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The impact of prebiotic supplementation in piglets and its effect on learning and memory

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Abstract

The microbiota-gut-brain axis is known to have the ability to influence host physiology and behaviour. Studies have demonstrated an impact from gut microbiota on a variety of behaviours, such as learning and memory, largely through the use of rodent models. These impacts can be observed through changing the gut microbiota composition in ways such as administration of prebiotics, which are defined as "selectively fermented ingredients that confer benefits upon host health". Beta-glucan is a known prebiotic that has been shown to promote beneficial microorganisms in the gut associated with effects on cognitive function. Pigs were used in this study to explore the impacts of early prebiotic supplementation on gut microbiota colonization and its subsequent effect on learning and memory. The effects on learning and memory were studied through the use of a standardized T-maze test. Supplemented pigs performed slightly better in the acquisition phase of the test, though control pigs were faster and performed better in the reversal phase. A possible sex and phase interaction was observed, as female pigs were faster and had more correct trials in the acquisition phase than males, though the opposite trend was seen in the reversal phase. Overall, trends observed were weak and likely would not have reached significance, apart from the reversal phase of mean trials correct per session, where pigs supplemented with beta-glucan performed worse than control pigs. This may indicate that supplemented pigs were less flexible and may have a greater difficulty in adapting to a changing environment. These results could have implications for on-farm practices, though how exactly requires further investigation. In conclusion, further research into the interaction of prebiotics and the microbiota-gut-brain axis is needed, as well as how this interaction impacts learning and memory in pigs.

1. Introduction

1. 1. The microbiota-gut-brain axis and behaviour

Gut microbiota play a fundamental role in regulating the physiology and health of their host. They are involved in a wide variety of activities that affect immune function, carbohydrate metabolism, fiber degradation and homeostasis maintenance (Cénit *et al.,* 2014; Clemente *et al.,* 2012). More recently it has become evident that gut microbiota also have the ability to influence host behaviour through bidirectional communication with the brain, a pathway commonly referred to as the microbiota-gut-brain axis. The effects of this pathway have been highlighted in various behavioural studies, many of which have involved the use of germ free mice. Several of these studies have shown a reduction in anxiety-like behaviours of germ free mice when compared to specific pathogen free mice raised with normal gut microbiota colonization (Heijtz *et al.,* 2011; Neufeld *et al.,* 2011). Depression, social and autism-like behaviours have also been manipulated in mice through altering the composition of their gut microbiota. Desbonnet *et al.* (2009) induced depression-like behaviours in rats as a result of maternal separation, which normalized after treatment with the probiotic *Bifidobacteria infantis*. In another study, valproic acid was introduced into the gut of mice to stimulate intestinal inflammation and resulted in reduced social behaviour (de Theije *et al.* 2014). Taken

together, these findings support the high capability of gut microbiota to influence not only host health, but behaviour and cognitive function as well.

Among the behaviours of interest in connection to the microbiota-gut-brain axis are those regarding learning and memory. Similar to other studies within the area, rodents have been a common subject choice when exploring potential impacts of microbiota on cognitive function. Encouraging results surrounding supplementation of probiotics to mice and rats have opened the doors to studying the role of microbiota on learning and memory behaviours in larger mammals, humans included. One such study demonstrated that rats with impaired learning and memory capabilities due to induced diabetes significantly improved in cognitive behavioural tests after treatment with probiotics (Davari *et al.,* 2013). Another study investigating the effects of certain strains of *Bifidobacterium* on cognition in a strain of anxious mice found that administration of the probiotic did indeed result in improved learning and memory behaviours when mice were assessed with a variety of cognitive tests (Savignac *et al.,* 2015). Additionally, Gareau *et al.* (2011) determined that mice infected with a pathogen and subjected to a stressor exhibited impaired cognitive function when compared to control mice. Interestingly, when infected mice were administered a probiotic, memory dysfunction was prevented. The same study observed poor learning and memory in germ free mice expressing no clear signs of anxiety, suggesting that commensal gut microbiota are essential for maintaining proper cognitive function. Of equal importance, it has been suggested that aspects of the microbiota-gut-brain axis related to cognitive function may operate in a sex-dependent manner (Clarke *et al.,* 2013). This information may be of particular consequence while studying the role that the microbiota-gut-brain axis plays in learning and memory processes.

Thus far, the vast majority of studies concerning the microbiota-gut-brain axis and learning and memory behaviours have been conducted on rodents, as previously mentioned. These studies have highlighted the influence that microbiota can have on cognitive function and as such, have provided the insight needed for scientists to begin moving forward and investigating this relationship in larger animal models. This has potentially significant implications for humans in regard to neuromedicine, and it is important that studies focusing on species physiologically similar to humans continue exploring this area. The pig is one such species that has been proven to be a promising candidate for the study of a wide array of human-related issues, including those involving intestinal gut microbiota, for reasons such as similarities in cognitive function, gut microbiota composition and gastrointestinal tract functions (Heinritz *et al.,* 2013; Lind *et al.*, 2007). Furthermore, investigating the relationship between the microbiota-gut-brain axis and cognitive function may prove to be of consequence for the pig, with the potential to improve both its health and welfare.

1.2. Prebiotics and behaviour

It is evident that microbiota-related changes to host physiology and/or behaviour typically arise due to alterations in gut microbiota composition. Consequently, in order to better understand the microbiota-gut-brain axis, it is imperative that factors affecting the composition of gut microbiota are thoroughly examined. In this regard, prebiotics have become of increasing interest for their known positive effect on gut health, and potential to target and support a wide range of beneficial gut microbiota (Douglas & Sanders, 2008). Though various definitions exist, a prebiotic is generally considered to be “a

selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health" (Gibson *et al.,* 2004).

One type of fibers in particular, the beta-glucans, have long been recognized for their health benefits, including their role in low cholesterol, heart health, diabetes prevention, immune function and a variety of other health aspects (Daou and Zhang, 2012). Some of the most common sources of beta-glucans include cereals, such as oats and barely, mushrooms, and yeasts (Zhu *et al.*, 2015). Beyond the general health benefits that they impose, it is evident that beta-glucans possess prebiotic properties as well. A comprehensive review written by Lam & Cheung (2013) highlights the bifidogenic and lactogenic properties of beta-glucans. These are desirable traits, as lactobacillus and bifidobacteria seem to be the dominant bacteria connected to influencing a multitude of cognitive behaviours, including learning and memory (Foster & Neufeld, 2013; Mayer *et al.,* 2015). These bacteria can also be found in both the pig and human gastrointestinal tract (Heinritz *et al.,* 2013). Futhermore, lactobacillus and bifidobacteria are two commonly used probiotics with health benefits of their own (Douglas & Sanders, 2008). This suggests that beta-glucans, which promote these bacteria, may have the ability to enhance learning and memory in pigs.

1.3. Life stage and microbiota composition

Life stage has been known to influence the ability of prebiotics to alter microbiota composition. Heijtz *et al.* (2011) highlighted that the ability of the microbiota-gut-brain axis to influence host behaviours may itself be sensitive to age. This was seen when altered behaviour in germ free mice could not be normalized with conventionalization of microbiota in adulthood, though it could be normalized through conventionalization early in life. Additionally, Mitsou *et al.* (2010) investigated the prebiotic potential of barley beta-glucan in a randomized, double-blinded, placebo-controlled study using healthy volunteers ranging from 39-70 years of age. In subjects 50 years and older, a strong bifidogenic effect of beta-glucan was found, though this effect was not apparent in subjects younger than 50 years of age, suggesting age to be a factor. Evidence also indicates that gut microbiota composition throughout early life is particularly vulnerable to environmental changes such as diet and the supplementation of prebiotics (Rodríguez *et al.,* 2015).

1.4. T-maze

The use of a T-maze has been shown to be an effective way of studying learning and memory in various animal models. The use of a spatial T-maze is particularly relevant for pigs due to their innate rooting and foraging behaviour (Gieling *et al.,* 2011). These exploratory behaviours have been important in ensuring that pigs in the wild are able to locate necessary resources crucial to their survival (Studnitz *et al.,* 2007). The T-maze utilized within this study was adapted from Elmore *et al.* (2012), which was the first learning T-maze task of its design applied to pigs. The acquisition phase of the T-maze measures the initial learning and memory of pigs trying to locate a reward in one of two reward arms within the maze. The reversal phase of the T-maze then measures the pigs' learning and memory flexibility/adaptability as they are taught to retrieve the reward in the opposite reward arm as previously required in the acquisition phase. The authors designed the maze with two starting gates, which they found was ideal for studying

learning using an allocentric mechanism (i.e. visual cues) that has been suggested to be hippocampal dependent, as opposed to an egocentric mechanism (repeatedly choosing left or right). This is an important aspect of the present study, as it has been suggested that the hippocampus can be affected by the microbiota-gut-brain axis (Dinan & Cryan, 2012; Foster & Neufeld, 2013). Thus, utilizing this type of learning may best highlight any changes via the microbiota-gut-brain axis that could occur as a result of supplementing pigs with oat beta-glucan.

Due to the information presented, we hypothesize that supplementing piglets with the prebiotic oat beta-glucan early in life will result in a change in microbiota composition, though complete results will not be available for this thesis. Due to oat beta-glucan possessing lactogenic and bifidogenic properties, an increase in these bacteria is expected, resulting in greater influence on cognitive function, leading to an improvement in learning and memory behaviour in supplemented pigs later on in life. It is expected that this improvement in learning and memory will be demonstrated through a superior performance in both the acquisition and reversal phases of the T-maze, when compared to control pigs. Furthermore, we hypothesize an effect of sex will be observed between the pigs, as the microbiota-gut-brain axis has been previously demonstrated to operate in a sex-dependent manner.

2. Purpose statement

The purpose of this study is to investigate the impact of prebiotic supplementation on learning and memory in pigs. This will be done through assessing mean trials to criterion completion, mean trials correct per session and mean latency to choice per session in a T-maze design. The main questions of interest are: Is an improvement seen in learning and memory when pigs are supplemented with prebiotics? Are any sex differences, in regard to learning and memory, observed within the pigs?

3. Materials and methods

3.1. Animals, housing, and supplementation

Forty-three Landrace-Yorkshire pigs from six litters were used in this study, including 24 females and 19 males. After farrowing, sows and piglets were housed with their litters in standardized open-farrowing pens. At seven days of age 50% of the litter was supplemented with an oat beta-glucan prebiotic, while the other half were used as control pigs that were given water. This was done pseudo-randomly by gender, resulting in male control, female control, male supplemented and female supplemented pigs. Supplemented pigs were given 40 milligrams of beta-glucan per kilogram of body weight throughout the duration of the supplementation period. This dosage was also applied to the volume of water control pigs received. When treatment was given, each supplemented piglet was gently lifted up by the use of a sling and given the supplement orally through a syringe. The other half of the litter was sham handled in the same way but given water. Supplementation continued three times a week for a period of four weeks. The last supplement was given one day before weaning, with weaning taking place when piglets were 34 days of age. After weaning the sow was removed and pigs remained within their

original home pens until maze habituation. Piglets were fed three times a day by an automated feeder and pens were cleaned daily by barn personal. All pigs had ab libitum access to water.

3.2. T-maze design

The T-maze used in this study was adapted from Elmore *et al.,* (2012). The main structure of the maze consisted of wood, while a transparent acrylic glass material was used for the walls. Guillotine style doors were used for the north and south entrances of the maze and both start boxes were made of wooden panels (Fig. 1 and Fig. 2). Rubber mats were fitted onto the floor of the maze and secured in place. Two identical plastic reward buckets were used in the maze, one in the east wing and one in the west wing, and were fastened to the floor. Both reward buckets had double bottoms that contained three marshmallows to control for pigs locating the reward due to smell. All marshmallows used in this study were Haribo Chamallows Barbecue. Visual cues in the form of four plastic coloured panels (two different colours for each reward bucket) were placed around the reward buckets to help enable the pig to distinguish between each reward wing, regardless of the direction it entered from.

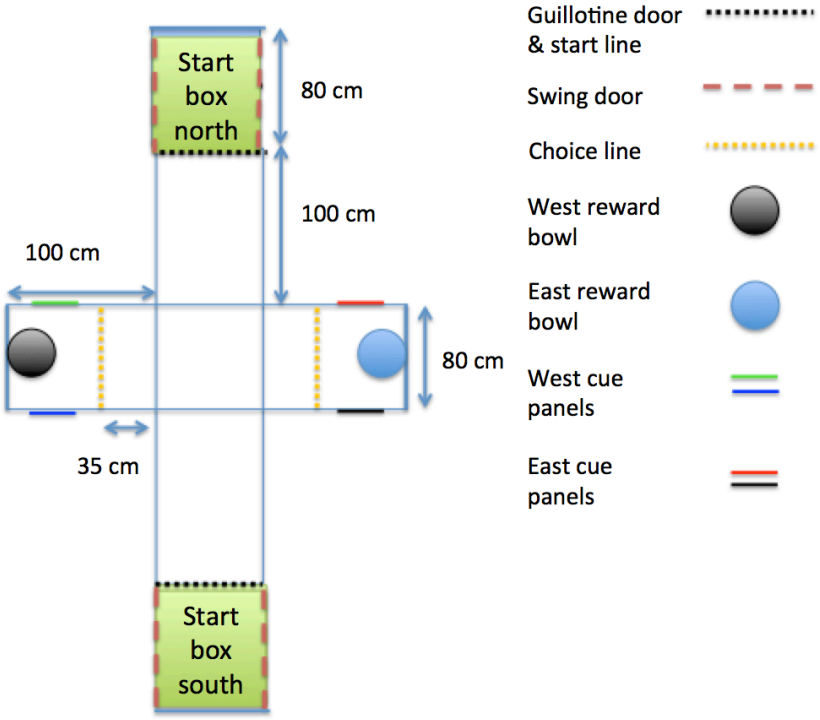

Fig. 1 Schematic diagram of T-maze used to examine learning and memory in pigs

3.3. Pen and maze habituation

Pigs were subjected to five days of habituation in the home pen, where the experimenter entered the pen and sat on the floor with the pigs for 15 minutes. The experimenter spoke softly, offered rewards in the form of marshmallows and gently touched the pigs that approached. The following week pigs were moved into the experiment wing of the barn and subjected to four days of habituation in a T-maze. Experimenters placed rewards of half of a marshmallow throughout the maze and inside the reward buckets in each wing before the pigs entered. On the first day of maze habituation pigs were led into the maze

Fig. 2 Photo of constructed T-maze used within the study

two times each, in groups of three and given time to search for rewards. Experimenters documented whether or not each piglet was calm and eating rewards, making note of any abnormal behaviour. After 10 minutes, or once all of the rewards had been eaten, pigs were let out of the maze. The following three days of maze habituation, pigs were led into the maze three times a day in groups of two, following the same protocol as previously described. On the fourth day of maze habituation, experimenters documented whether each pig was able to leave the start box unassisted and begin eating rewards within 15 seconds. Once this criterion was met, pigs were allowed to continue to maze training. In rare cases where pigs were unable to meet the set criteria, pigs continued with maze habituation until successful.

3.4. Maze training

Individual maze training began when pigs were around 63 days of age. The first task of acquisition learning consisted of each pig learning individually to locate a reward in a fixed arm of the maze. All rewards were given in the form of half of a marshmallow. Half of the pigs within each treatment were pseudo-randomly assigned to locate the reward in the east wing, while the other half were assigned to locate the reward in the west wing. Each pig was led into a start box before the beginning of each trial, alternating between the north and south entrance according to a pseudo-random pattern, and using the same entrance no more than three times. This was done to ensure that the pig did not locate the reward due to learning to turn right or left, instead of choosing the correct reward wing.

Once the experimenter had placed a reward in the reward bucket and returned to their position beside the start box, the guillotine door was lifted to allow the pig entrance into the maze. When the pig had entered the maze and both of its front legs had crossed the start line on the floor the trial began. Pigs were given 60 seconds to make a choice between the east or west reward arm. If the pig did not make a decision within the allotted time the experimenter recorded the trial as “no” for litters 1-4 and “no choice” for litters 5-6, and the trial was ended. A choice of east or west by the pig was defined as the

moment at which both of the pig's front legs crossed the choice line on the floor in front of the reward bucket. The pig was only allowed to make one choice per trial. If the pig chose correctly it was allowed to consume the reward inside the reward bucket before it was let out of the maze. If the pig chose incorrectly no reward was allowed. The trial was then complete and the experimenter recorded the latency of the pig's choice from start of trial until time of choice, and whether or not the pig chose correctly. If any urination or defecation occurred, experimenter cleaned the maze before the start of the next trial.

On the first day of maze training each pig completed five trials consecutively, which was considered "session 0". These acted as buffer trials to allow pigs to adjust to being in the maze individually. If the pig expressed clear signs of stress, such as frequent vocalization, defecation and/or urination, or jumping on the sides of the maze, a 10-20 minute break was given and remaining trials were completed afterwards. From the second day of maze training, each pig completed 10 trials per day, with every 10 trials considered as one session. The acquisition training was considered successful when the pig was able to locate the reward at least 80% of the time, for two consecutive sessions. When this criterion was met, the pig began its second task of reversal training in the maze. Here the pig was trained to locate the reward from the opposite wing that it had previously been assigned to, following the same protocol as described above. Once the pig was able to locate the reward at least 80% of the time for two consecutive sessions and had completed at least 50 trails (5 sessions), training was complete.

3.5. Data analysis

All data collected was processed in excel, where mean trials to criterion completion of treatment groups and mean trials to criterion completion of litters, mean trials correct per session of treatment groups and mean latency per session of treatment groups were calculated. Standard error for all data was also calculated. Clustered column graphs were then used to compare descriptive data.

4. Results

4.1. Piglet cognitive performance

All 43 pigs took part in the acquisition phase of this study. However, one pig did not participate in reversal training, as it became ill and was then excluded from the study, leaving a total of 42 pigs to complete this phase.

4.2. Mean trails to criterion completion

On average, all pigs were faster at reaching maze criterion in the acquisition phase when compared to the reversal phase (Fig 3a and 3b). Control pigs tended to reach criterion completion of the acquisition phase and the reversal phase of the T-maze with a slightly fewer number of trials when compared to beta-glucan pigs. A possible sex and phase interaction was observed, as on average, female pigs were faster than male pigs at completing the acquisition phase criterion, while slower than male pigs at completing the reversal phase criterion. However, all data for mean trials to criterion was extremely similar and no meaningful differences between the groups seemed to be present.

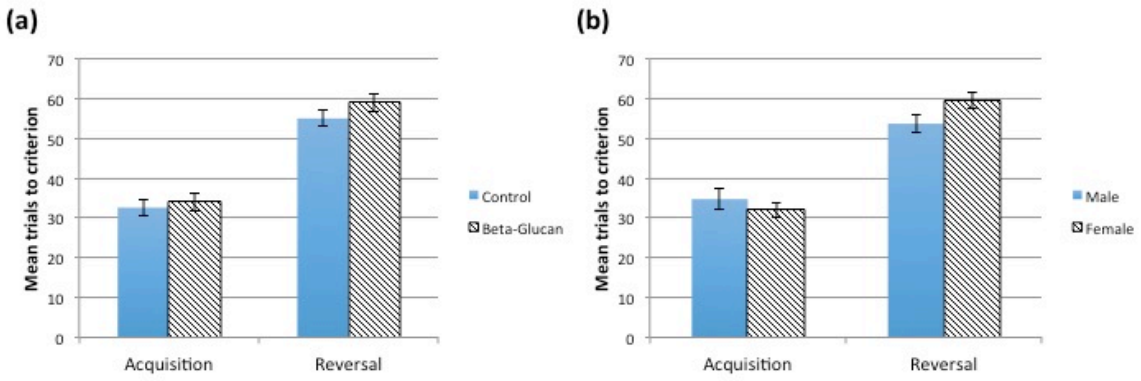

Fig. 3 Performance and standard errors of **(a)** control and supplemented (Beta-Glucan) pigs and **(b)** male and female pigs in mean trials completed in acquisition and reversal phase before reaching completion criterion.

4.3. Litter

The performance of pigs in litter one for mean trials to criterion (Fig. 4) appeared to be inferior during the reversal phase of the T-maze when compared to the other litters, a result that may reach significance. There did not appear to be any other major differences between the performances of individual litters during either the acquisition phase or the reversal phase of the T-maze.

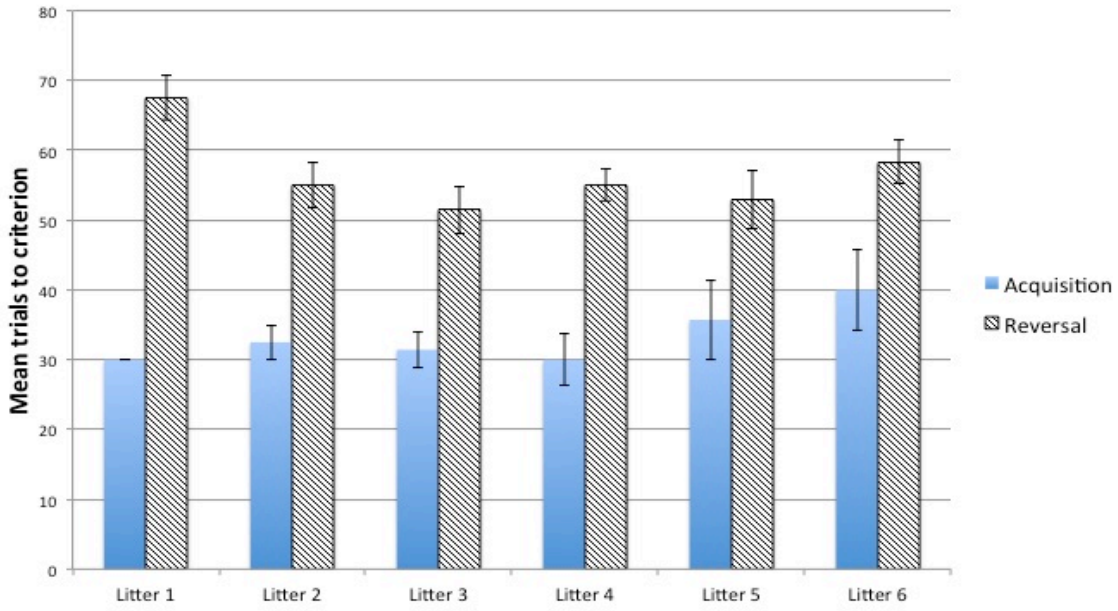

Fig. 4 Performance and standard errors of litters 1-6 in mean trials completed in acquisition and reversal phase before reaching completion criterion.

4.4. Mean trials correct/session

Mean trials correct per session (10 trials per session) showed that pigs improved throughout both acquisition and reversal sessions (Fig. 5). Across treatment groups, beta-glucan and control pigs had similar mean trials correct per session in the acquisition phase. During the reversal phase, control pigs completed more mean trials correct per session over all five sessions than beta-glucan pigs, which may be a meaningful difference. Female pigs completed slightly more mean trials correct per session during the acquisition phase compared to male pigs. The opposite trend between male and female pigs was observed over the five reversal sessions. However, it is important to note that the differences observed between male and female pigs in both the acquisition and reversal phase, were very small and would likely not reach statistical significance.

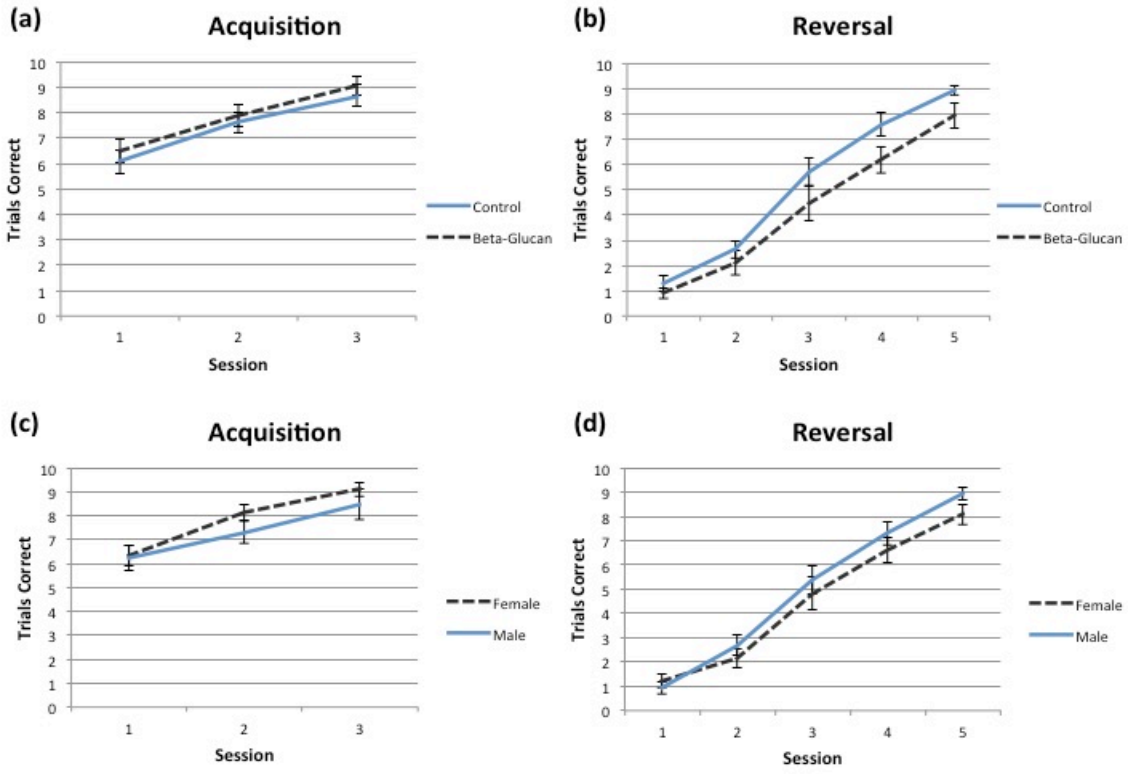

Fig. 5 Performance and standard errors of Control and Beta-Glucan pigs in the mean number of trials correct over each session during the acquisition phase **(a)** and the reversal phase **(b)** of the T-maze and Female and Male pigs in the mean number of trials correct over each session during the acquisition phase **(c)** and the reversal phase **(d)** of the T-maze.

4.5. Mean latency/session (s)

Mean latency to choice per session tended to decrease as sessions increased in the acquisition phase (Fig. 6a and 6c). During the reversal phase, all pigs showed an increase in latency to choice from session 1 in either session 2 or session 3, which then decreased in the following sessions. Overall, control pigs tended to be have faster latency to choice

in the acquisition and reversal phase when compared to beta-glucan pigs. A sex effect may have been observed during the reversal phase where male pigs had a faster mean latency to choice than females. However, it is important to note that for all latency data, differences observed in time to choice were small and variation was extremely high, suggesting that the data would not reach statistical significance.

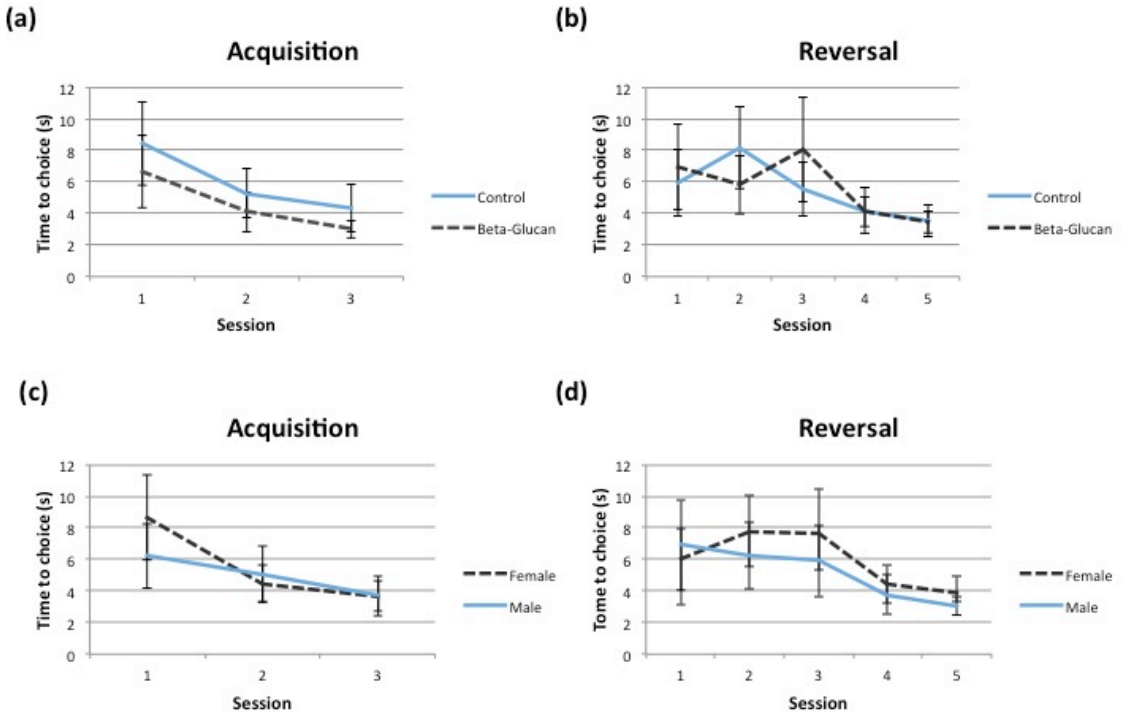

Fig. 6 Standard errors and mean time taken from the beginning of test until choice of reward arm for Control and supplemented (Beta-Glucan) pigs in **(a)** the acquisition phase and **(b)** the reversal phase of the T-maze and for Female and Male pigs in **(c)** the acquisition phase and **(d)** the reversal phase of the T-maze

5. Discussion

Through the use of a T-maze test, this study investigated learning and memory differences between control pigs and pigs supplemented with oat beta-glucan. Potential differences between male and female pigs were also analyzed and a trend suggesting a potential sex and phase interaction was observed during the acquisition and reversal phase of the T-maze. The reversal phase of the T-maze appeared to be more difficult for all pigs when compared to the acquisition phase, as mean trials to criterion increased, as well as a temporary increase in mean latency to choice during the reversal phase. Further analysis of mean trials to criterion did not indicate any significant differences between litters, apart from the inferior performance of litter one to all other litters during the reversal phase of the T-maze. As litter one was the first litter to be tested in the T-maze of

this study, the testing technique may have improved with the other litters, and could possibly explain the differences observed in litter one.

The majority of the data collected showed high levels of variation and did not appear to reach significant values. However, the larger differences and relatively low variation of mean trials correct per session during the reversal phase of the T-maze showed results that would likely have reached significance: control pigs appeared to outperform beta-glucan pigs in sessions 4 and 5 of the reversal phase, with a higher number of correct trails per session. The data obtained from "no choice" by pigs was not included in the results due to the low incidence that this occurred. Additionally, "no choice" for litter 1-4 and 5-6 was recorded differently, making comparisons of this data difficult.

The reason that all pigs in the study appeared to have more difficulty in the reversal phase than the acquisition phase of the T-maze was that once the pigs had become confident and accurate in their first training task, they were then made to learn the opposite of what was previously required. The challenge of the reversal task for the pigs was made even clearer through the observed mean latency to choice times. In the first reversal session the pigs took very little time to choose a reward bowl, as they were still performing as they had within the acquisition phase. When no reward was found in the expected bowl the pigs became more indecisive, which became evident from the distinct increase in mean latency to choice times for the pigs in session two or three. Experimenters also observed more behavioural signs of frustration from some of the pigs during this time, which mainly included floor biting, non-compliance to complete the task, and vocalization. The observed decrease in latency to choice times after these sessions highlights the learning that took place and the improvement in the task that the pigs experienced.

The meaningful results observed for mean trials correct per session during the reversal phase of this study between control and supplemented pigs is of interest, as in this phase, pigs were required to learn the opposite of what they had initially learned in the acquisition phase. This suggests that the pigs would need to be more flexible and readily adapt to the new task in order to be successful. The inferior performance of beta-glucan pigs was contrary to the hypothesis, and may have been due to an increased difficulty experienced by these pigs to change their previously learnt behaviour in order to learn a new task. Due to the unique circumstances created within this study, it is difficult to draw concrete conclusions from this data. However, these results could imply that certain prebiotic supplementation, or diet, may impact the ability of a pig to adjust to its environment, thereby having the potential to affect animal welfare. Consequently, an animal that is having difficulty to adapt to changes within its environment will likely become stressed, leading to production problems such as a decreased growth rate (Rutherford *et al.,* 2006), an outcome that is negative for both the animal, as well as the producer. This is especially relevant for pigs, as many farm practices result in changes to the pigs' environment, such as moving to new pens, mixing of piglets, introduction to new food, etc. Weaning, in particular, is known to be a stressful period for pigs due to a variety of sudden changes within the animals' environment (Campbell *et al.,* 2013). This makes these animals more vulnerable and highlights the important role that diet and gut microbiota composition could play in affecting health and welfare during this period. It also may have implications for pig management and for these previously described farm routines that require changes to the pigs' environment, an area that could prove to be of interest in future studies. While the supplemented prebiotics in this study did not positively support the pigs learning and memory as expected, that is not to say that

different results wouldn't be found under other circumstances or when supplementing different prebiotics, though further research is required.

A potential trend was observed in this study of an interaction between sex and phase. This was seen during mean trials to criterion completion and mean trails correct per session. Females tended to perform slightly better than males in the acquisition phase, and slightly worse than males in the reversal phase. Though results were not conclusive, this is of interest, as one study that used a spatial holeboard task to investigate cognitive abilities in pigs found that females performed better than males, but only during the reversal portion of the test (Roelofs *et al.,* 2017). Though this is in direct contrast to the trend observed in the current study, it supports the possibility of a sex effect involved in learning and memory processes. Research has also found evidence to suggest that the microbiota-gut-brain axis may be regulated in a sex-dependent way. This has most notably been seen in regards to the expression of brain-derived neurotrophic factor (BDNF), which among other places, is active in the hippocampus of the brain and is involved in memory function (Lu *et al.,* 2008). In particular, differences have been found in hippocampal BDNF of male and female germ free mice. Clarke *et al.* (2013) observed lower BDNF in male germ free mice, though not in female germ free mice. Though this interaction between sex and the microbiota-gut-brain axis cannot be explicitly implied for this study, it may be of significance if further research within this area is to continue.

Though control pigs performed better than supplemented pigs in the reversal phase of mean trails correct per session, no other significant data was observed. Due to this outcome, it appears that supplemented pigs did not experience improved cognitive function when compared to control pigs. This may have been observed for a number of reasons. Though numerous studies have observed changes in learning and memory through the manipulation of gut microbiota (Gareau *et al.,* 2011; Savignac *et al.,* 2015), Dvari *et al.* (2013) did not see the same positive effect of probiotic treatment on control rats as was seen for rats that had been made to be diabetic. It was concluded that if the subject in question already had an optimal activity of natural microbes within their gut, no further benefit on learning and memory could be obtained. Therefore, if the pigs within this study did not have a compromised gut microbiota composition, their learning and memory capabilities would potentially not have benefited from the supplemented prebiotic. Furthermore, it is also common for studies that explore the microbiota-gut-brain axis and effects on cognition to use germ free rodent models (Gareau *et al.,* 2011), anxious rodent strains (Savignac *et al.,* 2015) or to induce some form of gut dysbiosis (Fröhlich *et al.,* 2016). This brings to question whether these studies would have observed similar effects on cognitive function if animals that were not expressing signs of anxiety or that did not have induced gut dysbiosis had been used, and could help to explain the seemingly small differences between the control and supplemented groups of pigs within this study.

Another possible explanation for the lack of significant data is the effectiveness of the prebiotic itself. Due to time restraints, complete results on the control and supplemented pigs' gut microbiota profile were not available for analysis. Therefore, it was assumed that there was a difference between the gut microbiota composition of the control pigs and the gut microbiota composition of the supplemented pigs, as oat beta-glucan has been proven to increase counts of *Bifidobacterium spp.* and *Lactobacillus spp.* in the gut of pigs (O'Shea *et al.,* 2010). Futhermore, Murphy *et al.* (2012) determined beta-glucan derived from oats had superior effects on the *Lactobacillus* population in the porcine gastrointestinal tract when compared to barely beta-glucan. However, it is possible that

the oat beta-glucan did not successfully alter the gut microbiota of the supplemented pigs in our study. Though prebiotics have the ability to effect gut microbiota composition, various factors may determine the degree of their effectiveness. Source of beta-glucan has been shown to impact prebiotic potential, affecting oxygen radical absorbance capacity and ferric reducing antioxidant power (Du & Xu, 2014). In addition, studies have shown that content (high vs. low), solubility and form (purified vs. native) of beta-glucan can all impact possible prebiotic potential (Aumiller *et al.,* 2015).

Variation between individual personalities and coping strategies has been observed in many animal species, with two main coping styles emerging: proactive coping and reactive coping (Koolhaas, 2008). These same variations and coping styles may have played a role in the performance of individuals within this study, as they have been suggested to be present within pigs, though studies are somewhat inconclusive and seem to focus on aggressive behaviour (Janczak *et al.,* 2003). Additionally, animals expressing different aggression styles have not clearly been observed to contrast in their learning and memory performances (Koolhaas *et al.,* 2010). However, if high individual variation was a factor within this study, using a larger number of animals might help to achieve statistical significance in future studies.

Motivation to perform the task may have also impacted the results of some of the pigs, though this seems less likely, as pigs are natural foragers and appear to have a preference for sweets (Gieling *et al.,* 2011). This suggests that pigs should be highly motivated to locate a reward in the form of a marshmallow, which was inline with what the experimenters observed. During the reversal task, when the reward was not in the same reward bowl as the acquisition phase, pigs did express signs of frustration that somewhat hindered their progress, but all pigs eventually continued their trials and completed the task. Of further consideration, studies have shown that dietary fiber, especially that which is fermentable, has the ability to reduce physical activity and substrate-directed behaviour in pigs (de Leeuw *et al.,* 2008). This could account for a reduction in feeding motivation of the supplemented pigs. However, knowledge as to how early supplementation of fiber would affect piglet behaviour after weaning is limited and studies examining the relationship between dietary fiber and behaviour of pigs has thus far typically focused on sows.

It has been acknowledged that the bidirectional pathway established through the microbiota-gut-brain axis can play a large role in the development and activation of stress systems within the body (Dinan & Cyran, 2012). One way that this can be seen is through the role that early life stress plays on gut microbiota composition. Animals that have undergone perinatal stress have demonstrated altered microbiota composition and it has been suggested that these changes within the microbiota-gut-brain axis may influence the development of the animals' adult phenotype (Mayer *et al.,* 2015). Furthermore, a reduction in *Lactobacillus spp.* within the gut with exposure to stress has been repeatedly observed (Galley & Bailey, 2014) and a study on chronic stress found effects in the hippocampus and hypothalamus of rats that were suggested could have impacts on memory (Joëls *et al.,* 2004). These findings suggest a clear relationship between microbiota composition and stress, as well as cognitive function, which is relevant when studying the effects of early microbiota colonization and learning and memory. In this study, the piglets were orally administered prebiotics on a regular basis until time of weaning. Though all handling was done with care, the supplementation process required unavoidable stress to the piglets. This may have negatively affected microbiota composition from a young age in both sham handled and supplemented piglets. To limit

potential impacts on microbiota composition as a result of stress, future studies may benefit from exploring alternative prebiotic supplementation techniques that require minimal handling of the piglets and result in less stress. Furthermore, with all of the health benefits that prebiotics contain, a stress-free method of supplementing pigs will be highly desirable for use within a commercial setting.

In addition to the source of prebiotic, the quantity administered is known to impact potential benefits and overall effectiveness. The dose required to achieve adequate levels of prebiotics in the human diet can be wide ranging and often depends on the type of prebiotic being used and the fermentation profile of that prebiotic (Douglas & Sanders, 2008). In a study conducted by Modesto *et al.* (2009), pigs were administered three different prebiotics at two different doses, one at 1% of the diet and the other at 4% of the diet. The prebiotics promoted the growth of bifidobacteria in the caecum, which increased as the dose increased. Another study administered three different prebiotic doses to rats (0%, 10% and 20% of the diet) and observed a change in gut microbiota that was also found to be dose dependent (Parnell & Reimer, 2012). Due to the novelty of working with the microbiota-gut-brain axis and its interaction with prebiotics, it cannot yet be established what dose is required to observe subsequent effects on behaviour. There is a possibility that a higher dose than was used within this study is needed in order to adequately promote a shift in gut microbiota composition and impact learning and memory. More research between the microbiota-gut-brain axis and specific types of prebiotics may be helpful in the future in order to better clarify the proper dosage parameters to be working within.

There are many different methods that have been utilized to study learning and memory in animal models. Understanding the species that is being investigated is crucial for a successful study and there is an extensive list of such species appropriate methods for testing learning and memory in pigs (Gieling *et al.,* 2011). Various maze tests have been used with pigs and Elmore *et al.* (2012) found the design of the T-maze used within this study to be a valuable tool in assessing learning and memory. However, the current study did adjust some of the design aspects of the maze, such as the size, for practical reasons. This may have had an impact on the function of the maze or how the pigs made their choice of reward wing. It is also possible that the acquisition and reversal tasks were not challenging enough for the pigs, preventing any clear differences between treatment groups to be established. That being said, the addition of a reversal phase to a T-maze test has been suggested to be of adequate difficulty level for pigs (Gieling *et al,* 2011). In order to improve the T-maze design used within this study it may be beneficial to construct a larger maze with longer reward arms. This would make it easier to control for visual and olfactory cues, and the choice made by the pig would be much more distinct. It would also decrease the risk of the pig making a choice by chance, as longer reward wings would require a larger commitment and possibly a larger sense of motivation from the pig.

6. Conclusion

Supplementation with oat beta-glucan to piglets early on in life did not appear to improve cognitive function, as demonstrated through performance in a T-maze. However, results did indicate that pigs supplemented with oat beta-glucan performed inferior to control

pigs in mean trials correct per session during the reversal phase of the T-maze. As the reversal phase requires more flexibility from the animal, it is possible that these results indicate a reduced ability of the supplemented pigs to adapt to changes in their environment. This, in turn, suggests a connection between prebiotics/diet, environment, and animal welfare. These findings could have important implications for pig management and farm practices, as many common routines involve changes to the pigs' environment. Due to the complex relationship between microbiota, prebiotics and cognitive function, deeper investigation is required to determine the mechanisms behind these interactions. A possible sex and phase interaction may have been observed between male and female pigs, where female pigs tended to perform better in the acquisition phase of the T-test, while males performed better during the reversal phase, though results were likely not significant. This warrants further investigation, as the microbiota-gut-brain axis has been suggested to function in a sex-dependent manner. The area of prebiotics and the microbiota-gut-brain axis is novel and much has yet to be understood. Future studies may benefit from better understanding dosage requirements, improving supplementation techniques and considering the impact that the microbiota-gut-brain axis may have on how the pig responds to changes within its environment. The majority of research thus far has focused on the use of probiotics when investigating the microbiota-gut-brain axis, and encouraging progress has been made. However, prebiotics should not be overlooked, as they offer a wide range of health benefits, with much potential left to explore.

7. Popular scientific summary

The relationship between gut microbiota and the brain is commonly referred to as the microbiota-gut-brain axis. This axis has been thought to have the ability to influence a variety of behaviours of its host, such as learning and memory. Prebiotics are known as "selectively fermented ingredients that confer benefits upon host health". Oat beta-glucan is a prebiotic that supports the growth of particular gut microbiota. Some of these microbiota have been associated with the ability to influence learning and memory. Due to having a similar gut structure as humans, pigs were used in this study to observe the effect that early feeding of prebiotics has on gut microbiota, and how this impacts learning and memory later in life. A T-maze test with two phases was used to compare learning and memory abilities of pigs that had been fed the prebiotic oat beta-glucan to the pigs that had not. During the second phase of the test, the pigs that had been fed prebiotics did not perform as well as the pigs that hadn't and this result may have reached significance. The second phase of the T-maze is where more flexibility is required, as the pigs must learn the opposite task that they did in the first phase. This may suggest that the pigs fed oat beta-glucan had a more difficult time adapting to their changing environment. Many common farm practices today expose pigs to changes such as eating new food or moving pens. If the pig is not able to adapt to these changes its welfare may suffer, which is negative for both the farmer, as well as the pig. Through better understanding how altering the gut microbiota of pigs affects their learning and memory, and possibility their flexibility/adaptability, it may be possible to improve pig welfare. Further research is needed to investigate prebiotics and the microbiota-gut-brain axis, as well as how this impacts learning and memory in pigs.

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9. References

Aumiller, T., Mosenthin, R. and Weiss, E. (2015). Potential of cereal grains and grain legumes in modulating pigs' intestinal microbiota- A review. *Livestock Science,* vol. 172, pp. 16-32.

Campbell, J. M., Crenshaw, J. D. and Polo, J. (2013). The biological stress of early weaned piglets. *Journal of Animal Science and Biotechnology*, vol. 4, pp. 19. DOI:10.1186/2049-1891-4-19

Cénit, M. C., Matzaraki, V., Tigchelaar, E. F. and Zhernakova, A. (2014). Rapidly expanding knowledge on the role of the gut microbiome in health and disease. *Biochimica et Biophysica Acta*, vol. 1842, pp. 1981-1992.

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., Dinan, T. G. and Cryan, J. F. (2013). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, vol. 18, pp. 666-673.

Clemente, J. C., Ursell, L. K., Parfrey, L. W. and Knight, R. (2012). The impact of the gut microbiota on human health: an integrative view. *Cell*, vol. 148 (6), pp. 1258-1270.

Daou, C. and Zhang, H. (2012). Oat beta-glucan: its role in health promotion and prevention of diseases. *Comprehensive Reviews in Food Health and Food Safety*, vol. 11, pp. 355-365.

Davari, S., Talaei, S. A., Alaei, H. and Salami, M. (2013). Probiotics treatment improves diabetes-induced impairment of synaptic activity and cognitive function: Behavioral and electrophysiological proofs for microbiome-gut-brain axis. *Neuroscience*, vol. 240, pp. 287-296.

de Leeuw, J. A., Bolhuis, J. E., Bosch, G. and Gerrits, W. J. J. (2008). Effects of dietary fibre on behaviour and satiety in pigs. *Proceedings of the Nutrition Society*, vol. 67, pp. 334-342.

Desbonnet, L., Garrett, L., Clarke, G., Bienenstock and Dinan, T. G. (2009). The probiotic Bifidobacteria infantis: An assessment of potential antidepressant properties in the rat. *Journal of Psychiatric Research*, vol. 43, pp. 164-174.

de Theije, C. G. M., Koelink, P. J., Korte-Bouws, P. J., da Silva, S. L., Korte, S. M., Olivier, B., Garssen, J. and Kraneveld, A. D. (2014). Intestinal inflammation in a murine model of autism spectrum disorders. *Brain, Behavior and Immunity,* vol. 37, pp. 240-247.

Dinan, T. G. and Cryan, J. F. (2012). Regulation of the stress response by the gut microbiota: Implications for psychoneuroendocrinology. *Psychoneuroendocrinology*, vol. 37, pp. 1369-1378.

Douglas, L. C. and Sanders, M. E. (2008). Probiotics and prebiotics in dietetics practice. *Journal of the American Dietetic Association*, vol. 108, pp. 510-521.

Du, B. and Xu, B. (2014). Oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) of β-glucans from different sources with various molecular weight. *Bioactive Carbohydrates and Dietary Fibre*, vol. 3, pp. 11-16.

Elmore, M. R. P., Dilger, R. N. and Johnson, R. W. (2012). Place and direction learning in a spatial T-maze task by neonatal piglets. *Animal Cognition*, vol. 15, pp. 667-676.

Foster, J. A. and Neufeld, K. A. M. (2013). Gut-brain axis: how the microbiome influences anxiety and depression. *Trends in Neurosciences*, vol. 36 (5), pp. 305-312.

Fröhlich, E. E., Farzi, A., Mayerhofer, R., Reichmann, F., Jačan, A., Wagner, B., Zinser, E., Bordag, N., Magnes, C., Fröhlich, E., Kashofer, K., Gorkiewicz, G. and Holzer, P. (2016). Cognitive impairment by antibiotic-induced gut dysbiosis: Analysis of gut microbiota-brain communication. *Brain, Behavior, and Immunity*, vol. 56, pp. 140-155.

Galley, J. D. and Bailey, M. T. (2014). Impact of stressor exposure on the interplay between commensal microbiota and host inflammation. *Gut Microbes,* vol. 5 (3), pp. 390-396.

Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., MacQueen, G. and Sherman, P. M. (2011). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut*, vol. 60, pp. 307-317.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A. and Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews*, vol. 17, pp. 259-275.

Gieling, E. T., Nordquist, R. E. and van der Staay, F. J. (2011). Assessing learning and memory in pigs. *Animal Cognition*, vol. 14, pp. 151-173.

Heijtz, R. D., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., Hibberd, L., Forssberg, H. and Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences of the United States of America,* vol. 108 (7), pp. 3047–3052.

Heinritz, S. N., Mosenthin, R. and Weiss, E. (2013). Use of pigs as a potential model for research into dietary modulation of the human gut microbiota. *Nutrition Research Reviews*, vol. 26, pp. 191-209.

Janczak, A. M., Pedersen, L. J. and Bakken, M. (2003). Aggression, fearfulness and coping styles in female pigs. *Applied Animal Behavior Science*, vol. 81, pp. 13-28.

Joëls, M., Karst, H., Alfarez, D., Heine, V. M., Qin, Y., van Riel, E., Verkuyl, M., Lucassen, P. J. and Krugers, H. J. (2004). Effects of chronic stress on structure and cell function in rat hippocampus and hypothalamus. *Stress*, vol. 7 (4), pp. 221-231.

Koolhaas, J. M. (2008). Coping style and immunity in animals: Making sense of individual variation. *Brain, Behavior, and Immunity*, vol. 22, pp. 662-667.

Koolhaas, J. M., de Boer, S. F., Coppens, C. M. and Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology*, vol. 31, pp. 307-321.

Lam, K. L. and Cheung, P. C. K. (2013). Non-digestible long chain beta-glucans as novel prebiotics. *Bioactive Carbohydrates and Dietary Fibre*, vol. 2, pp. 45-64.

Lind, N. M., Moustgaard, A., Jelsing, J., Vajta, G., Cumming, P. and Hansen, A. K. (2007). The use of pigs in neuroscience: Modeling brain disorders. *Neuroscience and Biobehavioral reviews*, vol. 31, pp. 728-751.

Lu, Y., Christian, K. and Lu, B. (2008). BDNF: A key regulator for protein-synthesis dependent LTP and long-term memory? *Neurobiology of Learning and Memory*, vol. 89 (3), pp. 312-323.

Mayer, E. A., Tillisch, K. and Gupta, A. (2015). Gut/brain axis and the microbiota. *The Journal of Clinical Investigation*, vol. 125 (3), pp. 926-938.

Mitsou, E. K., Panopoulou, N., Turunen, K., Spiliotis, V. and Kyriacou, A. (2010). Prebiotic potential of barely derived β-glucan at low intake levels: A randomised, double-blinded, placebo-controlled clinical study. *Food Research International*, vol. 43, pp. 1086-1092.

Modesto, M., D'Aimmo, M. R., Stefanini, I., Trevisi, P., De Filippi, S., Casini, L., Mazzoni, M., Bosi, P. and Biavati, B. (2009). A novel strategy to select *Bifidobacterium* strains and prebiotics as natural growth promoters in newly weaned pigs. *Livestock Science,* vol. 122, pp. 248-258.

Murphy, P., Bello, F. D., O'Doherty, J. V., Arendt, E. K., Sweeney, T. and Coffey, A. (2012). Effects of cereal β-glucans and enzyme inclusion on the porcine gastrointestional tract microbiota. *Anaerobe*, vol. 18, pp. 557-565.

Neufeld, K. M., Kang, N., Bienenstock, J. and Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol Motil,* vol. 23, pp. 255-e119.

O'Shea, C. J., Sweeney, T., Lynch, M. B., Gahan, D. A., Callan, J. J. and O'Doherty, J. V. (2010). Effect of β-glucans contained in barley- and oat-based diets and exogenous enzyme supplementation on gastrointestinal fermentation of finisher pigs and subsequent manure odor and ammonia emissions. *Journal of Animal Science*, vol. 88, pp. 1411-1420.

Parnell, J. A. and Reimer, R. A. (2012). Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats. *British Journal of Nutrition*, vol. 107 (4). DOI:10.1017/S0007114511003163

Rodríguez, J. M., Murphy, K., Stanton, C., Ross, R. P., Kober, O. I, Juge, N., Avershina, E., Rudi, K., Narbad, A., Jenmalm, M. C., Marchesi, J. R. and Collado, M. C. (2015). The composition of the gut microbiota throughout life, with an emphasis on early life. *Microbial Ecology in Health & Disease*, vol. 26, 26050. DOI: http://dx.doi.org/10.3402/mehd.v26.26050

Roelofs, S., Nordquist, R. E. and van der Staay, F. J. (2017). Female and male pigs' performance in a spatial holeboard and judgment bias test. *Applied Animal Behaviour Sceince*, vol. 191, pp. 5-16.

Rutherford, K. M. D., Haskell, M. J., Glasbey, C. and Lawrence, A. B. (2006). The responses of growing pigs to a chronic-intermittent stress treatment. *Physiology & Behavior*, vol. 89, pp. 670-680.

Savignac, H. M., Tramullas, M., Kiely, B., Dinan, T. G. and Cryan, J. F. (2015). Bifidobacteria modulate cognitive processes in an anxious mouse. *Behavioural Brain Research*, vol. 287, pp. 59-72.

Studnitz, M., Jensen, M. B. and Pedersen, L. J. (2007). Why do pigs root and in what will they root? A review on the exploratory behaviour of pigs in relation to environmental enrichment. *Applied Animal Behaviour Science*, vol. 107, pp. 183-197.

Zhu, F., Du, B., Bian, Z. and Xu, B. (2015). Beta-glucans from edible and medicinal mushrooms: Characteristics, physiochemical and biological activities. *Journal of Food Composition and Analysis*, vol. 41, pp. 165-173.