare in agricultural production systems has become increasingly relevant to society and politics over the last decades (Lassen et al. 2006). It is still difficult to evaluate the welfare status of animals quickly at the farm level due to its multifactorial nature (Broom 1988; Hameenoja 2001) In addition to performance and health status, stable hygiene, quality of feed and quality of the pen and stable affect an animal's ability to engage in innate behaviours. Direct measurement of an individual animal's state of wellbeing is difficult with regard to neurological markers, and direct observation of behaviour is thereby a critical component of animal welfare assessment (Veissier and Forkman 2008). Ongoing structural changes in European pig production have led to increasing herd sizes on farms whilst numbers of supervising staff have remained constant or decreased (Eurostat 2019). This has led to a reduced amount of disposable time for animal observation. It has thus become increasingly important to develop an easily accessible measurement system that can be integrated into the daily workflow of the stable without requiring additional time.

Progressive digitalisation has enabled the near real-time assessment of a great deal of data in the modern stable, including air temperature, humidity, water efflux at the drinkers, and physiological measures of individual animals such as rumen pH in dairy cows (Cox 2007). The locomotion of individual animals within a pen can be examined under practical laboratory conditions (Ott et al. 2014), and even the vocalisation of pigs in pens is a viable measure of the animal group's wellbeing (Vandermeulen et al. 2015). The implementation of these data in [semi-]automatic animal welfare assessment systems will be an irreplaceable component of welfare control. However, many of these systems are still under development and will not be available for industrial use for several years.

In the area of pig nutrition, the focus was originally on meeting nutritional requirements to support animal health and performance. Over the course of the past decade, however, behavioural aspects related to nutrition have also aroused interest (Czycholl 2018; Preißinger 2018). Many recent husbandry challenges can be linked to restrictions of innate animal behaviours. The feeding behavioural axis in swine is complex and normally consists of foraging, grazing and some predating (Ballari and Barrios-García 2014). Under natural conditions, pigs spend 6 h to 7 h a day feeding and are not active at night (Signoret et al. 1975). The diets of wild boars are immensely diverse, consisting of herbs and grains, in addition to animal protein (Signoret et al. 1975; Ballari and Barrios-García 2014).

In modern intensive systems, however, pigs are fed highly concentrated, purely vegetarian feed. This has led to reduced feeding times, even under *ad libitum* conditions, with the pig needing other stimuli to fulfil its behavioural needs. The feeding patterns of conventionally housed pigs can provide many behavioural indicators besides the amount of feed intake, such as social status (Nielsen 1999).

Examination of feeding behaviours of pigs in practical housing conditions has historically utilised group-based, long-term data because of typical practical feeding techniques, such as trough feeding. Automatic single space feeders, however, allow the examination of an individual animal's feeding pattern without having to change its housing environment. Every single visit to the feeder by every individual animal is recorded, enabling assessments of feeding patterns of a group of housed pigs on the single day and at the single animal level. The goal of the present study was to evaluate whether group-housed pigs develop individualised constant feeding patterns. Additionally, typical short-term technical disturbances (< 48 h) were simulated to assess whether they altered regular feeding behaviour sustainably. If so, individualised feeding behaviour recording could be an additional indicator of an animal's wellbeing.

MATERIALS AND METHODS

Ethics

The presented experimental protocol was approved by the ethical committee of the Bavarian State Research Centre for Agriculture, Grub, Germany.

Experimental Design

A feeding trial using 96 fattening pigs [(German Large White × German Landrace) × Piétrain] was conducted at the experimental site of the Bavarian State Research Centre for Agriculture. One week before the start of the trial, piglets were placed in 8 separate pens (5.0 m × 2.6 m) with fully slatted floors to adapt themselves to the new feeding system.

The animals were distributed equally over the pens considering sex (females to castrated males 1 : 1) and litter (minimally four animals per litter). Each pen contained one single space automatic feeding system (Schauer Compident® MLP). These feeding stations documented the time when an animal entered the feeder and the consumed amount of feed.

For technical reasons, it was not possible to measure the duration of each visit to the feeder. The fatteners were fed three different weight-dependent diets *ad libitum* to meet the following nutritional requirements: starter diet from days 1 to 35; grower diet from days 36 to 63 and finisher diet from day 64 until the end of the experiment. The diets were fed dry as coarse meal and consisted of wheat (46–39%), barley (39–46%) and extracted soybean meal (18–12%). A standard macro premix with added synthetic amino acids was supplemented at a 3–2% inclusion rate. These adaptions, according to maturation, led to an analysed energy content of 13.8–13.5 MJ metabolisable energy and a protein content of 16.5–14.3%/kg feed during the fattening progress.

The experiment was designed to simulate short-term disturbances in technical housing management and to measure their possible influences on the animals (Table II-1). A pause of 21 days between interferences was considered enough for regeneration. Four experimental groups consisting of two pens each were created:

1. Control: animals experienced no artificial disturbances.
2. Starving: pigs were deprived of feed for 24 h (days 30–31, 12:00-12:00) and restrictively fed (<1.0 kg/d) for 48 h (days 51–53) to simulate defects in the feeding system. Due to technical reasons the feeders had to be turned off for the 24 h of deprivation.
3. Feed Change: animals were confronted with sudden, short (48 h) changes in feed composition. These were diets consisting only of cereals and macro-premix (days 30–32) and the Starter diet at the end of the Grower phase (days 51–53).
4. Social Stress: the fatteners were deprived of water (the efflux of the drinking nipples was reduced from 1.5 L/min to 0.8 L/min for 48 h, days 30–31). Additionally, on day 51, three animals from each pen were exchanged.

From day 77 onwards, animals having grown to 115kg–120kg live weight were slaughtered consecutively on a weekly basis. The last animals were slaughtered on day 105.

Table II-1. Experimental design and timetable

treatment		Control		Starving		Feed Change		Social Stress	
pen		1	2	3	4	5	6	7	8
experimental day	1	start of experiment, starter feed							
	30	---		no feed for 24 h		only cereals and macro-premix for 48 h		reduction of water efflux to 0.8 L/min for 48 h	
	35	change to grower feed							
	51	---		< 1kg feed per day for 48 h		first period feed fed for 48 h		exchanging of three animals	
	63	change to finisher feed							
	77	start of consecutive slaughtering							
	105	end of the trial							

Obtained parameters

Parameters were measured for each animal individually. In addition to zootechnical performance, weight (kg), daily weight gain (calculated from weekly weighins in g per day), daily feed intake (DFI, kg), feed conversion ratio (FCR, kg feed per kg gain) and meat quality figures [muscle and fat area in cm^2 of the chops, lean meat content in %, measured following the guidelines of the “Central Association of German Pig Production” (Zentralverband der Deutschen Schweineproduktion 2007) and other parameters of feeding behaviour were obtained from the single space feeders. These included the amount of consumed feed per visit to the feeder (g) and the number of feeder visits per day (n). As the third behavioural figure, the feeding action associated with the most consumed feed (g) was identified for each animal and day. For technical reasons, all feeding events of less than 5 g feed intake were not used for the analyses.

Statistical analyses

The animals were weighed on a weekly basis, and daily weight gain and FCR were calculated on a weekly basis. These data were summarised by individual animal for each fattening period. DFI, feed intake per visit to the feeder, count of daily feeder visits and feeding action with the highest feed intake were measured individually and summarised by day. The experimental day was the experimental unit. Two different models were designed to analyse the data.

Zootechnical performance data were analysed according to the following hierarchical model:

$$y_{ijkr} = \mu + treatment_i + pen_j\ (treatment)_i + sex_k\ (pen,\ treatment)_{ij} + e_{ijkr}\ (1)$$

Factors in brackets indicate nested parameters. Factor *sex (pen, treatment)* was tested against overall deviation. *Pen (treatment)* was tested against *sex (pen, treatment)* and *treatment* against *pen (treatment)*.

For feeding behavioural measures, data were analysed only for the first two fattening periods, because the first animals were slaughter shortly after the switch to the finisher period. This might have led to non-treatment-related effects. The following model was used:

$$y_{ijklr} = \mu + treatment_i + pen_j\ (treatment)_i + sex_k\ (pen,\ treatment)_{ij} + animal_l\ (sex,\ pen,\ treatment)_{ijk} + e_{ijklr}\ (2)$$

The *animal (sex, pen, treatment)* was then tested against residual deviation, and then factor *sex (pen, treatment)* was tested against *animal (sex, pen, treatment),* and so on.

To examine the persistency of feed intake parameters (DFI, feed intake per visit to feeder, count of visits to feeder and feed intake of most extreme feeding event per day) linear regressions were calculated using individual animal means for the respective fattening period: [*grower period = a + b*starter period*].

SAS 9.4 (SAS Institute, Cary, United States of America) was used for all statistical analyses and graphs. A *p*-value of less than 0.05 was considered statistically significant.

Significant differences between pens were identified using the Student Newman's Keul's test.

RESULTS

One animal was excluded from trial and analyses due to non-treatment related reasons.

Zootechnical performance

Table II-2 shows zootechnical performance parameters. *Treatment* did not affect any parameter other than FCR and grower periods. *Pen (treatment)* had no influence on any parameter. *Sex (pen, treatment)* was associated with expected differences.

Table II-2. Zootechnical performance results for all eight pens

Treatment		Control		Starving		Feed Change		Social Stress		over-all	SEM	*P-value*		
Pen		1	2	3	4	5	6	7	8			treat.	pen	sex
Animals	n	12	12	12	11	12	12	12	12	95	---	---	---	---
Feeding days of an individual animal (d)														
Duration	d	95	97	98	98	92	96	96	94	96	0.91	0.20	0.96	<0.01
Weight (kg)														
Day 1	kg	40.7	40.3	39.7	40.9	40.3	35.5	41.7	41.1	40.0	0.38	0.34	0.20	0.26
Day 35	kg	71.4	69.7	69.7	70.2	70.9	64.7	70.6	72.2	79.9	0.68	0.53	0.66	<0.05
Day 63	kg	94.6	93.0	92.3	90.6	96.6	90.5	92.1	96.0	93.2	0.90	0.71	0.82	<0.01
Final	kg	123.0	117.3	119.1	115.3	119.0	118.0	117.0	119.8	118.6	0.59	0.72	0.58	<0.01
Daily weight gain (g/d)														
Starter	g/d	879	838	857	838	876	832	827	887	854	12	0.96	0.87	<0.01
Grower	g/d	827	833	805	727	917	921	768	853	833	13	0.08	0.79	<0.01
Finisher	g/d	920	734	797	723	786	858	756	802	798	15	0.77	0.21	0.07
Overall	g/d	881	809	825	773	872	879	797	864	838	11	0.38	0.77	<0.01
Overall feed intake (kg)														
Starter	kg	66.7	64.0	63.1	63.2	62.6	59.0	60.8	66.2	63.2	0.02	0.32	0.92	<0.01
Grower	kg	64.5	59.8	61.2	54.6	63.1	61.9	59.5	62.9	61.0	0.03	0.28	0.98	<0.01
Finisher	kg	57.9	50.2	56.5	58.2	47.3	66.1	50.9	50.3	54.6	0.03	0.75	0.90	<0.01
Overall	kg	189.0	174.0	180.9	176.1	173.0	187.0	171.2	179.4	178.9	0.02	0.48	0.97	<0.01
Feed conversion ratio (FCR, kg feed intake per kg gain)														
Starter	kg/kg	2.2	2.2	2.1	2.2	2.1	2.0	2.2	2.2	2.1	0.02	<0.01	0.93	0.52
Grower	kg/kg	2.8	2.7	2.7	2.9	2.5	2.4	2.9	2.7	2.7	0.03	0.03	0.78	0.19
Finisher	kg/kg	3.0	3.2	3.1	3.4	3.2	3.0	3.4	3.3	3.2	0.04	0.49	0.07	0.80
Overall	kg/kg	2.6	2.7	2.6	2.8	2.5	2.5	2.7	2.7	2.6	0.02	0.07	0.30	0.56
Meat quality measures														
Muscle area	cm^2	59	60	60	61	60	62	61	61	61	0.48	0.63	0.90	0.02
Fat area	cm^2	16	14	15	14	15	15	16	16	15	0.28	0.34	0.96	<0.01
Lean meat	%	60.3	61.3	61.0	61.6	61.0	60.6	60.5	60.1	60.8	0.25	0.33	0.94	0.01

SEM = Standard Error of the Mean; *p*-values are from hierarchical ANOVA wherein each source of variance was tested against the column on the right; sex was tested against the overall data variation.

Feed intake measures

Distribution of daily counts of feeder visits and amounts of consumed feed. Figure II-1 shows histograms of the number of visits to the feeder per animal per day and the amount of consumed feed per visit. Median values were 12 visits per day and 105 g of feed per visit, whereas means were approximately 12.5 visits per day and 158.0 g per visit.

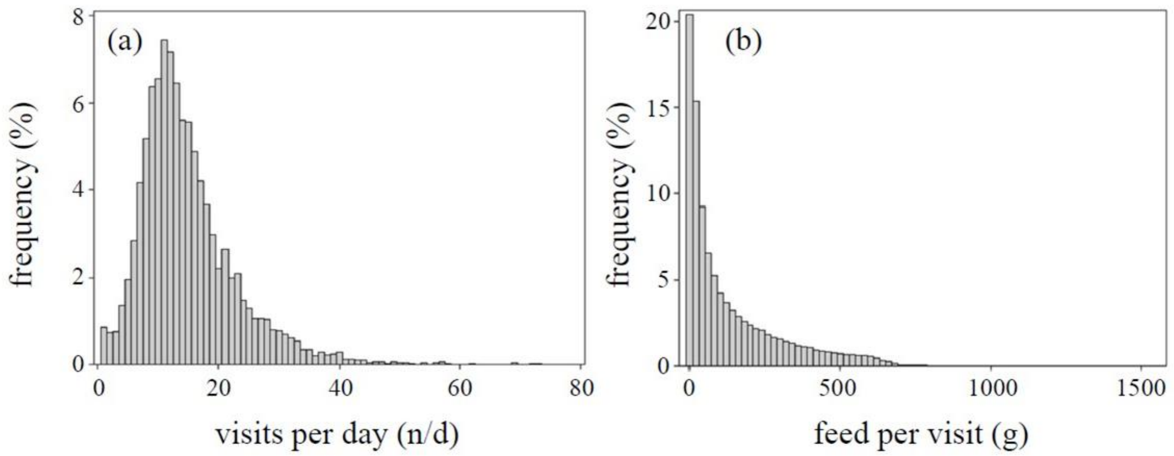

Figure II-1. Histograms of the distribution of the daily feeder visits per animal (a) and amount of feed consumed per visit (b)

Time patterns of assessed feeding behavioural measures. Figure II-2 presents feeding measures plotted per pen over the observation period. DFI increased over time in all pens. Feeding behavioural traits differed by pen independently of treatment group. Pen 2 showed consistently fewer daily visits to the feeder alongside higher feed intake per visit. Similar indications were evident for all pens.

The Starving treatment group showed evidence of behavioural impacts from both artificial disturbances. These disturbances directly influenced the amount of ingested feed, and impacts were expected as part of the methodology. All other pens showed fluctuations that were not statistically linked to the simulated treatments. By visual judgement, Pen 4 presented with the highest fluctuations in DFI and Pen 6 in amount of consumed feed per visit and feed intake during the feeding action with highest feed intake. Their partner pens did not change patterns.

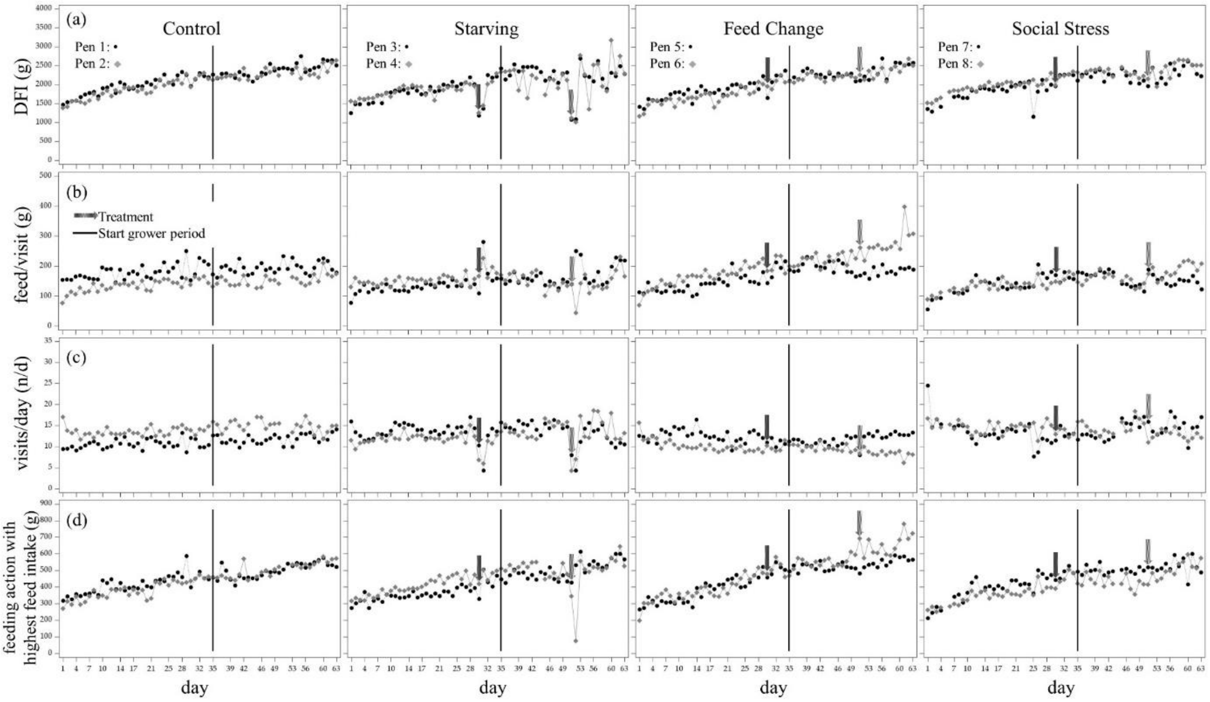

Figure II-2. Time series of daily feed intake (a), amount of consumed feed per visit (b), number of daily feeder visits (c) and the feeding action with highest feed intake per day (d); means per animal and day

Examination of grouping factors within the feeding behavioural data. Table II-3 shows mean values for feed intake parameters, DFI, feed intake per feeder visit, daily visits to the feeder, and feed intake of the feeding action with highest feed intake. *Treatment* did not affect any of the measurements. *Pen (treatment)* significantly influenced feed intake per visit during the Starter period, wherein Pens 2 and 3 showed the lowest feed intake per visit at 133g and 134g, respectively, and Pen 1 showed the highest average feed intake per visit at 179g. The pens with the lowest feed intake per visit tended to show an increased frequency of feeder visits (14 per day; $p = 0.06$), although the trend was not statistically significant. Pens with highest feed intake also showed decreased visit frequency (11 visits per day). *Sex (pen, treatment)* affected DFI at a rate of around approximately 2.0 kg/d; no other behavioural measures were affected. *Animal (sex, pen, treatment)* demonstrated highly significant effects on all measures ($p < 0.01$) throughout the study timeframe.

Table II-3. Overall results of feeding behavioural measures (daily feed intake, count of daily visits to the feeder per animal, overall amount of consumed feed per visit and amount of consumed feed of the single visit with highest feed intake per animal and day)

Treatment		Control		Starving		Feed Change		Social Stress		Over-	SEM	*P-value*			
Pen		1	2	3	4	5	6	7	8	all		Treat.	Pen	Sex	Anim.
Daily feed intake (kg/d)															
Starter	g	1.9	1.8	1.8	1.8	1.8	1.7	1.8	1.9	1.8	0.01	0.30	0.92	<0.01	<0.01
Grower	g	2.3	2.3	2.2	2.1	2.3	2.2	2.2	2.3	2.2	0.01	0.25	0.98	<0.01	<0.01
Days 1–63	g	2.1	2.0	2.0	1.9	2.0	1.9	2.0	2.1	2.0	0.01	0.37	0.97	<0.01	<0.01
Feed intake per visit (g)															
Starter	g	179^{a}	134^{e}	133^{e}	154^{c}	144^{d}	165^{b}	134^{e}	134^{e}	145	0.62	0.76	<0.05	0.48	<0.01
Grower	g	170	166	175	174	192	211	171	147	174	0.92	0.19	0.14	0.39	<0.01
Days 1–63	g	186	142	146	151	164	198	143	149	158	0.54	0.48	0.07	0.42	<0.01
Count of visits to the feeder per animal and day (n/d)															
Starter	n/d	11	14	14	12	12	10	13	14	12	0.07	0.55	0.06	0.40	<0.01
Grower	n/d	12	14	13	14	12	9	14	13	13	0.08	0.18	0.35	0.25	<0.01
Days 1–63	n/d	11	14	13	13	12	10	14	14	13	0.05	0.34	0.18	0.23	<0.01
Feed intake of the most extreme feeding action per animal and day (g)															
Starter	g	416	383	363	413	394	398	396	358	390	1.8	0.79	0.37	0.06	<0.01
Grower	g	502	495	503	503	534	599	506	461	513	2.1	0.13	0.35	0.21	<0.01
Days 1–63	g	454	433	425	452	456	487	445	404	445	1.6	0.30	0.49	0.10	<0.01

SEM = Standard Error of the Mean;
P-values are from hierarchical ANOVA wherein each source of variance was tested against the column on the right; animals were tested against the overall data variation.

Regression analyses of feeding parameters. Supplementary Figure II-1 through Supplementary Figure II-4 in electronic supplementary material (ESM) show individual mean values of the assessed feeding behavioural measures. The average standard deviations were around 18.3% for DFI, 90.5% for feed intake per visit to the feeder, 30.2% for count of daily feeder visits and 23.0% for feeding action with highest feed intake per day, relative to the respective means. This was despite the fact that from visual judgement, means appeared to persist from starter to grower period.

Figure II-3 shows the overall regression curves of the means overlaid on scatter plots of individual animals. All parameters showed a high correlation with determination coefficients ranging between 0.44 to 0.65, upholding this apparent persistency.

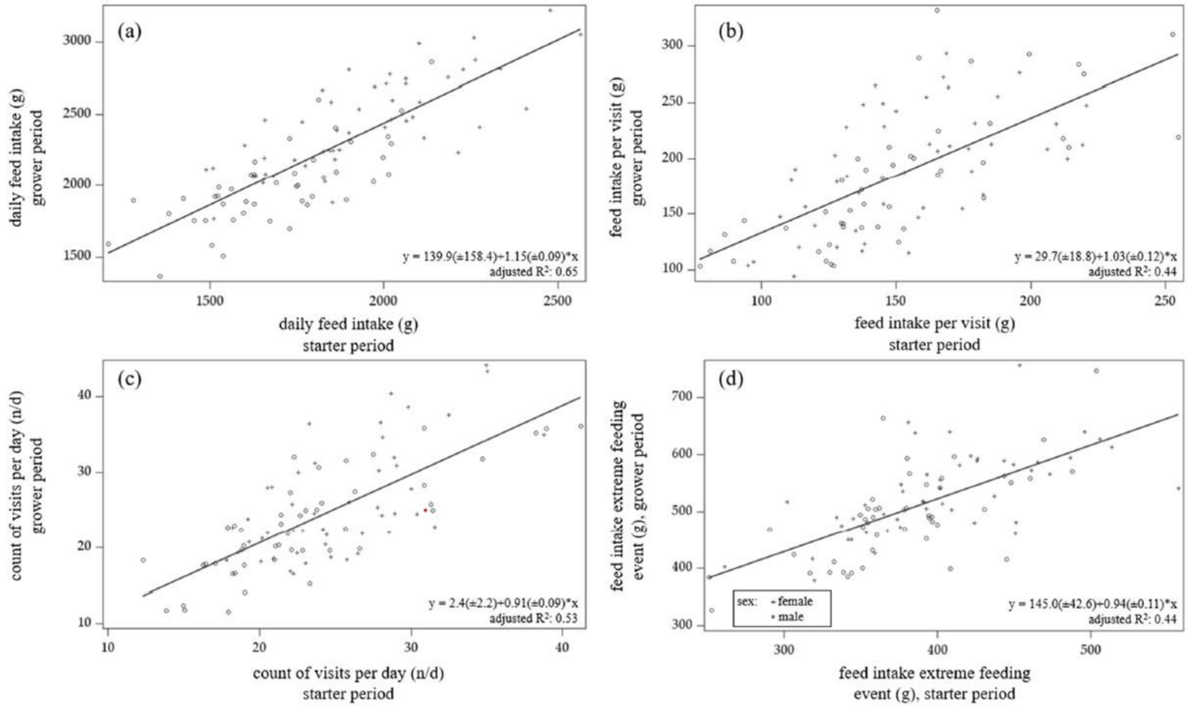

Figure II-3. Plots of regression curves for different feed intake parameters. (a) daily feed intake; (b) feed intake per visit; (c) count of visits per day; (d) extreme feeding events

DISCUSSION

This study investigated whether short-term technical fluctuations in stable routine altered zootechnical performance, with a focus on highly resolved patterns of an individual animal's feeding behaviour. As such, short-term disturbances were simulated to examine their potential effects on individual daily measures of feeding behaviour.

Zootechnical performance

As expected, *sex (pen, treatment)* significantly affected nearly all zootechnical performance parameters, excluding FCR. Literature contends that females and males (castrates) differ in feed intake resulting in differential weight development (Hale and Southwell 1967). Carcass parameters are known to differ between the sexes as well (Cahill et al. 1960). During the adaptation phase of the study, animals of pen 6 showed a slight delay in learning the feeder, which entailed the well-known phenomenon of compensatory growth with somewhat lower FCRs during the starter and grower period, respectively (Kirchgeßner et al. 2014). Consequently, the statistically significant effects on FCR observed in the Feed Change group seem to be artificial in nature. The factors *sex (pen, treatment)* and *pen (treatment)* did not mediate any deviation in FCR. Female pigs generally show the same FCR as males (Hale and Southwell 1967). In total, short-term disturbances were found to have little to no effect at all on zootechnical data.

Feeding behavioural measures

Several reports examining group-housed grower/finisher pigs fed with similar feeding techniques described similar feeding behavioural traits (Nielsen et al. 1995; Nielsen 1999; Kallabis and Kaufmann 2012). Another study (Nielsen et al. 1995) found that the pigs visited the feeder approximately 13 times per day, consuming similar amounts per visit.

Several methods for the examination of feeding behaviour have been published. Ethologists often use so-called 'meal criteria' for different species as the basis for their examinations (Tolkamp et al. 1998; Yeates et al. 2001). In assessing the duration of single feeding actions, a maximum pause based on the distribution of 'non-feeding time' is defined to summarise several feeding actions regarding a single meal. These datasets are compressed and can be more easily examined for rhythmicity, among other parameters, in time series analyses (Shono et al. 2000) or other statistical method. In the present study, the feeders did not document the lengths of these pauses. This is due to practical conditions, as the feeders used only recorded time of entering the feeder and amount of feed consumed. We thereby decided to investigate different deviation factors to determine which factor was causing the observed differences.

Animal (sex, pen, treatment) was the predominant source of variation regarding all feed intake measures. This parameter was highly significant over all four feeding parameters indicating strong behavioural differences between the individual animals. *Sex (pen, treatment)* was also significant regarding DFI, as discussed above. The feeding actions with highest feed intake also varied by *sex (pen, treatment)* (Starter period, $p = 0.06$; days 1–63, $p = 0.10$), indicating that the different sexes may have different maximum feed intake capacities. This phenomenon may also be explained by the slower growth seen in female fatteners that could underlie their lower feed intake potential (Cole et al. 1968).

Highly individualised feeding behaviours led to somewhat significant differences caused by *pen (treatment)* (feed intake per visit to the feeder in the Starter period, $p < 0.05$; days 1–63, $p = 0.07$; count of feeder visits in the Starter period, $p = 0.06$). The pen is equivalent to the feeder in this trial. Schamun and Hoy (2011) combining similar single space feeders with ethological analysis revealed that the group of fatteners within a pen developed a 'group' behaviour based on the constant behaviour of an individual pig that was presumably linked to the animal's rank within the group. Group dynamics may thereby affect other present findings. Highly individualised feeding behaviour led to immense variation among animals. Despite a range of mean values per pen (e.g., lowest daily feeder visit mean in the Grower period was 9 (Pen 6), and the highest was 14 (Pens 2, 4 and 7); however, no significant influence of *pen (treatment)* was found.

Across the two examined feeding periods, the count of daily feeder visits remained constant whilst feed intake parameters increased. Only Pen 6 showed a reduction in the count of daily visits. This is also indicative of effects on group behaviour caused by an individual in each group. Additionally, the animals appeared to react to increasing energy requirements over the course of maturation by increasing feed intake per visit rather than frequency of visits. Another report (Schamun and Hoy 2011) found similar results.

The development of feeding behavioural parameters over time (Figure II-2) was constant within a given pen over time. By visual judgement, the Starving group appeared to be slightly affected by the 48 h of restrictive feeding. After day 53, the mean DFI in Pen 4 began to fluctuate significantly. An effect of the 24 h of deprivation was not visible, however. Since the feeders were turned off to starve the animals, it could not be monitored if the animals tried to feed. Also, possible short-term increases in feed intake of individual animal following the starving period could not be identified as statistically relevant. All the other groups remained on a constant course of increasing DFI after each short-term disturbance. Looking at the standard deviations Supplementary Figure II-1 – Supplementary Figure II-4 (ESM)), it was found that detailed feeding parameters diverged immensely, in part due to high day-to-day fluctuation in measured parameters within individual animal data. This resulted in poor predictability of these measurements.

The exchange of animals did not lead to drastic changes in feeding behaviour, an unexpected result. Establishment of a new hierarchy within pig groups takes around 48 hours (Ewbank 1976). However, no drastic changes were visible in feeding behaviour even over this short timeframe.

The short-term disturbances simulated in the present study were insufficient to alter long-term feeding behaviours, and observed differences disappeared among the daily variation of the assessed parameters.

Regression analyses

Although individual animals showed significant day-to-day variation in their feeding behaviours, the means of the assessed factors remained similar throughout the Starter and Grower periods (Supplementary Figure II-1 – Supplementary Figure II-4

(ESM)). We thereby calculated linear regressions for these measures to assess their persistency (Figure II-3). Altogether, the slopes of the regression curves were significantly different from 0. An overall R^2 of 0.40–0.60 indicated that an individual animal's feeding behaviour persisted over the two feeding periods. However, due to the large daily variation in examined parameters, longer examination periods will be needed to assess any correlations.

One study (Schamun and Hoy 2011) did show that fatteners presented with rank-dependent feeding patterns. High-ranking animals showed significantly lower feeding frequency with significantly higher feed intake per feeder visit. By the regression curves shown in Figure II-3, one might assume that a similar situation was observed in the present study. However, due to the paucity of visual data, an individual animal's rank could not be conclusively determined. The same study (Schamun and Hoy 2011) also showed that due to rather constant hierarchy maintenance ($R^2 = 0.61$), all other traits, visits within 48 hours and feed intake per visit remained constant throughout the fattening period. In the present study, since *animal (sex, pen, treatment)* was found to be the only significant parameter affecting feeding behaviour, a constant hierarchy within the groups could be a major reason for this result. The short-term disturbances in this case did not alter the system sustainably. As such, the assessment of feeding patterns of individual animals does not appear to be a viable welfare indicator, as in this study, it was not sensitive enough to show an effect.

CONCLUSIONS

The present study found that group-housed fattening pigs fed with automatic single space feeders develop discrete individual feed intake behaviours. Individual parameters of feed intake per day, number of daily feeder visits, feed intake per visit and feeding action with highest feed intake per day were largely constant over time. The individual animal was the dominant factor that influenced these parameters. For DFI, sex was also found to have significant influence. However, even drastic short-term changes in stable routine such as exchanging a set of animals between pens did not significantly affect feeding behaviour.

Since individual feeding behaviour is consistent yet dispersive over time, the simulated artificial short-term (max 48 h) disturbances in stable routine did not produce any sustained effects. As such, feeding patterns of group-housed pigs are not an effective early warning system for short-term fluctuations in behaviour caused by technical problems.

Overall, pigs appear to quickly develop complex social structures and ranks within a group of animals that persist despite exogenous short-term impairments. Therefore, an animal group (in the present study, the animals housed in a single pen) seems to be the most suitable unit to study feeding behaviour documented by feeders.

Acknowledgement

The authors wish to thank the technical staff of the LVFZ Schwarzenau research station for all their practical on-site support during the trial.

Conflict of interest

The authors declare no conflict of interest.

ELECTRONIC SUPPLEMENTARY MATERIAL

Supplementary Figure II-1. Average daily feed intake per animal and fattening period; tick marks indicate standard deviations

Supplementary Figure II-2. Average daily feed intake per feeder visit, animal and fattening period; tick marks indicate standard deviations

Supplementary Figure II-3. Average daily count of visits to feeder per animal and fattening period; tick marks indicate standard deviations

Supplementary Figure II-4. Average daily feed intake of the single feeding action with highest feed intake per animal and fattening period; tick marks indicate standard deviations

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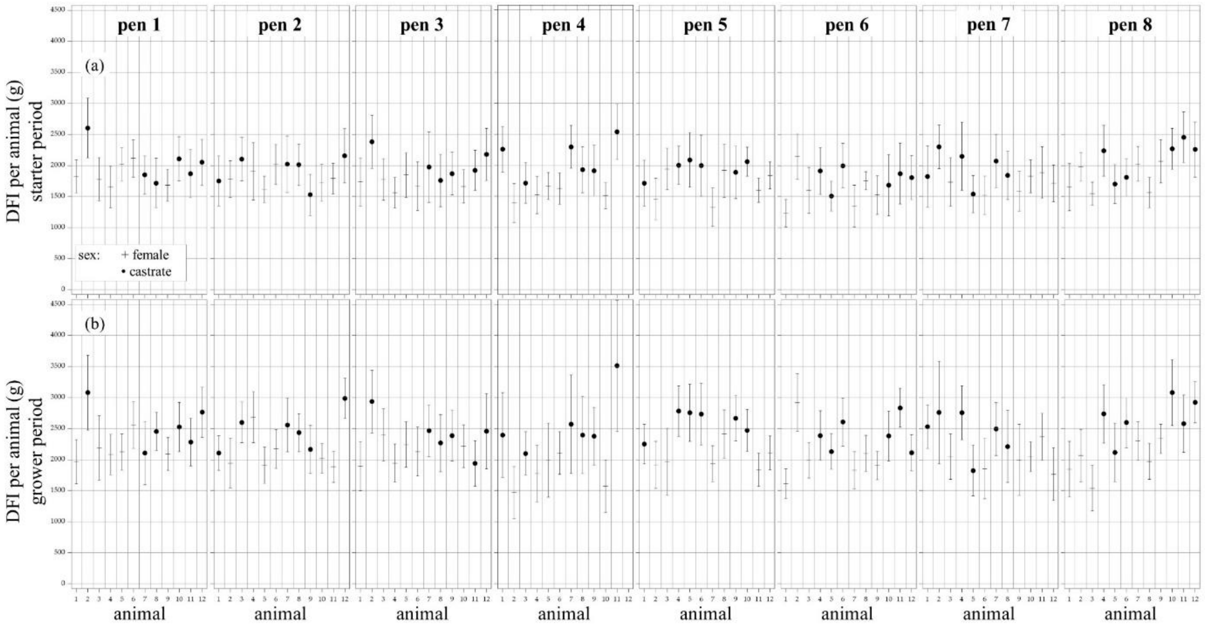
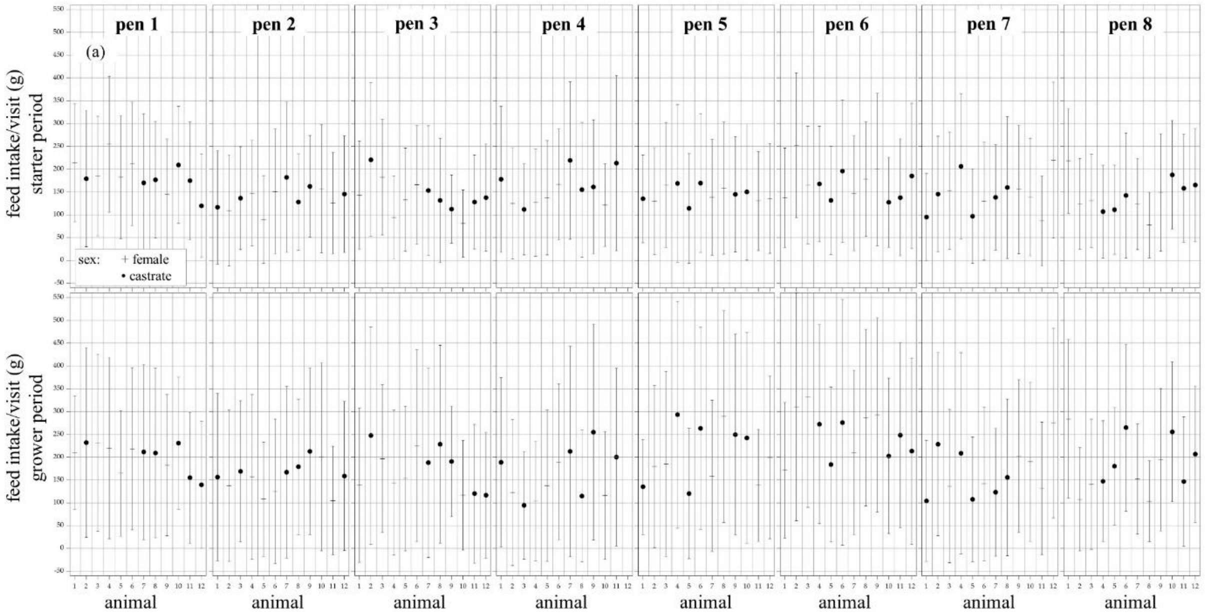
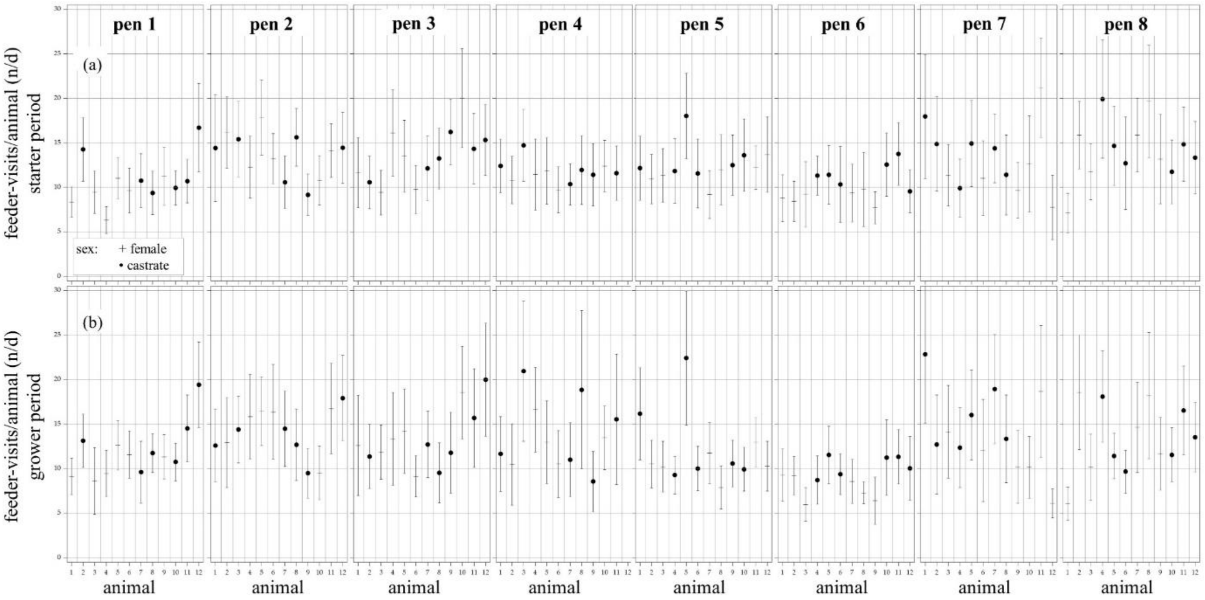
Electronic Supplementary Material



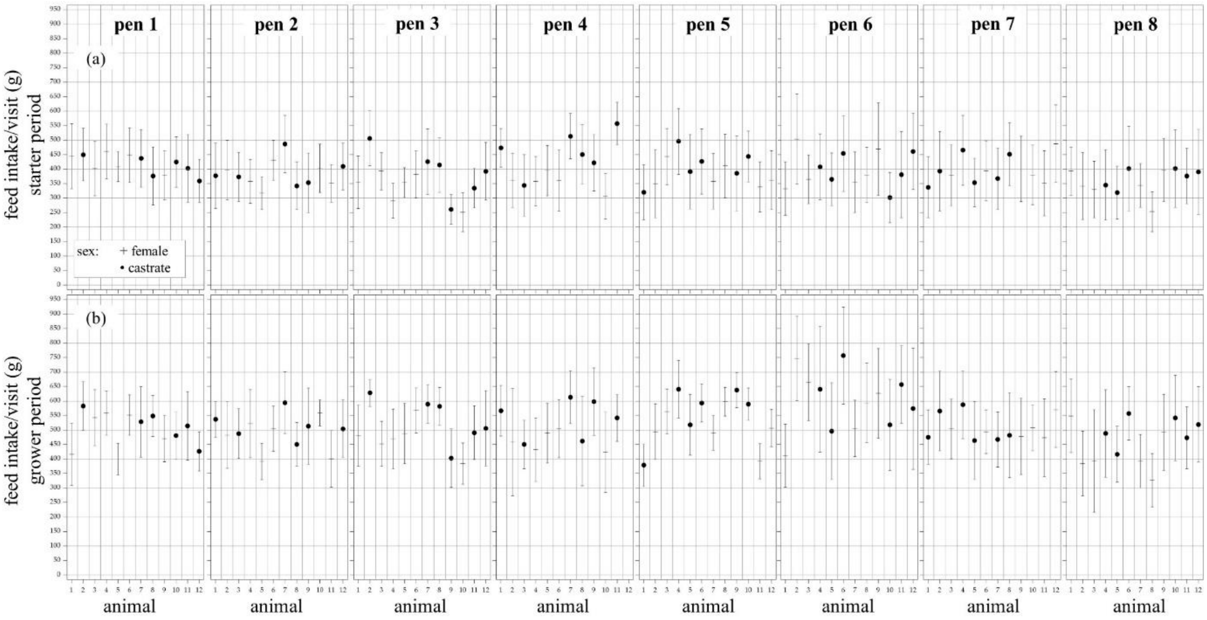
Supplementary Figure II-1. Average daily feed intake per animal and fattening period; tick marks indicate standard deviations



Supplementary Figure II-2. Average daily feed intake per feeder visit, animal and fattening period; tick marks indicate standard deviations



Supplementary Figure II-3. Average daily count of visits to feeder per animal and fattening period; tick marks indicate standard deviations



Supplementary Figure II-4. Average daily feed intake of the single feeding action with highest feed intake per animal and fattening period; tick marks indicate standard deviations

Chapter III. Examination of High-Resolution Feed Intake Data of Growing-Finishing Pigs Confronted with High Deoxynivalenol Contents Present in Their Feed

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Authors' Contributions

Peter Loibl contributed with trial design and supervision, data processing, development of the statistical model, calculation of the statistics and wrote the publication. Wolfgang Preißinger contributed with the general idea, support in trial design, and supervision of the practical feeding trial. Wilhelm Windisch delivered general support and ideas for statistical evaluation, and input on authoring the manuscript.

Summary

This publication presents the data of the second trial in fattening pigs which was part of this doctoral project. Since the first trial showed no recognisable effect of short-term disturbances on the feeding behavioural traits of group housed fatteners, it was decided to present the animals with a chronic feed-borne mycotoxin exposure. Due to wet harvesting conditions before the trial, Bavarian farmers were facing problems with Deoxynivalenol (DON)-contamination of their maize. Since the Bavarian State Research Centre for Agriculture is one of the main advisory organisations it was decided to use DON-contaminated maize to obtain additional data for consultancy next to the data focussing on feed intake. Therefore, it was decided to use two universally used mycotoxin binders. This way, four experimental groups were designed using different maize sources with either low (< 1 000 µg/kg) or high DON-contents (> 12 000 µg/kg): A control group

with lowest possible DON-contamination (resulting in on average < 900 µg/kg DON), a DON-group with highest possible DON-contamination (> 4 500 µg/kg DON) and two groups with the addition of two different mycotoxin binders to the DON-group.

The DON-contamination led to expected performance depression mostly caused by feed refusal of the animals. This way, the animals of all three DON-groups took > 30 days longer to reach slaughter weight than the control group's pigs. Interestingly, the mycotoxin binders showed no counteracting effect on the toxin. In some periods, they even significantly enhanced the depression of feed intake.

Regarding the assessed feeding behavioural traits (DFI, feed intake per visit, number of daily visits, and highest feed consumption in one visit per animal and day), however, only DFI was affect by the DON-treatments. All other parameters again were only influenced by the individual animal and the respective pen, namely the animal group of one feeder. An additional analysis of the Coefficients of Variation of the feeding data clearly showed that the addition of DON as well as mycotoxin binders led to higher variation and thereby more erratic and less consistent feeding behaviour of the animals. Therefore, DON did not influence the behavioural traits. They mainly were highly individual and depending on the animals rank within its group.

The format of text, tables, figures, and references in the following will differ from the original publication to maintain uniformity in this thesis. Some of the cited references might have been updated since the publication of the paper. The original publication is Open Access and can be downloaded under https://doi.org/10.17221/189/2020-CJAS. A copy of the publisher's permission to include the publication in this thesis can be found in the appendix.

Abstract: Modern single space-feeding systems for fattening pigs permit the detailed assessment of an individual animal’s feeding behaviour. In an experiment involving 96 fattening pigs, the influence of deoxynivalenol (DON)-contaminated feed (> 4 500 µg/kg DON) on the zoo-technical performance and feeding behaviour was compared to a feed with low DON concentration (< 900 µg/kg DON), this served as the control group. Additionally, in separate treatments, two commercial mycotoxin binders were added to the DON-contaminated feeds to assess if an expected DON effect could be attenuated. The high DON-content significantly ($P < 0.03$) reduced daily feed intake (500–600 g/d). The DON group showed 240 g less daily gain compared to the control with 728 g/d. Both mycotoxin binders were seen to have additionally depressed feed gain by approximately 65 g/d ($P < 0.01$). The treatment did not affect the individual feeding behaviour as assessed by daily visits to the feeder, feed intake per visit and the highest feed intake in one visit per day. These were influenced only by the pig and its pen, indicating that the animals developed a distinct behaviour within their respective groups. Behaviour analyses of persistency and day-to-day variation showed that the persistency was reduced and variation was increased when DON without or with binder was present. The DON-contents therefore seemed to lead to a more erratic and less consistent behaviour that remained dependent on the animal group.

Keywords: behaviour; DON; fattening pig; mycotoxin binder

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In modern swine husbandry, mycotoxins are a common feed-borne threat (Escrivá et al. 2015). The *Fusarium* family represents the most relevant source of the most hazardous mycotoxins, namely, the Trichothecenes. They include two of the most commonly observed mycotoxins, deoxynivalenol (DON) and zearalenone (ZEN) (Döll and Dänicke 2011). *Fusaria* strains mainly infect the commonly consumed feed cereals and produce either one specific toxin or several different toxins (Döll and Dänicke 2011). In feed for pigs, depending on the dose and duration of exposure, these toxins can increase the susceptibility to infectious diseases and impair reproductive performance (Antonissen et al. 2014). DON, however, primarily leads to feed refusal in pigs (Döll and Dänicke 2011). DON-contamination can cause vomiting resulting in its colloquial name 'Vomitoxin' (Young et al. 1983). The Commission of the European Communities (2006)) defines a guidance value for fattening pigs as 900 µg/kg DON above which feed intake, performance and animal health will deteriorate.

Next to cultivation, physical and chemical procedures such as washing, cleaning and the addition of e. g. ammonium hydroxide are effective ways of reducing mycotoxin contamination, but usually these measures are too costly to reduce the mycotoxin levels (Ramos and Hernandez 1997; McCormick 2013). Therefore, supplementing the feed with additives is the most common practice towards the prevention of mycotoxin intoxication. These additives utilise different modes of action. Many toxins can easily be bound by adhesion, which regularly shows no *in vivo* efficacy against deoxynivalenol (Dänicke et al. 2004). However, some *Coriobacteriaceae*-strains can metabolise DON to non-toxic metabolites, thereby reducing its hazard (Fuchs et al. 2002).

Since DON causes feed refusal in pigs, modern feeding systems could support the understanding of the mode of action regarding intoxication in its highest detail. Many state-of-the-art feeding systems allow the assessment of feed intake in the utmost detail. Automated single space feeders record every single feeding action of each animal within a group of pigs. Therefore, such a system enables the examination of possible alterations of the individual animal's feeding behaviour that will in turn decrease feed intake.

In this study, deoxynivalenol was used to examine the effect on both, zoo-technical performance and feeding behaviour of group-housed growing-finishing-pigs fed with automated single space feeders. Since probable alteration of performance and behaviour could be caused by either toxicity of DON, negative sensory properties, or other unknown effects, it was also pertinent to include two commercial mycotoxin binders in the study. The examination comprised a low-contaminated feed group, which served as a control and three highly DON-contaminated feed groups with or without mycotoxin binders.

MATERIAL AND METHODS

Ethics

The trial was officially approved by the responsible state authorities (reference number 2532-2-68, Government of Lower Franconia, Germany).

Experimental Design

Animal Material and Stable. A feeding trial was conducted using 96 fattening pigs [(German Large White x German Landrace) x Piétrain] at the experimental site of the Bavarian State Research Centre for Agriculture. For adaptation, pigs (27.7 ± 2.3 kg) were placed one week before trial commencement in 8 separate pens (12 animals each, 5.0 × 2.6 m) with fully slatted floors. Each pen contained one single space automatic feeding system (Schauer Compident MLP). The animals were distributed over eight pens in such a way that four pens each were filled with animals either above or below the median body weight, respectively, considering sex (female to male-castrated 1 : 1) and the litters were equally distributed over all pens.

In each pen one feeding station recorded the time when an animal entered the feeder and the then consumed amount of feed. The duration of a single visit could not be obtained due to technical reasons.

Feeds. The feeding procedure followed practical standards and included a starter diet from days 1 to 48, a grower diet from days 49 to 90, and a finisher diet from day 91 until the end of the experiment (day 153), respectively. Table III-1 shows the detailed diet compositions as well as the analysed nutrient contents of all experimental diets. The diets were fed as a dry coarse meal and consisted of maize (50.0%), Barley (28.0%, 34.5%, 39.0%) and soybean meal (48% CP, 19.0%, 13.0%, 9.0%). A standard macro-premix, which contained synthetic amino acids, was supplemented by 3.0%, 2.5% and 2.0% inclusion rates through starter, grower and finisher period. These adaptations to maturation led to an analysed energy content of 13.7 MJ–13.6 MJ ME standardised on 88 % DM and a crude protein content of 15.0%–13.5%/kg feed during the fattening process. In total, all diets met nutritional requirements (GfE 2006). The differences in nutrient content ranged within analytical tolerances.

The starter, grower and finisher diet were modified at four levels by using maize with low or high contamination (on average 1.0 and 12.1 mg/kg, respectively) of DON (deoxynivalenol) and dietary inclusion of mycotoxin binders (at the expense of barley) according to the following experimental design. Two pens each were assigned to the following treatments:

1. CONTROL (pen 1 and 2): Minimal content of DON, no addition of mycotoxin binders
2. DON (pen 3 and 4): > 4 500 µg/kg DON, no addition of mycotoxin binders

3. DON+Binder I (pen 5 and 6): > 4 500 µg/kg DON, addition of 4.0 g/kg Mycofix PLUS 3.EG (Biomin Holding GmbH, Getzersdorf, Austria)
4. DON+Binder II (pen 7 and 8): > 4 500 µg/kg DON, addition of 2.8 g/kg Mycofix PLUS BBSH (Biomin Holding GmbH, Getzersdorf, Austria)

Binder I primarily consisted of adhesive substances to bind mycotoxins, whereas Binder II also comprised a microorganism capable of producing a non-toxic DON-metabolite. The detailed diet formulas are shown in Table III-1.

Table III-1. Composition of the experimental diets and the respective analysed nutrient contents (standardised on 88% dry matter (DM)) of all treatments in the start, grower, and finisher phase

period	starter				grower				finisher			
treatment	Control	DON	DON+ BinderI	DON+ Binder II	Control	DON	DON+ BinderI	DON+ Binder II	Control	DON	DON+ Binder I	DON+ Binder II
diet composition (%)												
barley	28.0	28.0	27.6	27.7	34.5	34.5	34.1	34.2	39.0	39.0	38.6	38.7
maize (~1 000 µg/kg DON)	50.0	---	---	---	50.0	---	---	---	50.0	---	---	---
maize (~12 000 µg/kg DON)	---	50.0	50.0	50.0	---	50.0	50.0	50.0	---	50.0	50.0	50.0
soybean meal (48% CP)	19.0	19.0	19.0	19.0	13.0	13.0	13.0	13.0	9.0	9.0	9.0	9.0
macropremix[1]	3.0	3.0	3.0	3.0	2.5	2.5	2.5	2.5	2.0	2.0	2.0	2.0
Binder I	---	---	0.4	---	---	---	0.4	---	---	---	0.4	---
Binder II	---	---	---	0.28	---	---	---	0.28	---	---	---	0.28
analysed nutrient contents standardised on 88% DM												
ME (MJ)	13.7	13.8	13.7	13.8	13.6	13.6	13.6	13.7	14.1	13.4	13.6	13.7
CF (g/kg)	29	26	26	27	30	28	28	25	21	29	30	26
CP (g/kg)	148	148	153	150	142	146	142	142	126	140	138	136
Lys (g/kg)	9.7	11.1	9.9	10.7	8.5	8.3	8.1	8.9	7.6	7.2	6.7	7.5
Met+Cys (g/kg)	5.5	6.1	5.7	5.7	5.4	5.3	5.4	5.9	5	4.6	4.5	5.1
Thr (g/kg)	6.9	7.3	7.1	7.1	6.4	6.3	6.2	6.5	5.6	5.7	5.5	5.8
Trp (g/kg)	1.4	1.7	1.5	1.8	1.8	1.8	1.7	1.7	1.5	1.6	1.7	1.4
Ca (g/kg)	6.6	6.5	7.1	6.0	6.2	6.7	5.6	6.2	4.4	5.6	4.8	4.6
P (g/kg)	4.0	3.9	4.0	4.2	4.0	4.2	3.8	3.7	2.8	4.1	3.9	3.7

Ca = calcium; CF = crude fibre; CP = crude protein; Cys = cysteine; DM = dry matter; DON = deoxynivalenol; Lys = lysine; ME = metabolisable energy; Met = methionine; P = phosphorus; Thr = threonine; Trp = tryptophane

[1] mineral feed contained the following AA: 10% Lys, 3% Met, 4% Thr, 0.5% Trp

The feed was produced on site. The feed was sampled daily and merged weekly. These pooled samples were analysed for deoxynivalenol using HPLC-MS/MS at Romer Labs (protocol-number AT-SOP31, Romer Labs Diagnostic GmbH–Europe, Tulln, Austria). The inclusion of the additives was examined by the supplier. Nutrient contents were analysed at the institution's own laboratory following the respective VDLUFA (2012) methods.

The four experimental diets were distributed among the four pens with light as well as with heavy animals and were fed ad libitum throughout entire study. From day 91 onwards animals reaching 115–120 kg live weight were slaughtered in the institute's slaughterhouse. The experiment was concluded on day 153 and all remaining animals were slaughtered irrespective of live weight.

Experimental Parameters. All parameters were measured individually for each animal. The feeding stations recorded the number of visits at the feeder and the respective feed intake (g) within each visit. Daily feed intake (DFI) was calculated by summing up the feed consumption of daily visits. Feeding actions with less than 5 g were excluded from the analyses.

The pigs were weighed every second week to assess weight development. Thereof, daily weight gain (DWG, g) was calculated. The Feed Conversion Ratio (FCR, kg feed per kg gain) was calculated as quotient of DFI and daily gain.

Slaughter started on experimental day 91. Since the elimination of individuals from animal groups might affect social behaviour and hence also feeding behaviour, feeding actions at the feeders were assessed only until experimental day 91. Measurements included daily means of feed intake per visit, counts of daily feeder visits and the maximum feed quantity consumed within one visit in a day.

At slaughter, the muscle and fat area of the chops (cm^2) and the lean meat content (%) were measured according to the guidelines for assessment of fattening performance of the ‘Central Association of German Pig Production’ (Zentral Verband der Deutschen Schweineproduktion 2007).

Statistical Analyses

Zootechnical performance data were analysed according to the following hierarchical model:

$$y_{ijkr} = \mu + treatment_i + pen_j\ (treatment)_i + sex_k\ (pen,\ treatment)_{ij} + e_{ijkr}\ (1)$$

where:

y_{ijkr} – dependent performance variable;

μ – grand mean of the observations;

e – random error.

Factors in brackets indicate nested parameters. Factor *sex (pen, treatment)* was tested against overall deviation. *Pen (treatment)* was tested against *sex (pen, treatment)* and *treatment* against *pen (treatment)*.

For feeding behavioural measures, the following model was used:

$$y_{ijklr} = \mu + treatment_i + pen_j\ (treatment)_i + sex_k\ (pen, treatment)_{ij} + animal_l\ (sex, pen, treatment)_{ijk} + e_{ijklr}\ (2)$$

where:

y_{ijklr} – dependent behaviour variable;

μ – grand mean of the observations;

e – random error.

Animal (sex, pen, treatment) was tested against residual deviation, and then factor *sex (pen, treatment)* was tested against *animal (sex, pen, treatment),* and so on.

The persistency of feed intake parameters (DFI, feed intake per visit to the feeder, count of visits to feeder and highest feed intake within one feeding event per day) from the starter upon the grower period was assessed by calculating the Pearson's Correlation Coefficients. Therefore, means for the respective parameters and periods were calculated for each animal. The correlation was then analysed for each pen. Persistency was expected to result in a statistically significant correlation.

The coefficients of variation (CV) of feed intake parameters were calculated for each animal and averaged per fattening period. Therefore, a possible influence of DON levels and binder addition on persistency of feeding behaviour was assessed. Possible statistical effects were identified using the model (1).

SAS v9.4 (SAS Institute, Cary, United States of America) was used for all statistical analyses and graphs. An examination for homoscedasticity of the data was routinely conducted before statistical testing. In case of heteroscedasticity no test was conducted. A *P*-value < 0.05 was considered statistically significant. Significant differences between pens were identified using the Student Newman's Keul's test.

RESULTS

Five animals were excluded from the trial and statistical analysis. The exclusion was not treatment related.

Deoxynivalenol Contents

Figure III-1 presents the time course of analysed DON concentrations in feed samples. The control feed contained on an average 904 µg/kg DON (min: 624 µg/kg, max: 1 316 µg/kg). The DON levels of treatment groups DON, DON+Binder I, and DON+Binder II ranged around 5 609 µg/kg, 6 370 µg/kg, and 6 446 µg/kg with lowest levels approximately 4 000 µg/kg between experimental days 71 to 91. In total, dietary

DON concentrations of DON groups exceeded the control level by factor 7 on average ($P < 0.01$). The recovery of the binder products was on target in all feed samples (94–107%)

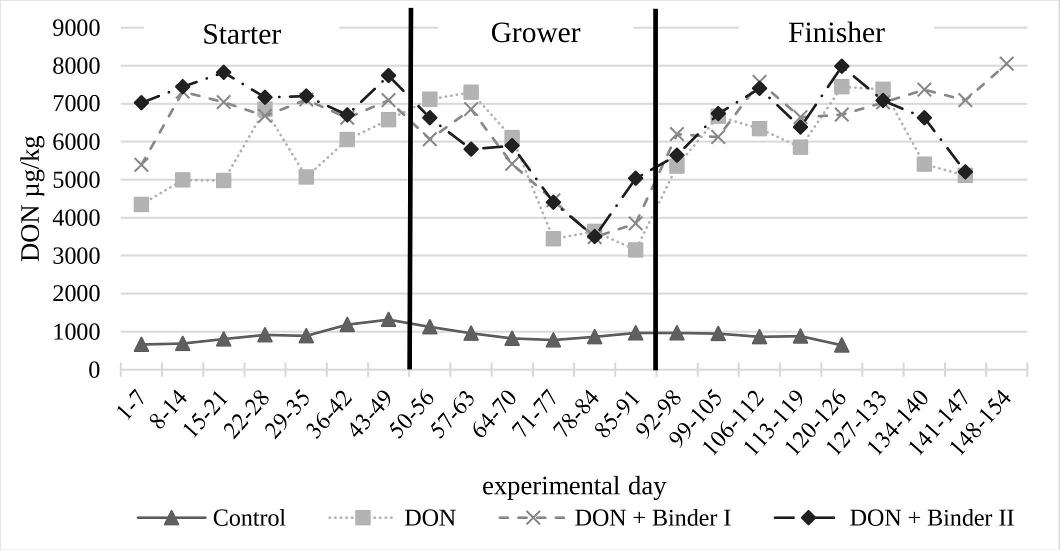

Figure III-1. Time course of concentrations of deoxynivalenol in the control group, DON, DON+Binder I, and DON+Binder II diets

Zootechnical Performance

Table III-2 summarises the zootechnical performance of the four treatment groups. Treatments DON, DON+Binder I, and DON+Binder II had a reduction in DWG, DFI, meat and fat area in the chops and declined the FCR in the grower period. In comparison to DON, the addition of Binder I and Binder II further depressed the animal weight at the end of the grower and finisher phase as well as final weight and overall DWG.

The statistical factor *pen (treatment)* was found to be statistically relevant only for the FCR in the grower phase. As expected, the pens' starting weight differed significantly since half of the pens were preselected for either light-weight and or heavy-weight animals. Factor *sex (treatment, pen)* was statistically relevant in parameters exhibiting sex dimorphism (weight, DWG, overall feed intake).

Grouping Factors within the Feeding Behavioural Data

Table III-3 presents the feeding behavioural patterns at pen level. DFI was depressed by DON, DON+Binder I and DON+Binder II. Within treatments, the pens showed no difference.

For the other feeding behavioural parameters, however, the pen showed prominent, significant effects ($P < 0.05$). For example, pen 5 showed the lowest feed intake per visit (86 g/visit from day 1 to 91) whereas pen 7 showed the highest (202 g/visit). Subsequently, these two pens also showed the highest (15 visits per day, pen 5) and lowest counts of daily feeder visits (8 visits per day, pen 7). This indication found for feed intake per visit was also found for the highest feed intake within one daily visit. Pen 5 averaged at the lowest value (280 g per visit, day 1–91) whereas pen 7 reached the highest value (516 g per visit, day 1–91).

Sex (treatment, pen) did not affect behavioural parameters except DFI. *Animal (treatment, pen, sex)* demonstrated highly significant influence on all parameters presented in Table III-3.

Correlation Analyses and Examination of CVs of Feeding Parameters

Supplementary Figure III-1 to Supplementary Figure III-4 in electronic supplementary material (ESM) show means and standard deviations of each pig of the assessed feeding behavioural measures, DFI, feed intake per visit to the feeder, count of daily feeder visits and the highest amount of feed consumed in one visit per day. Standard deviations ranged on average at 29% for DFI, 98% for feed intake per visit to the feeder, 33% for the count of daily feeder visits and 348% for most consumed feed in one visit per day, relative to the respective means.

Table III-2. Zootechnical performance results (weight, daily weight gain, overall feed intake, feed conversion ratio, meat quality) for the low DON-contaminated feed Control, and the highly-DON-contaminated feed DON groups with respective mycotoxin binders

treatment	Control	DON	DON+ Binder I	DON+ Binder II	overall	SEM	*P-value*		
							treatm.	pen	sex
number of animals, individual fattening period									
Animals (n)	24	22	22	23	91	---	---	---	---
Duration (days)	115^{b}	148^{a}	149^{a}	145^{a}	137	0.75	<0.01	0.95	<0.01
Weight (kg)									
Day 1	33.0	33.0	32.6	32.6	32.8	0.22	1.00	<0.01	0.21
Day 49	67.9^{a}	54.2^{b}	47.5^{c}	46.9^{c}	54.3	0.63	<0.01	0.79	<0.01
Day 91	100.9^{a}	79.8^{b}	74.3^{c}	73.5^{c}	82.4	0.91	<0.01	0.86	<0.01
Final	114.6^{a}	103.8^{b}	94.7^{c}	93.8^{c}	102.0	1.09	<0.01	0.91	<0.01
Daily weight gain (g/day)									
Starter	712^{a}	441^{b}	302^{c}	282^{c}	439	12.19	<0.01	1.00	<0.01
Grower	785^{a}	608^{b}	639^{b}	634^{b}	669	11.14	0.04	0.87	<0.01
Finisher	595^{a}	431^{b}	360^{b}	378^{b}	443	12.73	<0.01	0.78	0.08
Overall	728^{a}	488^{b}	424^{c}	423^{c}	519	8.77	<0.01	1.00	<0.01
Overall feed intake (kg)									
Starter	77.9^{a}	50.9^{b}	40.4^{c}	41.2^{c}	53.0	1.11	<0.01	0.60	0.02
Grower	90.1	69.1	69.9	76.2	76.6	1.26	0.13	0.69	<0.01
Finisher	48.7^{b}	92.6^{a}	82.1^{a}	83.3^{a}	75.3	1.64	<0.01	0.93	<0.01
Overall	213.7	212.6	192.4	200.7	205.0	3.01	0.36	0.38	0.15
Feed conversion ratio (kg/kg)									
Starter	2.3	2.5	3.1	3.5	2.8	0.09	0.27	0.07	0.19
Grower	2.6^{a}	2.0^{b}	1.9^{b}	2.1^{b}	2.2	0.05	0.04	<0.01	0.99
Finisher	3.8	4.2	5.7	5.1	4.7	0.19	0.11	0.68	0.03
Overall	2.7	3.0	3.3	3.5	3.1	0.05	0.10	0.28	0.14
Meat quality measures									
Muscle area (cm^2)	55^{a}	51^{b}	45^{c}	45^{c}	49	0.62	0.01	0.75	0.17
Fatty area (cm^2)	15^{a}	13^{b}	12^{b}	12^{b}	13	0.30	<0.01	1.00	<0.01
Lean meat (%)	60	60	60	59	60	0.19	0.69	0.82	<0.01

DON = Deoxynivalenol, SEM = Standard Error of the Mean;
$^{a\text{-}c}$superscripted letters indicate significant *pen* differences ($P < 0.05$)
p-values are from hierarchical ANOVA where each source of variance was tested against the column on the right;

Table III-4 presents the persistency assessment of feeding parameters from starter to grower period as performed via correlation analyses within pens. The Pearson Correlation Coefficients ranged between −0.18 and 0.91. The control and DON pens 1 through 4 showed a correlation higher than 0.58 in nearly all parameters almost all the time. For the DON+Binder I and DON+Binder II pens the situation was more diverse. Pen 6 (Binder I), 7, and 8 (Binder II) did not show a significant correlation for both, counts of daily visits at the feeder and highest feed intake within one visit per day. The DFI of animals of pen 7 and 8 did not correlate as well as feed intake per visit for pen 4 and 6.

Table III-3. Overall results of feeding behavioural measures (daily feed intake, number of daily visits to the feeder per animal, the overall amount of consumed feed per visit and highest consumed amount feed per visit per day) for the low DON-contaminated control group and the highly-DON-contaminated feed DON groups with respective mycotoxin binders

Treatment	Control		DON		Binder I		Binder II		Overall	SEM	*P-value*			
Pen	1	2	3	4	5	6	7	8			treatm.	pen	sex	animal
Daily feed intake (kg/d)														
Starter	1.6^{A}	1.7^{A}	1.1^{B}	1.0^{B}	0.9^{D}	0.8^{D}	1.0^{C}	0.8^{C}	1.1	0.006	<0.01	0.41	0.03	<0.01
Grower	2.2	2.2	1.7	1.7	1.8	1.7	2.1	1.6	1.9	0.008	0.13	0.70	<0.01	<0.01
Days 1–91	1.9^{A}	1.9^{A}	1.4^{B}	1.3^{B}	1.3^{C}	1.2^{C}	1.5^{B}	1.2^{B}	1.5	0.007	0.03	0.51	<0.01	<0.01
Feed intake per visit (g)														
Starter	168^{a}	125^{b}	87^{d}	91^{c}	69^{e}	94^{c}	121^{b}	70^{e}	103	0.47	0.14	<0.01	0.70	<0.01
Grower	182^{c}	157^{d}	127^{f}	140^{e}	100^{g}	190^{b}	312^{a}	156^{d}	160	0.79	0.48	0.01	0.12	<0.01
Days 1–91	175^{b}	140^{c}	105^{e}	115^{d}	86^{f}	139^{c}	202^{a}	108^{e}	130	0.47	0.44	0.01	0.08	<0.01
Count of visits to the feeder per animal and day (n/d)														
Starter	10^{c}	13^{a}	13^{a}	11^{b}	13^{a}	9^{d}	8^{d}	11^{b}	11	0.06	0.79	<0.01	0.69	<0.01
Grower	12^{c}	14^{b}	13^{b}	12^{c}	18^{a}	9^{e}	7^{f}	10^{d}	12	0.06	0.51	<0.01	0.63	<0.01
Days 1–91	11^{d}	14^{b}	13^{b}	11^{c}	15^{a}	9^{e}	8^{f}	11^{d}	11	0.05	0.64	<0.01	0.68	<0.01
Feed intake of the most extreme feeding action per animal and day (g)														
Starter	392^{a}	353^{b}	285^{c}	260^{d}	202^{f}	225^{e}	341^{b}	168^{g}	281	1.82	0.22	<0.01	0.44	<0.01
Grower	528^{b}	494^{c}	460^{d}	442^{d}	371^{f}	462^{d}	707^{a}	393^{e}	488	2.50	0.71	0.03	<0.01	<0.01
Days 1–91	455^{b}	418^{c}	365^{d}	344^{e}	280^{f}	334^{e}	516^{a}	272^{f}	376	2.00	0.58	<0.01	0.05	<0.01

DON = Deoxynivalenol, SEM = Standard Error of the Mean;

$^{A\text{-}D}$Superscripted capital letters indicate significant *treatment* differences; $^{a\text{-}g}$superscripted letters indicate significant *pen* differences;

p-values are from hierarchical ANOVA where each source of variance was tested against the column on the right; the animal was tested against the overall data variation.

Table III-5 summarises the average CVs of feeding behavioural parameters of the individuals per pen. Overall, the coefficients ranged between ~17% to ~55%. For DFI, feed intake per visit and highest feed intake per feeding event per day the control's CV was always lowest. For count of daily feeder visits the observation was more diverse. The control, DON and DON+Binder I group ranged on a similar level (~31%) while DON+Binder II group was approximately 7% higher. Pen 7 showed the highest variation (24.3%–68.1%) in nearly all parameters throughout the experimental time and pen 1 the lowest (17.7%–34.4%)).

Table III-4. Pearson Correlation Coefficients for the individual animal's means of feeding behavioural parameters (daily feed intake, feed intake per visit, number of daily visits, and maximum amount of feed consumed per visit) from starter to grower period averaged over each pen

Treatment	Pen	Pearson Correlation Coefficient	Pen	Pearson Correlation Coefficient
		daily feed intake (g)		feed intake per visit to feeder (g)
Control	1	0.77*	1	0.68*
	2	0.91*	2	0.85*
DON	3	0.64*	3	0.87*
	4	0.67*	4	0.40
DON+Binder I	5	0.87*	5	0.79*
	6	0.70*	6	0.08
DON+Binder II	7	0.47	7	0.72*
	8	-0.18	8	0.65*
overall		0.68*		0.54*
		count of visits to feeder (n/d)		highest feed intake in one visit (g)
Control	1	0.75*	1	0.58*
	2	0.81*	2	0.69*
DON	3	0.80*	3	0.69*
	4	0.61*	4	0.60*
DON+Binder I	5	0.68*	5	0.73*
	6	0.19	6	0.18
DON+Binder II	7	0.38	7	0.44
	8	0.35	8	0.30
overall		0.70*		0.59*

DON = Deoxynivalenol

*significant correlation coefficient $P < 0.05$

Table III-5. Means of coefficients in variation of the feeding behavioural traits (daily feed intake, feed intake per visit, number of daily visits and maximum amount of feed per visit) of the low DON-contaminated feed control and the highly DON-contaminated feed DON groups with added respective mycotoxin binders

treatment	Control		DON		Binder I		Binder II		Over-all	*P*-value		
pen	1	2	3	4	5	6	7	8		treat.	pen	sex
daily feed intake (kg/d)												
Starter (%)	25.1^{C}	23.7^{C}	25.3^{C}	25.0^{C}	37.9^{B}	35.9^{B}	50.5^{A}	42.3^{A}	33.2	<0.01	0.69	0.02
Grower (%)	18.0^{C}	21.2^{C}	33.4^{A}	33.7^{A}	33.4^{AB}	28.4^{AB}	24.3^{B}	29.5^{B}	27.5	0.02	0.26	0.44
Days 1–91 (%)	21.6^{C}	22.4^{C}	29.3^{B}	29.4^{B}	35.7^{A}	32.2^{A}	37.4^{A}	35.9^{A}	30.3	<0.02	0.90	0.11
feed intake per visit (g)												
Starter (%)	34.4^{c}	37.3^{c}	37.6^{c}	35.1^{c}	40.1^{bc}	44.8^{bc}	68.1^{a}	48.8^{b}	43.4	0.10	<0.01	0.99
Grower (%)	27.1^{C}	27.5^{C}	39.2^{B}	35.7^{B}	39.7^{B}	38.5^{B}	47.4^{A}	46.5^{A}	37.5	<0.01	0.87	0.67
Days 1–91 (%)	$30.7^{f, D}$	$32.4^{ef, D}$	$38.4^{cd, C}$	$35.4^{de, C}$	$39.9^{cd, B}$	$41.6^{c, B}$	$57.8^{a, A}$	$47.7^{b, A}$	40.5	0.02	<0.01	0.89
count of visits to the feeder per animal and day (n/d)												
Starter (%)	31.3^{b}	36.9^{b}	33.1^{b}	29.2^{b}	31.9^{b}	33.6^{b}	59.3^{a}	30.6^{b}	35.9	0.60	<0.01	0.93
Grower (%)	29.4	29.9	31.5	29.4	27.4	28.9	35.9	32.4	30.6	0.08	0.36	0.64
Days 1–91 (%)	30.4^{b}	33.4^{b}	32.3^{b}	29.3^{b}	29.7^{b}	31.2^{b}	47.6^{a}	31.5^{b}	33.3	0.48	<0.01	0.93
maximum feed intake on one visit per animal and day (g)												
Starter (%)	27.2^{e}	32.0^{de}	35.2^{cd}	34.6^{cd}	40.9^{bc}	40.7^{bc}	62.8^{a}	45.6^{b}	39.9	0.06	<0.01	0.33
Grower (%)	$14.7^{d, D}$	$19.4^{cd, D}$	$24.8^{bc, C}$	$22.5^{bc, C}$	$28.1^{b, B}$	$37.6^{a, B}$	$37.5^{a, A}$	$38.2^{a, A}$	27.8	0.02	0.04	0.60
Days 1–91 (%)	$20.9^{f, D}$	$25.7^{ef, D}$	$30.0^{de, C}$	$28.5^{de, C}$	$34.5^{cd, B}$	$39.2^{bc, B}$	$50.1^{a, A}$	$41.9^{b, A}$	33.8	0.01	0.04	0.61

DON = Deoxynivalenol; SEM = Standard Error of the Mean;

$^{A\text{-}D}$Superscripted capital letters indicate significant *treatment* differences; $^{a\text{-}f}$superscripted letters indicate significant *pen* differences

P-values are from hierarchical ANOVA where each source of variance was tested against the column on the right; the animal was tested against the overall data variation.

DISCUSSION

This study investigated the effects of high deoxynivalenol-contents in diets on the zoo-technical performance as well as feed intake behaviour of fattening pigs. Three feeding groups with either high deoxynivalenol contents (> 4 500 µg/kg) or additionally supplemented with two commercial mycotoxin binders were designed and compared to a low-contaminated control group (< 900 µg/kg). High resolution feeding data of group-housed grower-finishers were used to assess the feeding behaviour of each pig. The data were analysed using nested ANOVA models to determine the main sources of deviation. The nutrient composition where on similar levels, therefore, an effect of the diet other than the DON-contamination can be excluded (Table III-1).

Zootechnical Performance

The control group's overall zoo-technical performance was quite low (728 g/d). Loibl et al. (2020) using the same stable and genetics reached higher gains (overall ~860 g/d). The still high DON levels could be one reason for this reduction. They ranged around ~900 µg/kg, which is exactly the threshold of possible alterations regarding performance (The Commission of the European Communities 2006).

Sex (pen, treatment) was seen to significantly influence nearly all parameters excluding FCR. Male-castrates and females differed in feed intake, thereby resulting in different DWG, whereas feed conversion remained unchanged (Hale and Southwell 1967; Pichler et al. 2020). Also, the carcass parameters differed between the sexes (Hale and Southwell 1967). This was the case for all carcass traits excluding the muscular area.

A significant *Pen (treatment)* effect became visible regarding FCR in the grower period. Pen 6 showed significantly lowest FCR in that period (1.79 kg/kg, data not shown in detail) probably caused by compensatory growth (Kirchgeßner et al. 2014) due to reduced DON levels following day 71. Starting weights of pens were significantly different since the animals of each treatment were divided into a light-weight and a heavy-weight group. This is common practice in feeding trials to increase the homogeneity and decrease initial variation of the animal material to emphasise the recovery of possible treatment effects (Köhler et al. 2007). After day 35, no statistical difference was recognisable, probably due to increasing weight variation within the respective animal groups.

Next to the expected effect of *sex (pen, treatment)* the presence of DON and mycotoxin binders (statistical factor *treatment*) seemed to have a prominent effect on nearly all performance parameters. The high DON-contents significantly impaired DWG by > 200 g/d throughout the examination period. This led to significantly longer fattening periods as well as reduced slaughter weights. DON typically causes feed refusal as well as immunological problems, vomiting, or skin dermatitis (Pestka 2007). Goyarts and

Dänicke (2005) postulated that the reduced feed consumption caused by DON-contamination of feed subsequently reduced daily gains. This was observed in the present study as well. More severe reactions, however, were not noted. The control's higher overall feed intake led to increased DWG throughout the trial. The control pigs were slaughtered ~30 days earlier than the rest explaining the reduced feed intake in the finisher period. Overall, all animals consumed nearly the same amount of feed.

Treatment affected FCR only in the grower period. The DON groups showed low ratios around 2.00 kg/kg, whereas the control group ranged on a usual 2.57 kg/kg. This seems to contradict previous observations that DON impairs FCR (leads to higher ratios) (Döll and Dänicke 2011). However, dietary DON-contents decreased during the last three weeks of the grower period below 4 000 µg/kg in all three DON groups (Figure III-1). As a consequence of relief from exposure to DON, animals seemed to exert compensatory growth, which also entails reduced FCR (Kirchgeßner et al. 2014). Additionally, the FCR increases with maturation and the change in composition of the accreted body mass (Kirchgeßner et al. 2014). Since the animals fed with DON contaminated feed were significantly lighter this also might be a reason for the reduced FCR in the grower phase.

The faster growth of the control animals led to differences in maturity (at slaughter, DON animals weighed ~97.4 kg, whereas control pig reached 114.6 kg) and thereby altered carcass composition, especially the reduced fat area in the chops. During growth, protein accretion switches toward fat accretion in pigs (Kirchgeßner et al. 2014).

The addition of mycotoxin binders further decreased DWG, overall and in the starter period. Although, the products differ in composition, statistically their effect was identical. The DON-detoxifying potential of most commercial adhesive mycotoxin binder products (like Binder I) is described to be somewhat limited (Dänicke et al. 2004). *Coriobacterium* BBSH 797 (included in Binder II) metabolises DON to non-toxic De-Epoxy-deoxynivalenol (Schatzmayr et al. 2006). However, compared with Binder I, Binder II did not result in any improvement. One could hypothesise that DON and the detoxifying additives affect taste with the same effect on feed intake. In a review of previous studies by Döll and Dänicke (2004), no significant additional negative effects on feed intake due to the addition of similar mycotoxin binders to DON-contaminated pig diets were noted. Alternatively, as another possible explanation, the high DON levels in the DON groups might have exceeded the detoxifying potential of applied dose of *Coriobacteria*.

Time Patterns and the Examination of Grouping Factors within the Feeding Behavioural Parameters

The observed behavioural traits except DFI, ranged on similar levels as previously published (Kallabis and Kaufmann 2012). Loibl et al. (2020) examined the feeding behaviour of fattening pigs confronted with short term deviations in stable routine and observed similar behavioural traits in the same stable with identical genetics.

As observed by Loibl et al. (2020), *animal (sex, pen, treatment)* was found to be the predominant cause of variation in all feeding behavioural parameters.

Sex (treatment, pen) influenced DFI significantly and feeding actions with highest feed intake per day during the grower period and overall. This was expected as female pigs show slower growth and reduced feeding capacity than male -castrates (Cole et al. 1968). A very similar situation was recently found by Pichler et al. (2020).

The DON levels of > 4 500 µg/kg of feed with or without inclusion of binders (factor *treatment*) clearly influenced DFI in the starter period and over the first 91 days. Despite its clear effects on zoo-technical performance, no treatment effect was found for all other behavioural parameters. Döll and Dänicke (2011) reviewed several dose response studies and found that per 1 000 µg/kg feed intake was reduced by 5.4%. Levels below the European Commission’s guidance value of 900 µg DON per kg feed (The Commission of the European Communities 2006) did not alter feed intake. The reduction in feed intake during the starter period and overall met this correlation. The average weekly pooled contents ranged > 7 000 µg/kg DON significantly decreased feed intake by ~43% in the three DON groups. From day 1–91, feed intake was reduced by 30%. In the second half of the grower phase the mycotoxin-contents in the DON groups dropped to ~4 000 µg/kg, which seemed to immediately lead to higher feed intake and subsequently the depression of feed intake ranged at insignificant 18%.

The addition of mycotoxin binders seemed ineffective. In fact, the Binder I product was seen to even further cause a reduction in feed intake. Since the product was added according to the supplier’s recommendation no decrease in feed intake was expected. The causes of this observation therefore remain uncertain although Dänicke et al. (2004) using a precursor of the Binder I product found an insignificant but similar effect on the DFI. The different modes of actions of both binders again showed no improvement in comparison to the DON group.

Pen (treatment) was a significant source of variation of all other behavioural traits, such as feed intake per feeder visit, count of daily feeder visits per animal and highest feed consumption within one visit per day. Loibl et al. (2020) observed a remarkably similar situation. In both studies pen equalled the feeder and comprised a group of 12 animals. Pigs quickly develop a clear and constant hierarchy within a group (Ewbank 1976). Even when confronted with chronic Vomitoxin intoxication this trend seems to remain the same. The factors *animal (sex, pen, treatment)* and *pen (treatment)* were therefore the most prominent sources of variation. Probably, the individual animal developed a distinct feeding behaviour according to its rank in its respective groups. The recently published study of Pichler et al. (2020) seems to confirm this finding. In their study, on average only five animals were allotted to each feeder. The numbers of daily visits were much higher and, correspondingly, feed intake per visit was lower than in our study. This again suggests that group feeding behaviour depends on (hardly predictable) somewhat persistent behavioural patterns of individual animals.

Correlation Analyses and Examination of the CV of Feeding Parameters

The individual animals showed wide deviations in the mean values of the assessed behavioural traits (Supplementary Figure III-1 to Supplementary Figure III-4 (ESM)). To examine the persistency from starter to grower period of feeding behaviour the Pearson's Correlation Coefficients were calculated for each pen (Table III-4). They ranged between −0.18 and 0.91 and 28% of the correlations were insignificant. The two control pens showed significant and highest correlation in almost all parameters, whereas pen 7 and pen 8 showed the lowest, often insignificant, correlations. The exposure to diets with high DON contents therefore might have led to more erratic and less persistent feeding behaviour of the individual animal, probably due to the increased variation.

Looking at detailed mean values including standard deviations (Supplementary Figure III-1 to Supplementary Figure III-4 in ESM) all parameters showed comparably high day-to-day-variation. The addition of DON alone or combined with a binder might have led to increased day-to-day variation. Consequently, the CV of DFI, feed intake per visit, and highest consumed amount of feed on one feeder visit was increased (Table III-5). However, the count of daily visits was influenced only by the pen. We observed prominent effects of DON and binders on zoo-technical performance and DFI. This leads to the assumption that the increased levels of deoxynivalenol with or without binder caused a directed alteration of the animals' behaviour that could only be identified by examining the means of the observed parameters. This combined with the distinct difference between the CVs of the treatment groups (the control mostly lowest, DON+Binder II highest) raises the conclusion that the animals' feeding behaviour became more erratic due to the contamination of DON or the addition of a mycotoxin binder.

CONCLUSION

This study examined the influence of DON-contaminated maize based diets (either without or with two binders) on the zoo-technical performance and feeding behaviour of fattening pigs. It was shown that chronic DON-intoxication (irrespective of added mycotoxin binders) reduced the zoo-technical performance significantly but did not influence feeding behavioural traits such as daily feeder visits, feed intake per feeder visit and highest feed consumption in one visit. However, the DON-contamination and binder addition significantly increased the day-to-day variation in these parameters. This led to less persistency of the animals' behaviour as well as increased day-to-day variation. The behaviour of the fatteners, therefore, became more erratic due to the addition of DON independent of the addition of the mycotoxin binder.

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Conflict of interest

The authors declare no conflict of interest.

ELECTRONIC SUPPLEMENTARY MATERIAL

Supplementary Figure III-1. Average daily feed intake per animal and fattening period; tick marks indicate standard deviations

Supplementary Figure III-2. Average daily feed intake per feeder visit, animal and fattening period; tick marks indicate standard deviations

Supplementary Figure III-3. Average daily count of visits to feeder per animal and fattening period; tick marks indicate standard deviations

Supplementary Figure III-4. Average daily maximum feed intake within one feeding action per animal and fattening period; tick marks indicate standard deviations

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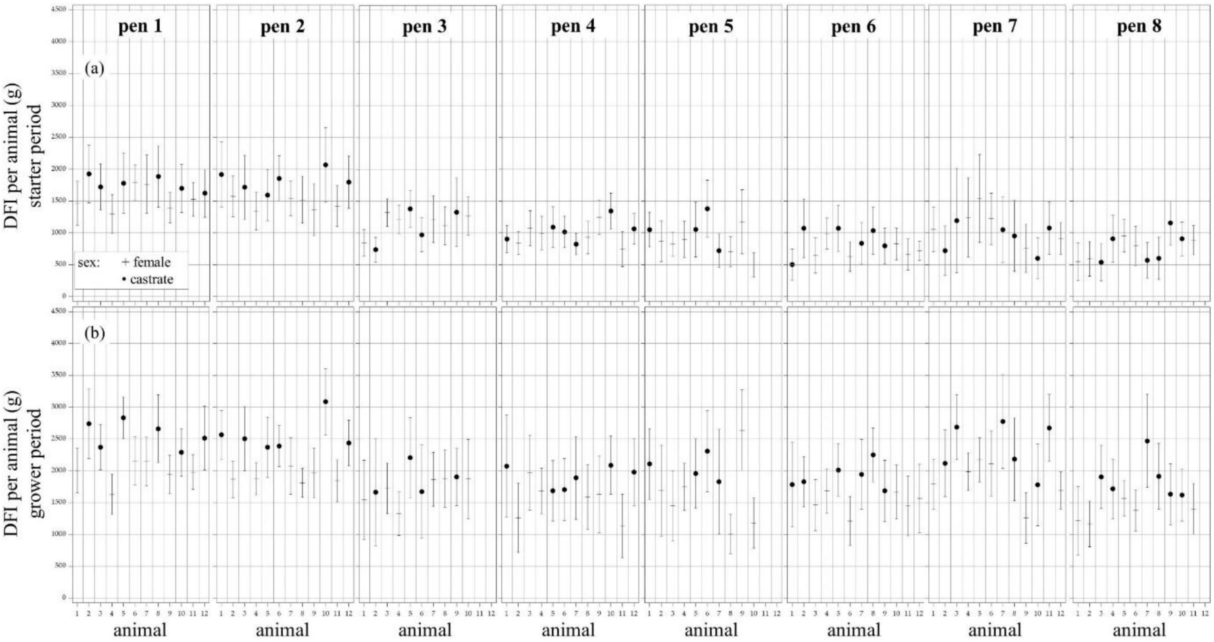
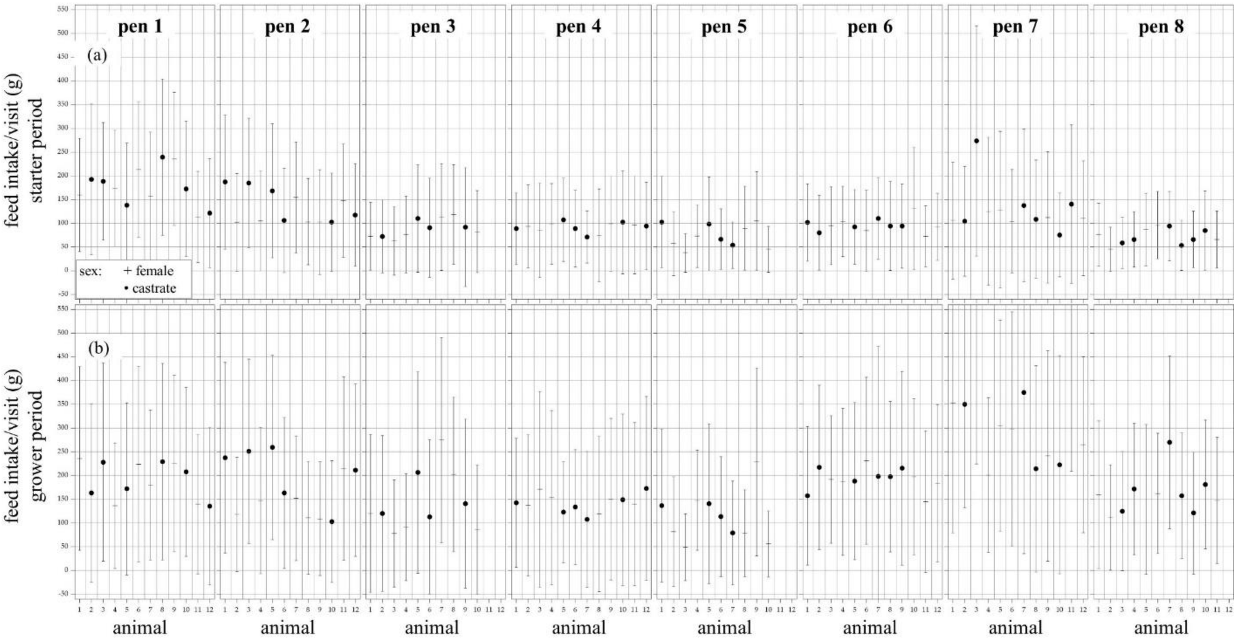
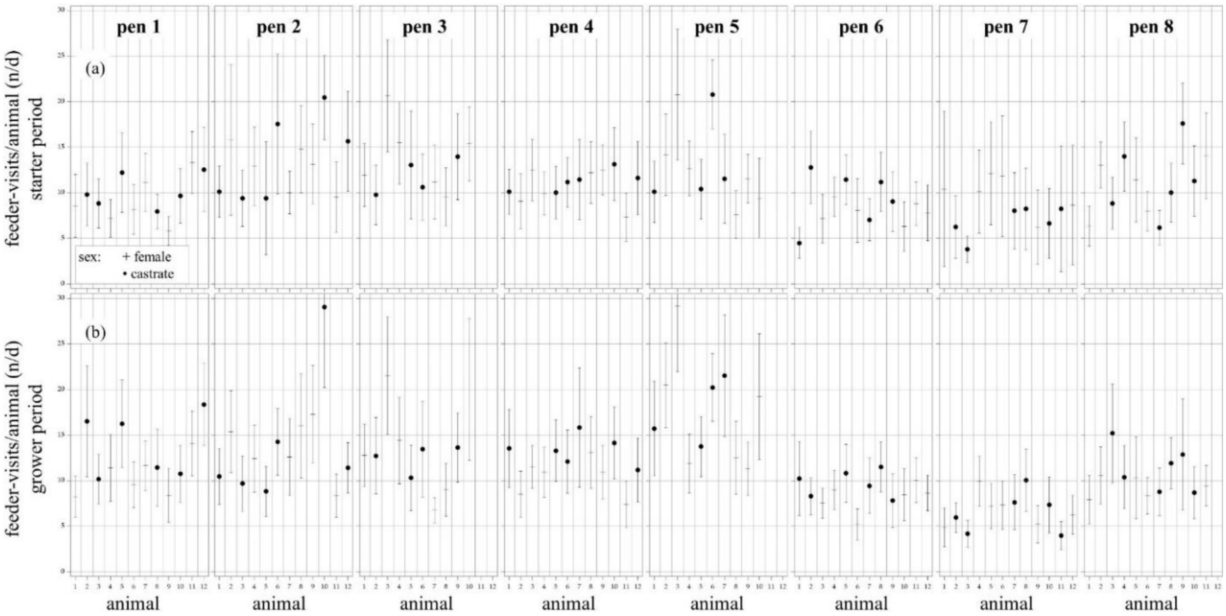


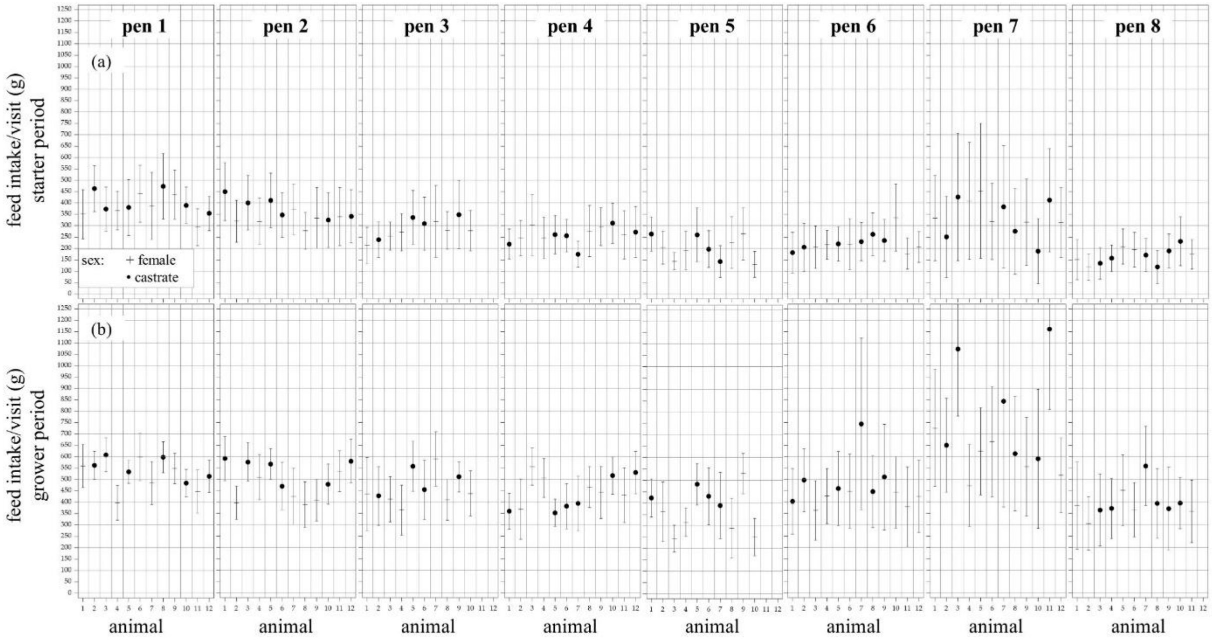
Supplementary Figure III-1. Average daily feed intake per animal and fattening period; tick marks indicate standard deviations



Supplementary Figure III-2. Average daily feed intake per feeder visit, animal and fattening period; tick marks indicate standard deviations



Supplementary Figure III-3. Average daily count of visits to feeder per animal and fattening period; tick marks indicate standard deviations



Supplementary Figure III-4. Average daily maximum feed intake within one feeding action per animal and fattening period; tick marks indicate standard deviations

Chapter IV. Results of an unpublished study in piglets

Both fattener studies indicated that pigs developed highly individual feeding behavioural patterns that were not influenced by short-term disturbances or long-term exposure to mycotoxins. In order to verify these results, a third study in weaned piglets was designed. The weaning phase is the most crucial phase of a piglet's life as it delivers many distressful changes. After the withdrawal from the sow, piglets are usually put into new environments, litters are often mixed, the feed immediately changes from essentially sow's milk towards a mostly plant-based liquid or pulp (Campbell et al. 2013). This often leads to a fasting phase of up to three days affecting the gut morphology and ultimately leading to post weaning diarrhoea (Rhouma et al. 2017). The general situation of distress around weaning often makes piglets more sensitive to external stressors.

Regarding feeding behaviour, piglets synchronise their behaviour and are generally accustomed to synchronised drinking at the sow right up until weaning (Lewis and Hurnik 1985; Wattanakul et al. 1997). It imposes additional stress to the piglets when this need is impaired by mixing with other piglets and changing to a feeding system of any kind. Therefore, detailed assessment of animal individual feeding behaviour could support managing these problems.

The experiment in weaned piglets was designed to examine the effect of several short-term disturbances similar to Loibl et al. (2020a) rather than feed hygienical problems. As the focus in piglet feed lies on best quality and hygiene, such problems occur far less in practice than short-term aberration do.

1. *Experimental design*

The experimental stable comprised a nursery compartment equipped with similar feeding technology adjusted to the size of weaners (Figure I-5, Figure I-6). The piglets originated from a sow trial that tested two different boar lines, either Piétrain (Pit) or Duroc (Dur). It was decided to include this in as an additional parameter in the design. Table IV-1 summarises the experimental design. Two undisturbed boar line control groups (CON) were compared to the respective treatment groups. A total of 80 piglets originating from either Dur or Pit boars, were distributed over 8 pens (4 pens for each boar line) to spread sexes (50% male-castrated and 50% females) and litters equally. The piglets were fed two different weaner diets (weaner feed 1 and 2 with 40% wheat, 34.5%-36.5% barley, soybean meal 19%-17%, macro premix 4% resulting in analysed 13.6 MJ-

13.8 MJ ME, 17.0%-17.5% crude protein) for three weeks each. On day 15 the feeders were turned off for 12 hours in both treatment groups and from day 29 the water efflux of the nipple drinker was reduced to 0.4 l/min for 48 hours. These disturbances were designed to have no effect on zootechnical performance, similar to Loibl et al. (2020a) and adjusted to the younger age of the animals. Experimental parameters (weight development, DWG, FCR, DFI, feed intake per visit, number of visits per day, most consumed feed in one visit per animal and day) and statistical evaluation followed the same regimen as described above, focussing on the hierarchical ANOVA (Loibl et al. 2020a; Loibl et al. 2020b).

Table IV-1: Experimental design of the unpublished piglet study.

	Treatment	Pit Control		Pit Treatment		Dur Control		Dur Treatment	
	Pen	1	2	3	4	5	6	7	8
Experimental day	1	Start of experiment, weaner feed 1							
	15	---		No feed for 12 h		---		No feed for 12 h	
	28	Change to weaner feed 2							
	29	---		reduction of water efflux to 0.4 L/min for 48 h		---		Reduction of water efflux to 0.4 L/min for 48 h	
	42	End of nursery phase							

2. *Results and Discussion*

One animal had to be excluded from trial and analyses due to non-treatment related reasons.

Table IV-2 shows the zootechnical performance results of all four treatment groups. On average, the piglets grew from 9.7 kg to 29.6 kg, gained roughly 490 g/d with an FCR of 1.47 g/g which represents solid intermediate performance levels routinely observed in this stable compartment (e. g. Preißinger et al. (2019)). As expected, the *treatment* had no effect on any of the assessed parameters´. Regarding the factor *pen*, the animals of pen 7 showed significantly increased FCR (not shown in detail) during the first 21 days of the trial. These animals seemed to struggle to adapt to the new environment after weaning. The factor *sex* showed a significant effect on daily weight gain in the second half of the nursery period. This might already hint to the sex dimorphism (Hale and Southwell 1967). Significant increases in FCR were also found over the whole experimental period (day 1-42). In pigs, FCR usually is not influenced by sex, at least in the nursery stage (Colson et al. 2006). Therefore, this might partly have been caused by the difficulties in adaption of pen 7.

Table IV-2. Zootechnical performance results (weight, daily weight gain, feed conversion ratio) for the Piétrain (Pit) and Duroc (Dur) control groups and the Pit and Dur groups confronted with stable routine disturbances (Loibl, 2014, unpublished)

Treatment	Pit Control		Pit Treatment		Dur Control.		Dur Treatment		Overall	SEM	*P*-value		
Pen	1	2	3	4	5	6	7	8			Treat.	Pen	Sex
Animals (n)	10	10	10	10	10	9	10	10	79	---	---	---	---
Weight													
Day 1 (kg)	9.8		9.4		9.4		10.2		9.7	0.1	0.22	0.33	0.23
Day 21 (kg)	16.6		16.1		16.0		17.0		16.4	0.2	0.63	0.26	0.37
Day 42 (kg)	29.7		29.1		29.8		30.0		29.6	0.4	0.89	0.56	0.13
Daily weight gain													
Day 1-21 (g/d)	323		317		314		326		320	9	0.98	0.32	0.53
Day 22-42 (g/d)	655		651		690		668		666	11	0.80	0.55	0.03
Overall (g/d)	480		485		498		493		489	8	0.90	0.70	0.12
Feed conversion ratio (FCR)													
Day 1-21 (g/g)	1.36		1.39		1.43		1.66		1.48	0.03	0.35	0.01	0.64
Day 22-42 (g/g)	1.46		1.46		1.50		1.50		1.48	0.01	0.79	0.26	0.38
Overall (g/g)	1.42		1.44		1.45		1.54		1.47	0.01	0.51	0.04	0.02

SEM = Standard Error of the Mean;
p-values are from hierarchical ANOVA where each source of variance was tested against the column on the right.

Table IV-3 presents the results of the feeding behavioural traits. DFI lay on standard levels for the compartment with around 446 g/d – 700 g/d (Preißinger et al. 2019) and was not influenced by the short-term disturbances. From day 1-21 piglets of pen 6 fed significantly less (388 g/d) and the animals in pen 7 significantly more (569 g/d) than the other groups. This was probably due to problems in adaption to the feeders, where pen 6 supposedly struggled to start a normal feeding regimen whereas piglets in pen 7 could have played a lot with the feed. This then resulted in the above-described increase FCR. Overall, the piglets frequented the feeder 22 times per day and fed around 31 g/visit. Feed intake per visit increased twofold during the nursery period (20 g to 41 g) whereas the number of daily visits remained on a level of 22 visits per day. The highest amount of consumed feed in one visit per animal and day also increased roughly by the factor 2 (86 g to 175 g). The pens differed only in the first nursery phase, after that no differences were observed. *Sex* seemed to influence the highest feed consumption in one visit in the second nursery stage. The *animal* had a highly significant influence on feeding behavioural traits throughout the nursery phase. Following day 21 neither pen nor treatment affected any behavioural parameter.

The piglets frequented the feeders nearly twice as often as the fattening pigs of Loibl et al. (2020a) (on average 13 times) and fed only a fifth of the volumes of the fattening pigs (154 g/visit). These fattening pigs were confronted with similar disturbances and did not show any reaction to these treatments either. A reduction in feeding frequency during maturation has been described in literature. Young piglets feed with high frequency (Bigelow and Houpt 1988) whereas sows only take a few large meals per day (Auffray et al. 1980). The frequency reduction of course needs increased feed intake to maintain the same feed volume. Also, older pigs need to feed more feed per day. Therefore, the feed volume of fatteners was five times higher than in piglets, whereas frequency was only reduced twofold. This effect of age was also observed looking at the highest feed consumption in one visit per animal and day in the present experiments. The piglets increased the intake from 86 g to 175 g from the first to the second nursery stage whereas the fatteners showed 358 g and 513 g (Loibl et al. 2020a) and 281 g and 488 g (Loibl et al. 2020b) in the respective starter and grower periods. Particularly when comparing to Loibl et al. (2020a) this represents roughly a doubling every three to four weeks, at least until a weight of 60 kg (end starter period). The two examined fattening periods lasted 63 days in total (Loibl et al. 2020a).

Table IV-3: Overall results of feeding behavioural measures (daily feed intake, number of daily visits to the feeder per animal, the overall amount of consumed feed per visit and highest consumed amount feed per visit per day) for the Piétrain (Pit) and Duroc (Dur) control groups and the Pit and Dur groups confronted with stable routine disturbances

Treatment	Pit Control		Dur Control		Pit Treatment		Dur Treatment		Overall	SEM	*p-value*			
Pen	1	2	3	4	5	6	7	8			Treat.	Pen	Sex	Anim.
Daily feed intake (g/d)														
Day 1-21 (g)	401^{bc}	456^{b}	416^{bc}	441^{bc}	440^{bc}	388^{c}	569^{a}	442^{bc}	446	5	0.41	<0.05	0.48	<0.01
Day 22-42 (g)	911	928	576	971	917	1058	891	968	940	7	0.73	0.30	0.09	<0.01
Overall (g)	669	701	663	709	689	732	730	723	702	6	0.42	0.77	0.16	<0.01
Feed intake per visit (g)														
Day 1-21 (g)	16^{e}	18^{d}	17^{d}	20^{c}	20^{c}	23^{b}	32^{a}	19^{d}	20	0.2	0.54	<0.01	0.64	<0.01
Day 22-42 (g)	36	37	38	38	40	53	46	44	41	0.3	0.31	0.43	0.12	<0.01
Overall (g)	26	28	28	30	31	39	39	32	31	0.2	0.35	0.16	0.23	<0.01
Number of visits to the feeder per animal and day (n/d)														
Day 1-21 (n/d)	26^{a}	25^{a}	24^{a}	22^{b}	22^{b}	17^{c}	18^{c}	24^{a}	22	0.2	0.30	0.04	0.68	<0.01
Day 22-42 (n/d)	25	25	23	26	23	20	19	22	23	0.2	0.14	0.66	0.22	<0.01
Overall (n/d)	25	25	24	24	22	19	19	23	22	0.1	0.19	0.31	0.31	<0.01
Feed intake of the most extreme feeding action per animal and day (g)														
Day 1-21 (g)	59^{b}	78^{b}	62^{b}	68^{b}	86^{b}	71^{b}	176^{a}	78^{b}	86	2	0.39	<0.01	0.92	<0.01
Day 22-42 (g)	121	148	186	188	191	153	182	228	175	1	0.14	0.19	0.02	<0.01
Overall (g)	92	114	128	129	141	113	179	158	132	2	0.05	0.21	0.22	<0.01

SEM = Standard Error of the Mean;
$^{a\text{-}c}$superscripted letters indicate significant *pen* differences ($P < 0.05$);
p-values are from hierarchical ANOVA where each source of variance was tested against the column on the right.

The factor *pen* had a significant influence on all behavioural traits in the first three weeks of the trial mainly caused by the high DFI of pen 7 (569 g/d) and the low DFI of pen 6 (388 g/d). All other parameters followed logically, i. e. depending on the frequency the amount of feed intake per visit was higher or lower resulting in the respective DFI. In contrast to standard weaner feeding systems the automatic single space feeders do not allow synchronised feeding which would be natural part of feeding behaviour of pigs (Gonyou 2001). Coming from such a synchronised and regular feeding scheme it is not surprising that some piglets and therefore feeding groups (i.e., *pen*) struggled to adjust to the single space feeding system, with only one feeding space located inside a feeding station (see Figure I-6). Synchronisation during the lactation phase is common, as free-roaming sows would even synchronise their nursing times (Newberry and Wood-Gush 1985). Therefore, newly weaned piglets are used to suckle all at once according to the sows lactation rhythm of around once per hour (Lewis and Hurnik 1985; Wattanakul et al. 1997). In comparison, under constant DON exposure, the factor pen was also significant throughout the whole examination period in Loibl et al. (2020b). Possibly, great distress leads to a more consistent group reaction. This, however, requires further investigation. In the second nursery phase no effect of *pen* was observed. This could be an indication for the piglets' accomplished adaption to the new stable and therefore a higher importance on the individual feeding pattern. The significant influence of *sex* on highest feed consumption in one visit in the second half of the weaner period could have already been caused by early sex dimorphism as male pigs show higher feed intake capability resulting in higher gain (Hale and Southwell 1967).

Table IV-4 shows the means of coefficients of variation (CV) for the feeding behavioural measures. In contrast to Loibl et al. (2020b) the CVs were not normally distributed in this study, probably due to lower observation number and days. Therefore, no statistical post-hoc test was conducted, and only descriptive means are shown. These ranged between 31.8% (number of daily visits) and 39.1% (DFI). Numerically, no directed effect of the treatment-groups, but differences between the pens could be observed. Pen 6 and 7 showed the highest CVs in all parameter in the first experimental weeks. Also, the CVs decreased by 3%-10% from nursery phase one to two. CVs ranged on remarkably similar levels to Loibl et al. (2020b). There the overall maximum and minimum were 40.5% (feed intake per visit) and 30.3% (DFI), respectively. In contrast to the piglets, however, the fatteners were facing feed-borne mycotoxin exposure throughout the trial, which led to a clear treatment effect. The similar CV-levels indicate that piglet feeding behaviour is still quite erratic. The main numerical differences in piglets derived from the animal group (pen). The most prominent differences between the pens were visible during the first three weeks. This might indicate as well that some pens struggled more than other to adapt to the new environment, as mentioned above.

Table IV-4. Means of coefficients in variation of the feeding behavioural traits (daily feed intake, feed intake per visit, number of daily visits and maximum amount of feed per visit) of the Piétrain (Pit) and Duroc (Dur) control groups and their respective treatment groups.

treatment	Pit Control		Dur Control		Pit Treatment		Dur Treatment		Overall
pen	1	2	3	4	5	6	7	8	
daily feed intake (g/d)									
Day 1-21 (%)	37.7	34.9	43.0	39.8	42.1	50.0	58.3	44.9	43.9
Day 22-42 (%)	31.8	32.8	31.3	31.6	33.5	22.5	33.4	33.3	31.3
Overall (%)	34.7	33.8	36.3	35.7	37.8	37.1	45.8	39.1	37.6
feed intake per visit (g)									
Day 1-21 (%)	31.5	26.5	34.9	29.5	32.3	63.4	57.2	38.0	39.3
Day 22-42 (%)	35.6	32.2	37.6	42.3	30.7	27.7	34.4	36.4	34.5
Overall (%)	33.5	29.3	36.2	35.9	31.5	45.5	45.8	37.2	36.9
count of visits to the feeder per animal and day (n/d)									
Day 1-21 (%)	30.9	28.7	32.0	30.9	33.6	42.2	30.4	35.2	33.0
Day 22-42 (%)	29.8	33.1	31.4	33.9	35.5	18.3	33.3	29.5	30.6
Overall (%)	30.3	30.9	31.7	32.4	34.5	30.3	31.8	32.4	31.8
maximum feed intake on one visit per animal and day (g)									
Day 1-21 (%)	41.1	40.8	36.0	37.2	38.9	65.5	113.3	43.3	52.2
Day 22-42 (%)	32.3	28.7	38.3	41.3	27.9	30.5	29.8	33.3	32.7
Overall (%)	36.7	34.8	37.2	39.3	33.4	48.0	71.5	38.3	42.4

Similar to Loibl et al. (2020a) and Loibl et al. (2020b), the main cause of deviation in feeding behaviour was the individual animal. A clear effect of the pen could not be found in this study as in Loibl et al. (2020a). One reason for this could have been the lower stocking density of only 10 animals per pen compared to 12 fatteners in the fattener studies. This gave more freedom to the individual piglet to exert its behavioural pattern independently. Also, the general variation of the data was increased which in turn reduced statistical precision. The SEM of the behavioural parameters was nearly ten times higher than the one shown by Loibl et al. (2020a). Partly, the shorter examination period of two times three weeks might have caused this finding too. Freshly weaned piglets quickly develop distinct hierarchies in their groups that stay rather stable after maximum 7 days after birth (Ewbank 1976). Therefore, a connection of the individual animal's feeding behaviour and its rank within a group cannot be excluded, although it was not observed in this study.

3. Conclusions

Short-term disturbances did affect neither piglets' zootechnical performance nor feeding behaviour. The piglets therefore seemed to react in a similar manner as fattening pigs and could compensate short aberration of their stable routine quickly.

Some piglet groups seemed to struggle with adaption to the single space feeders, however, once they had adapted, clear individual feeding patterns were developed and remained constant. The variation of these patterns was on the same level as the variation of fattening pigs chronically exposed to DON. The piglets' feed intake behaviour therefore still was quite erratic and variable.

The addition of individual animal feeding data to practical piglet feeding systems, therefore, seems not feasible for now. Due to the clear animal-individual pattern and the high variability, feeding behaviour of weaned piglets lacks necessary persistency and predictability.

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Chapter V. General Discussion

This doctoral project investigated the feasibility of highly detailed animal individual feed intake measurement to identify the influence of a variety of rather typical disturbances and feed hygienical problems occurring in husbandry routine. Possible effects on the zoo-technical performance were evaluated as well. The animals were kept under practical conditions in pens equipped with single space automatic feeders. These allowed the examination of the feeding behaviour of group-housed individual animals while maintaining a practical husbandry environment. To do so, a statistical model for detailed examination of the factors influenced by the stressors was established.

This thesis comprises two recently published studies in growing/finishing pigs and one unpublished study in weaned piglets. During the initial fattener experiment different short-term disturbances were simulated (Loibl et al. 2020a). Thus, potential sustained effects on zootechnical performance and/or feed intake behaviour of stressors, like deprivation of feed or water could be examined. One of the specific goals of this study was to evaluate whether such detailed feeding data of individual pigs could feasibly be implemented as a real-time animal welfare indicator. Since feeding systems become increasingly precise and, depending on the general stable system, increasingly focussed on the individual animal, feeding data could be an easily accessible early warning system for newly occurring stress within a stable compartment, pen etc.

In the second study, three dietary treatments containing high levels of the mycotoxin DON with or without addition of two different commercial mycotoxin binders were designed. Next to an expected effect on zootechnical performance it could have been interesting to see whether a persistent chronic distress through DON-exposure via feed altered the feeding patterns of the fatteners (Loibl et al. 2020b).

A third study in weaner piglet aimed to verify the initial indications and identify potential differences in weaned piglets. These animals originated from different boar lines which could lead to differing reactions to short-term disturbances (feed deprivation for 12 h and reduced water efflux of the nipple drinkers, Chapter IV).

Thanks to the establishment of a hierarchic ANOVA model, we were able to examine in detail, which of our fixed variables were influenced by the designed treatments and other observed parameters.

1. Experimental set up and technology

1.1. Experimental facility

The stable compartments of the LVFZ Schwarzenau assigned to animal nutrition were designed to combine precise assessment of nutritional data while keeping animals in practical housing conditions. This comparability enables a transfer of knowledge to practical German pig farmers. All compartments follow the legal requirements regarding conventional husbandry environment, such as flooring, stocking density, etc. (TierSchNutztV 2006). This way, broad practical German pig husbandry conditions are represented.

Regarding the two barns used in this doctoral project, the only difference to practical conditions was the feeding system. The automatic single space systems are regularly used in progeny testing and other applied scientific approaches (Nielsen et al. 1995b; Zentral Verband der Deutschen Schweineproduktion 2007). Their use in practical stables is rather uncommon, however. Using these stations allowed the best compromise between practical housing conditions and best feeding data assessment. For this reason, these barns were best suitable for this project.

1.2. Data generation and processing

The state-of-the-art single space automatic feeders gave access to several feeding parameters of each animal. Besides the time when an animal entered the station the feed intake of each visit was assessed. All feeding data was uploaded automatically on a daily basis at midnight. The upload to the central server-infrastructure worked well. Feeding data of only very few days were incomplete due to network failures. In these cases, the whole dataset of the respective day was withdrawn from analysis to avoid misinterpretation. The few data gaps were the result of the network infrastructure required for experimental purposes especially with regards to a central server located off-site. Under practical conditions, such collection of data would not be necessary. Therefore, similar feeding systems could easily be implemented in practical stables without the risk of incomplete measurements and possible misinterpretations.

Animals were weighed every week (Loibl et al. (2020a) and Chapter IV) or every second week (Loibl et al. 2020b) to calculate daily weight gain and FCR. In the experimental stable of the LVFZ Schwarzenau, all animals are always weighed by highly qualified personnel and back-checked already during the weighing process for misreadings or mismeasurements. The scales were linked to the network and thereby the weights were uploaded directly to the server to avoid data loss of any kind. This way, precise assessment of zootechnical performance was achieved.

All feeding actions with less than 5 g feed consumption were excluded from analyses due to lack of weighing-precision. Pichler et al. (2020) also suggested, that feeding acts with low feed intake (< 10 g) can be considered as playing or “gambling”. The visits with sufficient feed consumption were summed up per day to obtain daily feed intake. For Loibl et al. (2020a) and Loibl et al. (2020b), the feeder visits with < 5 g intake accounted on average for roughly 0.2% (cumulated < 5 g/d) of daily feed intake and 4% of daily visits (~ 0.5 visits per animal and day; unpublished). These feeding actions did not contribute distinctively to the feeding behaviour, therefore, keeping utmost weighing precision was prioritised.

All stable compartments were fitted with data loggers that recorded air temperature and humidity. The experiments were run during autumn, winter, or spring; therefore, stable climate remained constant. By this an influence of external temperature or humidity could be excluded which was proven by the data loggers. Consequently, these data were not considered in the examinations.

With all these measurements it was possible to generate highly detailed data for the examination of group housed fattening pigs living in near practical housing environment.

1.3. Development of the analytical model

A hierarchical ANOVA model allowed the combined statistical evaluation of nearly all observed fixed variables, the individual animal, its sex, its pen and the respective treatment. Hierarchical or nested ANOVAs allow testing the different sources of variation against each other, thereby enabling the identification of the main effects. In the present studies, the highest resolving fixed variable was sex in the case of zootechnical performance and CVs and the individual animal for all the other parameters, was tested against residual deviation. Then, step by step, the variables with lower resolution were tested against the higher one. The main factors causing the observations could therefore be identified with reasonable effort and described in an easy and comprehensive way. Also, no additional aggregation of data was necessary. This guarantied analyses in utmost detail without the loss of valuable information. To our knowledge, similar statistics in similar experiments have not been done before.

The model even allowed to include the time-dependency of behaviour to a certain extent. All parameters measured several times per day for the individual animals were summarised per day. Therefore, the day-to-day variation of feeding behaviour is an essential part of the variable *animal*.

The hierarchical ANOVA, therefore, allowed to draw relevant conclusions regarding feeding behaviour of group-housed pigs without losing valuable information and keeping sufficient precision. Also, results have been shown in a comprehensive way.

2. *Examining feeding behavioural traits with automatic single space feeders*

2.1. *Comparison of the experiments of this doctoral project*

The above-described studies Loibl et al. (2020a), Loibl et al. (2020b), and Chapter IV were all analysed using the established model to gain utmost information out of the high quality dataset.

The simulation of short-term disturbances in stable routine in Loibl et al. (2020a) and Chapter IV should simulate standard practical husbandry problems. Hereby, one of the original hypotheses was that feed intake is a sensitive marker for animal stress which otherwise would not be seen with routinely assessed zootechnical performance data. Thereby, feed intake behaviour could be used as a welfare indicator.

Both studies confirmed that such short-term aberrations in pigs' routine did not cause any sustained effect on zootechnical performance. Even feed deprivation for 12 h (piglets) or 24 h (fatteners), respectively, or exchange of animals were compensated within reasonable time. As the pigs in these studies were weighed every week, the resolution to detect effects of these treatments was far higher than under practical circumstances. In practice, pigs usually are only weighed when placed into the stable and when marketed in the end. Since we did not measure any treatment effects on performance, they would not have been observed in practice either. This was a goal of the treatment design.

The third study (Loibl et al. 2020b) aimed to investigate the effects of chronic exposure to Deoxynivalenol (DON) in fattening pigs. Mycotoxin contamination is a common problem for pig husbandry all over the world and depends mostly on the harvest situation of the feed crops (Döll and Dänicke 2011). Therefore, three treatments with DON contents of about five times the European guidance value (The Commission of the European Communities 2006), ~ 4500 µg/kg DON, and two different mycotoxin binders were implemented to measure the effects on zootechnical performance and feeding behaviour. As expected, all animals reacted with feed refusal of about 30% compared to the control group. Subsequently DWG was reduced too. Feed refusal is a well-known reaction of pigs to DON (Döll and Dänicke 2011). These results hence were to be expected. Interestingly, the mycotoxin binders did not improve the performance.

In total, simulated disturbances showed the expected effects on zootechnical performance. The short-term disturbances did not alter the results, whereas DON-exposure reduced performance drastically and could be identified with routinely assessed performance data already.

Regarding feeding behavioural patterns of fattening pigs, the results showed similar levels with the ones presented in literature when stocking density or feeder design were similar (Nielsen and Lawrence 1993; Nielsen et al. 1995b; Pichler et al. 2020). Details are discussed in the respective papers. To our knowledge, similar studies in weaned piglets have not been published yet.

Animal behaviour is generally highly individual. That could be seen in our studies. The individual pig was the highly significant main source of variation in all examined data of all three studies.

It was not possible to identify typical short-term disturbances on a pen level. This interferes with the standard "way of thinking" in animal nutrition. Usually, the examination object when feeding livestock is a feeder and not the individual animal. Comparable behavioural examinations on a group level are scarce.

Optical assessment of the timeseries of feeding data of an animal indicated that the individual pig showed a reaction and then returned to its original pattern. Figure V-1 shows exemplarily cumulative daily feeding curves of one animal of the starving group of the Loibl et al. (2020a) trial, three days before the feeder was turned off, then two days of feed withdrawal (the feeder was turned off from 13:00 to 13:00 o'clock) and then the three following days. The pig started feeding often around 00:00 o'clock and fed until around 12:00 o'clock, paused and then fed again between 18:00 o'clock and 21:00 o'clock. After the feeder was turned on again, the pig fed only twice around 21:00 o'clock. On the first day after feed withdrawal the animal already returned to the original pattern and maintained this. On the 2nd day after deprivation, it seemed to have compensated the lost feed intake to a certain extent.

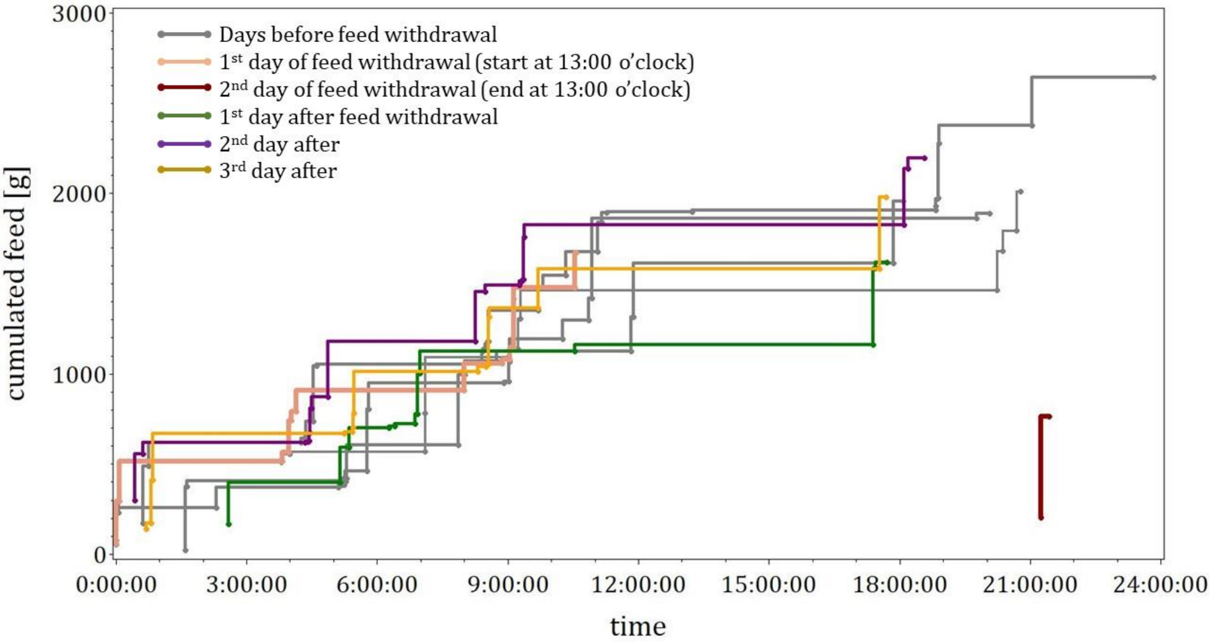

Figure V-1: Cumulative daily feed curves of one animal of the starving group of the first fattener study (unpublished)

Regarding this animal, the feeding pattern goes in accordance with published literature. de Haer and Merks (1992) or Hyun and Ellis (2000) e. g. showed that pigs have two peaks in feed intake, one in the morning and one in the evening. However, in Loibl et al. (2020a) there were several pigs reacting in completely different manner, e. g. feeding only at night (not shown in detail). Nielsen (1999) postulated that feeding frequency on automatic feeders relates to social constraint of pigs. This probably was one of the reasons for the observed differences in feeding patterns, as the stocking density might have made it necessary for some animals to alter from their inert feeding patterns. However, this was not further investigated, since it was not possible to evaluate the hierarchical rank of the pigs. These individual behavioural patterns, cross-linked to the rank of the animal, were probably the reason for the factor *animal* being the only consistently significant influence on behaviour. Due to the immense influence of the individual animal, it consequently became difficult to detect differences between the groups as the patterns fluctuated in between animals as well as from day to day.

In Loibl et al. (2020b), animals exposed to DON also developed individual patterns. In contrast to Loibl et al. (2020a) and Chapter IV, for all feeding behavioural traits except DFI, the factor *pen* was consistently significant as well. We hypothesised, that the mycotoxin enhanced the behavioural patterns of the group as feeding behaviour is part of pigs' social behaviour (Gonyou 2001). If this hypothesis is correct, similar situations were observed in the piglet study. There, a significant pen effect was found for all behavioural parameters in the first period. It seems as if moments of distress (in piglets the phase after weaning, in fatteners the chronic exposure to a mycotoxin) led to a more prominent group-behaviour. In Loibl et al. (2020b), the exposure to DON increased the experimental timeframe by about 30 days. Therefore, more data was included in the analyses which might have improved the recovery of statistical results.

Generally, the animals developed individual patterns which were not influenced by the treatments. Especially when animals faced drastic stress (DON-exposure, weaning) the pen (group)-dependency of behaviour increased.

Loibl et al. (2020b) found that three treatment groups facing different DON dietary treatments developed more erratic behaviour. Increasing CVs were found in all of the behavioural parameters in these groups. The CVs of the piglets ranged on a similar level (Chapter IV). 1 shows the CVs of the feeding behavioural traits of the first study. Generally, the CVs were roughly 10% lower compared to the ones presented in Loibl et al. (2020b) and Chapter IV. Only the feeding action with highest feed intake showed a significant treatment effect during the respective starter period. Significant differences of pens were only observed in the grower period in DFI and in the feeding action with highest feed intake, and during the whole observation period for DFI. Also, CVs ranged on similar levels over the whole observed period, while they decreased in both piglets and the DON-exposed pigs. The weaning stress probably caused this finding in piglets. These animals needed some time to adjust to the new living situation (Campbell et al. 2013). The DON-confronted fatteners probably reacted with increased irregularity in their feeding patterns to the DON-contents in the feed.

Table V-1. CVs of the feeding behavioural parameters of the first study

Treatment		Control		Starving		Feed Change		Social Stress		overall	*p-value*		
Pen		1	2	3	4	5	6	7	8		treat.	pen	sex
Daily feed intake (kg/d)													
Starter	%	18.4	19.8	22.2	19.5	20.5	20.5	21.8	17.0	20.0	0.80	0.14	0.42
Grower	%	18.1^{bcd}	15.5^{d}	22.7^{b}	28.6^{a}	16.9^{cd}	15.2^{d}	21.4^{bc}	18.8^{bcd}	19.6	0.06	<0.01	0.95
Days 1–63	%	18.3^{b}	17.6^{b}	22.4^{a}	24.1^{a}	18.7^{b}	17.9^{b}	21.6^{ab}	17.9^{b}	19.8	0.07	0.03	0.91
Feed intake per visit (g)													
Starter	%	25.1	25.6	31.4	27.2	32.5	36.0	32.9	30.2	30.1	>0.05	0.30	0.19
Grower	%	23.3	29.6	31.9	34.8	25.8	32.6	35.0	30.3	30.4	0.37	0.11	0.14
Days 1–63	%	24.2	27.6	31.7	31.0	29.1	34.3	34.0	30.3	30.3	0.19	0.07	0.27
Count of visits to the feeder per animal and day (n/d)													
Starter	%	24.7	26.3	29.0	27.7	28.5	30.4	35.2	31.0	29.0	>0.05	0.27	0.47
Grower	%	25.7	30.2	33.2	37.8	27.1	29.8	36.6	31.0	31.3	0.17	0.12	0.17
Days 1–63	%	25.2^{d}	28.3^{bcd}	30.6^{bc}	32.7^{ab}	27.8^{cd}	30.1^{bc}	35.9^{a}	31.0^{bc}	30.2	0.15	0.01	0.81
Maximum feed intake on one visit per animal and day (g)													
Starter	%	22.1^{B}	22.2^{B}	21.6^{B}	22.4^{B}	27.3^{A}	29.0^{A}	28.1^{A}	29.8^{A}	25.3	<0.01	0.87	0.10
Grower	%	17.2^{bc}	18.3^{bc}	17.9^{bc}	23.3^{ab}	13.9^{c}	25.8^{a}	22.8^{ab}	26.8^{a}	20.7	0.59	0.03	0.09
Days 1–63	%	19.7	20.2	19.7	22.9	20.6	27.4	25.4	28.3	23.0	0.23	0.06	0.15

SEM = Standard Error of the Mean;

$^{A\text{-}B}$superscripted capital letters indicate significant treatment differences;

$^{a\text{-}d}$superscripted letters indicate significant differences between pens;

P-values are from hierarchical ANOVA where each source of variance was tested against the column on the right; the animal was tested against the overall data variation.

Short term disturbances did not alter the variation of feeding behaviour in contrast to DON exposure. The animals compensated the short-term effect quickly whereas chronic mycotoxin exposure led to more erratic behaviour. Piglets likely need time to develop persistency.

In total, our studies indicate that the examination of animal feeding behaviour in a practical group-context needs an immense number of replicates to recover reproducible patterns. Using 24 pigs in two pens (groups) per treatment only showed that the animals developed a supposedly group dependent behaviour. The two groups within a treatment, however, did not react similarly to any of the posed disturbances which is shown by the lack of significant treatment-effects. An increased number of replicate pens could be useful to reach statistical evidence of a treatment effect. Based on our findings, it was not possible to identify influences of short-term distress or mycotoxin exposure on animal behaviour of pigs housed under practical conditions. Therefore, a transfer to and implication into more practical feeding systems does not seem reasonable at this point. To further investigate our results, it would be necessary to evaluate possible concordant behavioural schemes of groups and animals. To achieve this, an increase in animal numbers as well as groups would be essential.

2.2. Comparison with literature

Automatic single space feeders have now been present in pig production for several decades (Nielsen et al. 2016). Their predominant use is, however, still the observation of feed intake of progeny of breeding boars or other rather scientific approaches with pigs kept in small groups (Zentral Verband der Deutschen Schweineproduktion 2007). Nearly all studies using similar feeding technique examine fattening pigs, data on weaned piglets fed by automatic single space feeders seem very scarce. A detailed assessment of the influencing factors on feeding behaviour using similar analytical approaches to our knowledge has never been done.

Studies conducted in the 1990ies and early 2000s examined general feeding patterns (Hyun et al. 1997), different feeder-designs (with low or high protection of the feeding pig (Nielsen et al. 1995b)), group sizes (Nielsen and Lawrence 1993; Nielsen et al. 1995a; Hyun and Ellis 2001), different environments (concrete floor or straw bedding (Morgan et al. 1998), as well as linking feed intake patterns to performance (Hyun and Ellis 2000). Interestingly, group sizes similar to the ones described above showed similar feeding parameters. E. g. in the study comparing different feeder designs, ~9 fattening pigs were kept per pen and their number of visits ranged between 10.4 – 14.0 on near identical level as above described (Nielsen et al. 1995a). Hyun and Ellis (2000) using 10 animals per feeder found an average of 14.8 visit per day. The feed intake per visit was logically cross-linked to the number of visits, as described above, i.e. the higher the count of daily visits the lower the feed intake per visit. When increasing the group size per feeder to 20 pigs the count of visits was significantly decreased to 7.1 visits per day with a feed intake of 214 g/visit whereas groups of 5 – 15 pigs showed 14.3 visits per day with 119 g/visit (Nielsen et al. 1995a). The variance of these measurements within the treatments, however, was not stated in any of these studies. Taking our findings into consideration, the significant differences in literature could well be a result of differing behaviours of the animals in the context of the respective group rather than a pure effect of the treatments. However, since we did not examine different counts of animals per pen, this conclusion is not entirely reasonable.

In the most recent study, Pichler et al. (2020) conducted a nutrient density choice-feeding study with 5 or 10 animals per pen in the low or high nutrient density treatment or the nutrient choice treatment, respectively. Although, in contrast to our study all feeding actions below 10 g/visit were defined as "gambling" and excluded from statistical analysis, they measured an average of 25.5 visits per day. This was, probably caused by the lower number of animals per pen. As Nielsen et al. (1995a) indicated, a reduction of animals per pen increases the feeding rate. Interestingly, in contrast to our studies, Pichler et al. (2020) found a significant effect of sex regarding the feed intake per visit, similar to Hyun and Ellis (2000). As they used a different statistical approach, however, this also cannot quite be compared to our findings. Pichler et al. (2020) did not analyse the effect of the individual animal, which in the case of our studies presented the most prominent source of variation, thereby possibly resulting in insignificant sex-effects.

Overall, published data shows that the stocking density per feeder has a strong influence on the feeding rate of the animals. This makes a comparison with literature difficult. Under similar conditions, our observations generally ranged on similar levels. Since, to our knowledge no other study took the behavioural impact of an individual animal on the observed parameters into account, different significant effects might have been caused rather by the unique group behaviour based on the pen-mates' behavioural patterns than by a treatment effect.

3. Limitations of the experimental design

The main goal of this doctoral project was to examine possible implementations of a feeding system measuring feeding data of the individual animal as an addition to the practical routine measurements of piglets and fattening pigs. In these animals feeding data is usually measured for entire groups only. The animals were housed under practical conditions (group housed, fully slatted floors, stock density according to the legal requirements) and fed with sophisticated feeding systems. This combination led to some limitations in the generated data and ultimately some interferences with specific ethological methods to examine animal behaviour.

The practice-oriented feeding system did not allow a recording of the time when an animal finished a feeding action as feeding duration is not assessed in practical stables. Group housed fattening pigs feed highly concentrated practical feed around 0.5 to two hours per day depending highly on the stocking density and offered feed (Nielsen et al. 1995a; Pichler et al. 2020). Since the measured values regarding frequency and feed intake ranged on similar levels to these studies, one can assume that the animals fed for a similar duration. However, as we did not measure the endpoint of each feeding act, it was decided not to take any feeding duration into account.

Several methods for the examination of feeding behaviour of different species are established in literature and already described in Chapter I. Several of these methods were applied with the data set of Loibl et al. (2020a), i.e. in fattening pigs confronted with short-term disturbances. The outcomes will be outlined in the following.

Many ethologists calculate meal criteria to summarise several individual feeding actions to one meal (de Haer and Merks 1992; Tolkamp et al. 1998; Tolkamp and Kyriazakis 1999; Morgan et al. 2000a; Morgan et al. 2000b; Tolkamp et al. 2000; Tolkamp et al. 2011). A "meal", therefore, comprises several feeding actions which can be separated by pauses of different lengths. The maximum pause length can be identified by log-normalising the pauses and fitting up three Gaussian or other curves to the histograms of the normalised pauses (Tolkamp and Kyriazakis 1999; Yeates et al. 2001; Yeates et al. 2003; Howie et al. 2009). Per definition of the respective method, a point of intersection between any of these curves is then defined as the meal criterion.

Meal criteria were extensively calculated based on differing principals for evaluation (e. g. individual animal, pen, or treatment over different periods such as weeks, months, or fattening phases), because the data generally fit this method. However, not the actual pauses were considered, but the time between the starting points of each feeding act. This approach aimed to achieve the examination of feeding behaviour in a time-dependent manner. Analytical methods based on timeseries-analysis benefit from condensed data which could be attained by summarising the individual feeding actions to meals.

Most calculations, regardless of comprised number of experimental days, animals or pens, condensed the count of daily visits to the feeder (ranging around 10-14 visits per animal and day, see Loibl et al. (2020a), Loibl et al. (2020b)) to three to six meals per day. This of course indicated that many visits were likely connected and that the ethological assumption, a meal can comprise several independent feeding acts (Slater and Lester 1982), should be correct. The resulting meal data, however, differed drastically with deviation partly greater than 100% depending on the reference point (timeframe, animal, pen, treatment, etc.). To further evaluate the feasibility of this method and the dataset, meals calculated based on meal criteria calculated for each pen and fattening period were used for further analyses.

Animals seem to develop harmonic rhythms in their behavioural patterns connected to the circadian (24 h) rhythm (Berger et al. (1999), Berger et al. (2003), Berger (2011), and Scheibe et al. (1999); for details see Chapter I). Using the condensed meal data, we conducted an examination for rhythmicity shown exemplarily in Figure V-2 for one animal of the undisturbed control group of Loibl et al. (2020a) over a period of 14 days (Loibl et al. 2016). This pig showed the main feed intake regularly around the beginning or the end of the light day as it is common for pigs (de Haer and Merks 1992). Therefore, the spectral density peak following the 24-hour peak (not shown) was at 12 hours. Also, quite prominent peaks were found at four, three and two hours. These peaks indicated the inert rhythms of this animal's feed intake timeseries.

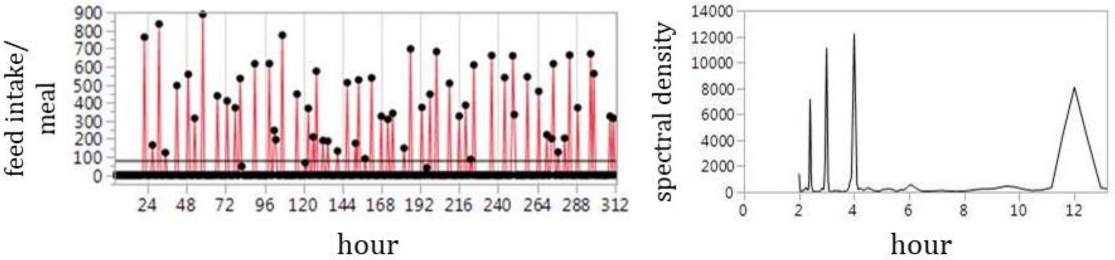

Figure V-2: Exemplary timeseries of feed intake per meal and the respective wave's spectral density of one animal over 14 days in study 1 (Loibl et al. 2016)

When examining the crude data, i. e. the timeseries of the individual feeding actions, the spectral peaks were not nearly as clear (data not shown). However, using methods to statistically identify significant spectral density peaks (Fisher 1929), the observed spectral peaks could not be proven significant in both, crude data, and condensed meal-data. Also, a model to evaluate all pigs separately at the same time could not be established. This meant, we were only able to look at individual pigs and could not include the context of its group. This was possible to a certain extent in the above-described hierarchical ANOVA model. Therefore, this analytical method was not elaborated any further.

In addition to not delivering statistically sufficient results in rhythmicity analysis, several problems regarding meal criteria were observed. As mentioned, depending on period and data basis used, deviations were partly greater than 100% in meal criteria and number of meals. When calculating meal criteria for each individual animal, meal data were deviating similarly to the parameters observed based on crude data. Also, the actual pauses between the feeding events were not available since it was not possible to measure the duration of the feeding actions. This might have been one cause for the immense deviations between calculation periods. As the data clearly indicated that the individual's behaviour was a prominent factor in the observed parameters, it was concluded that condensing the data based on pen, treatment, or even the whole observed pig herd, important information would be lost. Therefore, it was concluded that a different approach was needed, since both crude and condensed data seemed to not fit rhythmicity analysis.

4. Outlook

The highly detailed data generated under near practical conditions, was not fitting established ethologic analytical methods. Future studies could change this by adding the duration of a feeding act or other additional measurements to the dataset. This should improve the practicability of feed intake data of group housed pigs for classic behavioural examination under practical conditions. Also, a model for inclusion of the individual animals into groups and their interaction therein should be developed to include the social context into the analytical model. This would probably need additional observation technique, such as modern automatic video systems, to assess the hierarchy and social status of the animals as this is one of the most important influences on the behaviour of an individual.

The disturbances in stable routine did not influence the feeding behaviour of the piglets and fattening pigs consistently. To elaborate whether the animals show even a concordant reaction, examination of more animals and groups would be necessary. Increasing the numbers of observed pigs with a reduction in treatments could support such an experimental approach. The chronic exposure to DON (Loibl et al. 2020b) and the stressful period after weaning (Chapter IV) already indicated that phases of distress seemed to harmonise the behaviour of an animal group to a certain extent. As pigs in practical husbandry conditions will continue to be group housed in the future and changes in legislation increasingly emphasise group-housing systems in sows, understanding the behaviour of swine groups will be crucial. Feed intake is measured in all stables routinely, therefore, this parameter still could be the easiest measurement to evaluate the health and welfare status of the animals.

5. Conclusions

The goal of this doctoral project was to determine whether detailed measurement of individual feed intake of pigs under practical housing conditions offered a valuable addition to the routinely recorded nutritional parameters. These potentially new indicators could then support the pig industry in reacting to its current main challenges, especially animal welfare and resource efficiency. Therefore, several traits of feeding behaviour of piglets and fattening pigs confronted with short term disturbances in stable routine and fattening pigs persistently exposed to feed-borne mycotoxins were examined. The detailed feeding data was generated by automatic single space feeders.

The developed hierarchical ANOVA model delivered good insight in the data's main sources of variation. Thereby, the most important factors (treatment, animal group (pen), sex, or individual animal) influencing performance and feeding behaviour were identified with reasonable effort and precision.

The measurement of zootechnical performance and feeding behavioural traits (daily feed intake, feed intake per visit to the feeder, number of daily feeder-visits, and most consumed feed in one visit per animal and day) worked well with the used technique. If necessary, such technique could be implemented in practical feeding systems.

Short-term disturbances, like feed deprivation, changes in feed composition, or exchanging of animals, did not have sustained effects on the zootechnical performance of piglets and fattening pigs. The animals were able to quickly compensate these disturbances in the following days.

Pigs confronted with long-term mycotoxin (Deoxynivalenol) exposure reduced feed intake by over 30%, resulting in reduced gain and longer fattening periods. The two different mycotoxin binders used did not improve performance. These results were expected and go hand in hand with literature. We verified that exposure to DON causes feed refusal. Also, the effects of mycotoxin were easily accessible with performance measurement routine.

The treatments did not affect the feeding behavioural traits, except for the reduction in daily feed intake when animals were exposed to DON. Therefore, the animals did not react concordant to the same disturbance. The pigs developed highly individual feeding patterns probably cross-linked to the hierarchical rank within the group. These patterns underlay a great day-to-day variation interfering with statistical precision. The presence of high levels of DON in feed even increased the variation leading to even more erratic behaviour.

Feeding behaviour of pigs is subject to several social factors. Therefore, the animals developed their persistent patterns within the context of their group, i. e. the pen. This was particularly demonstrated when fattening pigs were exposed to mycotoxins. Although, no treatment influence was measured on feeding behaviour, the groups' influence was highly significant. Presumably, the animals developed their individual behavioural pattern depending on their rank in the hierarchy of their respective groups.

Therefore, “group behaviour” was linked rather to the individual animals within the group than to external influences.

Ethologist have developed several methods to observe and analyse behaviour and its aberrations over time focussing on individual animals. These include rhythmicity or spectral density analyses with or without prior calculation of meals or timeseries analysis. This way, a time dependency in behaviour should be considered. However, the high day-to-day variation in the behavioural traits of the group-housed pigs seemed to interfere with these methods. Furthermore, the duration of the single feeder-visit could not be measured. That reduced the feasibility of the dataset for several of the analytical methods. Follow-up studies in such practically housed pigs should focus more on establishing data that could be used for these analytical methods taking the time-factor into account.

In total, adding the individual animal as a secondary observation to feeding assessment in practical housing does not result in an added value compared to the already available data of animal groups. Individual animals develop highly variant behavioural patterns depending on the context of their rank within a group. Individual behaviour’s volatile nature interferes with possible implication of these parameters in identification of distress. Future studies should focus on increasing animal as well as group (pen) numbers to examine whether conform reactions to disturbance can be observed. This way, the time-dependency of feed intake behaviour could be investigated as well.

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