Journal of Animal Science, 2025, **103**, skaf118
https://doi.org/10.1093/jas/skaf118
Advance access publication 17 April 2025
Housing and Management

Restricted vs. *ad libitum* feeding during sow gestation affects piglet performance, behavior, and fecal microbiota composition

Marinus F.W. te Pas,[†] Marion Kluivers-Poodt,[‡] Johan W. van Riel,[†,‖] Dirkjan Schokker,[$,1] and Johanna M.J. Rebel[$,¶]

[†]Animal Breeding and Genetics, Wageningen Livestock Research, Wageningen, The Netherlands
[‡]Animal Ecology, Wageningen Environmental Research, Wageningen, The Netherlands
[‖]Department of Animal Health and Welfare, Wageningen Livestock Research, Wageningen, The Netherlands
[$]Epidemiology, Bioinformatic, Animal models, and Vaccine Development, Wageningen Bioveterinary Research, Lelystad, The Netherlands
[¶]Adaptation Physiology Group, Department of Animal Science, Wageningen University, Wageningen, The Netherlands

[1]Corresponding author: dirkjan.schokker@wur.nl

Abstract

This study investigated how the nutrition of gestating sows affects piglet performance, behavior, and fecal microbiota. Twenty-four sows were divided into 2 feeding groups: those on a restricted diet (once a day) and those fed ad libitum (as much as they wanted), with all receiving the same diet during lactation. The piglets were categorized based on their feeding groups; RG-RL: Piglets born and nursed by restricted-fed sows with restricted feeding, AG-AL: Piglets born and nursed by ad lib-fed sows, RG-AL: Piglets born by restricted-fed sows and nursed by ad lib-fed sows, and AG-RL: Piglets born by ad lib-fed sows and nursed by restricted-fed sows. Performance traits were analyzed using the model including treatment, switching piglets, and room effects. Piglet behavior was evaluated with a generalized linear mixed model (GLMM) using binomial distribution, testing interactions of treatment, switching, gender, and other factors while accounting for random effects related to room, pen, and sow. Results showed that ad lib-fed sows had higher feed intake ($P < 0.001$), weight gain ($P = 0.04$), and backfat gain ($P = 0.01$) compared to restricted-fed sows. They also had lower cortisol levels during gestation ($P = 0.02$) and lactation ($P = 0.04$). Restricted-fed sows displayed more air-chewing behavior ($P = 0.002$), while ad lib-fed sows were more active ($P = 0.03$) and engaged in eating ($P < 0.001$). Birth weights and the number of piglets were similar across groups. During lactation, backfat loss varied among piglet groups, with the highest loss in AG-RL ($P < 0.01$). Piglets from ad lib-fed sows performed better overall, regardless of nursing source. At weaning, piglet weights ($P = 0.01$) were highest in AG-AL, followed by RG-AL, RG-RL, and AG-RL (interaction effect $P = 0.006$). Switching piglets initially reduced their playtime but later increased it ($P = 0.04$). The novel object test indicated that gilts became more active, resembling boars. Additionally, gut microbiota composition varied among sow groups during gestation ($P = 0.04$) and lactation ($P = 0.02$), suggesting that maternal diet influences piglet gut health. Overall, these findings highlight the potential role of epigenetic mechanisms in shaping these traits.

Lay Summary

This study examined how a sow's diet during pregnancy impacts her piglets' growth, behavior, and gut bacteria. Some sows had ample food, while others had less. Those with sufficient feed consumed more, gained more weight, and developed more backfat. Additionally, differences in behavior were noted; stress levels were lower during the birthing process, and there was less abnormal behavior observed. The researchers also investigated how the piglets grew when they consumed milk from their own sow or from a sow with a different diet. They discovered that piglets from well-fed sows grew better, regardless of whose milk they drank. The piglets' weight at weaning varied based on their sow's diet and whether they nursed from their own sow. Furthermore, piglets that switched sows exhibited changes in their activity levels. Interestingly, there were variations in gut bacteria at birth and during suckling among the piglet groups and the sows' diet groups, indicating that a sow's diet during pregnancy may influence her piglets' gut bacteria. This study suggests that a sow's diet during pregnancy can affect her piglets' growth and behavior through a process known as epigenetics.

Key words: epigenetic effects, gestation, lactation, pig (*Sus scrofa*), sow feeding

Abbreviations: Ad lib: ad libitum; PCoA: principal coordinates analysis

Introduction

Events early in life can determine performance, stress, and activity phenotypes and responses in later life. These events may be as early as prenatal development (Roseboom et al., 2001).

Gestational nutrition affects prenatal development. For example, gestating sows fed different diets showed differences in muscle fiber cellularity of the fetuses, and thereby growth rate and muscle / meat production capacity of the pigs in later life (Rehfeldt and Kuhn, 2006; Rehfeldt et al., 2011). Furthermore, the gestating sow diet also affects the birth weight of the piglets, which is related to piglet survival (Campos et al., 2012). Thus, gestational nutrition predisposes to specific performance levels during the productive life of the offspring

Received November 27, 2024 Accepted April 9, 2025.

(Pond, 1973; Foxcroft et al., 2006, 2009). Gestational nutrition, rather than early lactation nutrition, is the driving mechanism underlying the changes (Clouard et al., 2016; Johnston et al.).

Diet composition affects the composition of the gut microbiome (Flint, 2012; Voreades et al., 2014; Wilson et al., 2020). Lactation and postweaning nutrition also affect the development of the gut microbiota of both the sows and the piglets (Schokker et al., 2015b). The composition of the gut microbiota is important for the feed efficiency of the piglet (Gardiner et al., 2020), and it is a major component in determining the piglet's gut health, immunology, and productivity (Fouhse et al., 2016; Gardiner et al., 2020). Dietary restriction can be an effective inducer of stress and (dietary) stress-related behavior (Toth and Gardiner, 2000). Major effects on stress have been reported to be related to the gut microbiome-brain axis (Foster et al., 2017; Liu, 2017).

Diet composition and restriction, stress, gut microbiome composition, and epigenetic modifications due to programming are all connected to each other. Early nutrition during prenatal life influences the gut microbiome (Indrio et al., 2017). This latter effect already starts with early gut microbial colonization (Pan et al., 2018). The triangle between (gestational) diet, gut microbiome, behavior, and stress has also been proven.

Our hypothesis is that gestational nutrition influences sow feed intake, behavior, which subsequently affect the performance and behavior (stress response) of the offspring (in later life). The specific research objectives of this study were to investigate what the effect of dietary restriction or ad libitum (**ad lib**) feeding of gestating sows was on 1) the feed intake, stress-related cortisol levels, the backfat metabolism, and the gut microbiome of the sows, and 2) the performance, stress response, behavior, and gut microbiome composition of suckling and postweaning piglets. Furthermore, in the offspring, we studied the potential differences between the sexes.

Materials and Methods

Ethics approval

The absence of invasive sampling or treatment techniques meant that specific ethical approval was not necessary.

Experimental design

The experiment was conducted at the Pig Innovation Centre Sterksel (Sterksel, The Netherlands). Gestating sows were housed in groups of 4 and lactating sows were housed individually. Sows and piglets were housed in pens enriched with a burlap sack and a chain with bolts attached to the floor. Four days after artificial insemination, a total of 24 Topigs20 (TZZNN) sows were homogeneously distributed between 2 experimental groups, according to body weight and backfat thickness. In the gestation room, the sows were allocated 6 pens (3 pens per experimental group), each one housing 4 animals. The sows received either a restricted diet (control group—Welzijnskorrel Garant, ABZ, Nijkerk, The Netherlands), or an ad lib diet (Drachtkorrel AdLib, ABZ) (Supplementary material 1). The ad lib diet contained a total estimated crude fiber of approximately 18% to ensure satiety, whereas crude fiber was approximately 10% the restricted diet. The estimated energy density was approximately 2.9 kcal/g for ad lib diet and approximately 3.1 kcal/g for the restricted diet. Calculations were made (data not shown) to ensure that the expected intake of the ad lib feed would still provide sufficient vitamins and minerals. The sows in the control group were fed once a day (approximately 0800 hours) via floor feeding. For the control group, various feeding schemes, based on the period in days and backfat thickness (Renco lean meter), were generated beforehand for sows with a parity of 2 or higher (Table 1). The sows in the saturation group were offered ad lib diet via a dry feed trough. Water was supplied ad lib. The average parity of the sows was 4.3 for the control group, and 4.0 for the ad lib-fed group. A week before the expected delivery date sows were moved to the gestation pen, which is 1.80 m wide and 2.40 m deep. The floor consists of a 1.85 m plastic slatted floor and a 0.55 m metal slatted floor. The floor section under the sow is 0.65 m wide. In all gestation departments, the fresh air enters via a lowered air inlet under the slurry pans. The performance of sows was measured with average daily feed intake, weight gain, and back fat thickness. Hair cortisol was used as a marker for stress, and behavioral traits such as eating, chewing air, and no activity were measured during gestation and shortly before farrowing (Fig. 1, sampling moments 1 and 2). The number of piglets and the total birth weight were recorded (Fig. 1, sampling moment 2).

During lactation, all sows (*n* = 24) received the same commercial lactation diet. The piglets either continued to receive milk from their sow, or from a sow of the other feeding group. Therefore, 4 groups of piglets could be defined (R = restricted-fed sows (i.e., control); A = ad lib-fed sows; G = gestation; L = lactation) (Fig. 1): 1) Piglets, born and nursed from sows with restricted feeding during gestation (RG_RL: Restricted-fed sows during gestation, same sow during lactation); 2) Piglets, born and nursed from sows with ad lib feeding during gestation (AG_AL: ad lib-fed sows during gestation, same sow during lactation); 3) Piglets, born from sows with restricted feeding during gestation and nursed by a foster sow that received ad lib feeding during gestation (RG_AL: Restricted-fed sows during gestation, after birth switched to sows of the other group); 4) Piglets, born from sows with ad lib feeding during gestation and nursed by a foster sow that received restricted feeding during gestation (AG_RL: ad lib-fed sows during gestation, after birth switched to sows of the other group). Four piglets from each litter were selected to be followed throughout the rest of the trial.

After birth, the piglets received pellets (diameter 4 to 6 mm) in a container (quantity depending on consumption speed; increasing with age) and had a burlap bag and a rope available. From approximately 7 d of age the piglets switched to a pre-starter diet, i.e., pellet mixed with pre-starter. Subsequently, approximately 1 wk before weaning piglets were gradually switched to their postweaning diet, i.e., pre-starter

Table 1. Feeding scheme per sow per day (in kg) for control[1] animals

	Parity ≥ 2		
Period (d)	Normal	Thin	Thick
1 to 40	3.0	3.2	2.8
41 to 72	2.6	2.8	2.5
73 to 108	3.0	3.1	2.8

Normal: backfat thickness 12 to 15 mm; thin: backfat thickness < 12 mm; thick: backfat thickness > 15 mm.
[1]Control refers to restrictedly fed sows, which was applied during gestation.

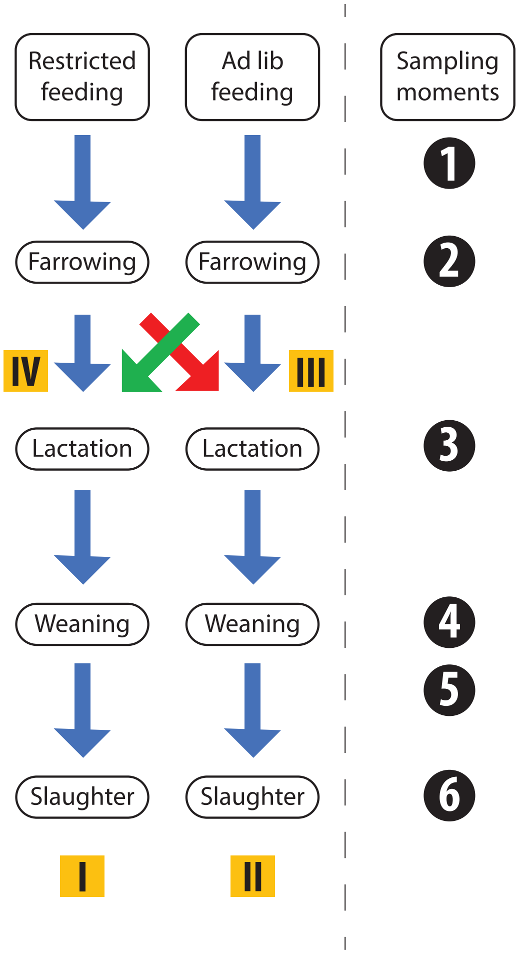

Figure 1. Experimental design of the study. Starting with 2 groups of gestating sows, either fed restricted or *ad lib* (12 sows each). During lactation the newborn piglets received either milk from the same sow, or from a sow from the other feeding group, groups I to IV. Group I (RG-RL): piglets born from sows restricted-fed during gestation and fostered from their own sow during lactation; group II (AG-AL): piglets born from sows *ad lib* fed during gestation and fostered from their own sow during lactation; group III (RG-AL): piglets born from sows restricted-fed during gestation and after birth switched to sows *ad lib* fed during gestation; group IV (AG-RL): piglets born from sows *ad lib* fed during gestation and after birth switched to sows restricted-fed during gestation. Sows and piglets were sampled, and performance was analyzed at 6 sampling moments, referring to the period of the day.

mixed with weaning feed. At 28 days of age, all the piglets were weaned and moved to the weaning department, where pens were 2.35 m by 2.20 m and housed 12 piglets. Weaned piglets are provided with standard 'VIC Sterksel' weaning diet for the first 8 days after weaning. They are then gradually switched over 3 days to the standard piglet-rearing pellet, which they receive until they are laid up in the fattening pigsty. The piglets are fed unlimitedly via a 2-compartment dry feed trough.

Piglet performance was measured at birth, weaning, and postweaning, and subsequently at 5, 7, and 9 wk of age. Feed intake was recorded for the entire lactation period, and on a weekly basis after weaning. Subsequently, feed conversion was determined. After weaning the piglets continued as fattening pigs in the experiment until 5 wk after weaning. The pigs were sampled near the end of the lactation phase, after weaning, and during the postweaning growing phase (Fig. 1, sampling moments 3 to 6). Table 2 provides an overview of the sampling moments, and the analyses performed.

Performance measurements

The feed intake was measured with electronic sow feeding stations (Insentec BV, Marknesse, the Netherlands) and calculated via back-weighing. Weight and growth determination were performed by weighing the sows at placement in the gestation room, day of transfer to the farrowing room, and at weaning of the piglets. Newborn piglets were weighed within 16 h after birth. The sow back fat was measured at the P2 position (65 mm down the left and right side from the midline at the level of the head of the last rib).

Hair cortisol and behavioral analyses

Hair samples for cortisol measurements were taken by shaving hairs at the lumbosacral part of the back (i.e., after the last rib and before the tail) and analyzed according to Greff et al., where the complete methodology is explained, from sampling the hair to data analysis (Greff et al., 2019). For sows, hair samples were collected at 4 time points, 1) shortly after being placed in the gestation pens, 2) at week 10 of the gestation, 3) immediately after being moved to the farrowing unit, and 4) at weaning. Whereas for piglets, hair samples were collected at birth, 2 days after weaning, and at 9 wk of age.

Behavioral analyses were performed by video observations (Xue and Henderson, 2006). Eating times and air-chewing times were recorded. Playing of piglets was investigated using the novel object test (Bracke and Spoolder, 2008). The sows were observed in their home pen at week 10 of gestation, and the day before and after being moved to the farrowing unit. They were observed during 5 intervals of 1 h using 4 minutes instantaneous scan sampling. Live observations started at 0900, 1015, 1130, 1330, and 1445 hours. The posture of each sow and the performed activity were recorded for each scan, using the following ethogram—Postures: total lying (sleeping and lying while awake); lying lateral (sleeping or awake but inactive); lying ventral (sleeping or awake but inactive); standing and walking; Behavioral activities: explorative behavior (nosing or rotting a substrate and manipulating parts of the pen); chewing (air and objects); feeder-directed behavior (eating or manipulating the feeder); total oral behavior (chewing behavior, explorative behavior, feed-directed behavior, manipulative behavior directed at pen mates, and drinking).

Piglets' behavior was observed in 3 different moments: the day where the switch of the piglets between sows occurred, the day before weaning, and at the end of the trial. Piglets were observed in their home pen during 6 intervals of 1 h. Live observations started at 0900, 1000, 1100, 1300, 1400, and 1500 hours. During the nursery phase, the behavior of the piglets was evaluated according to the following ethogram: total inactive behavior (sleep and awake but inactive); walking and playing; total nursing behavior (looking for a teat and suckling); explorative behavior (nosing a substrate and manipulating parts of the pen); total ingestive behavior (suckling and eating solid feed). During the postweaning phase, the ethogram considered: total inactive behavior (sleeping, lying inactive, standing inactive); eating and drinking; explorative behavior (nosing a substrate and manipulating parts of the pen); total oral behavior (eating and drinking, explorative behavior, and manipulation directed toward pen mates). The recordings of these behavioral observations were made by an experienced researcher.

Table 2. Sample collection and analyses. The sampling moments refer to Fig. 1

Sampling moment no.	Life stage	Sow	Piglet
1	During gestation (days 28, 66, and 75)	Feed intake; weight gain; back fat; cortisol (hair); behavior	
2	(Shortly before) Parturition (farrowing) (days 109 to 114)	Feed intake; weight gain; back fat; cortisol (hair); behavior; farrowing data; feces	
3	Day 4 after birth		Feed intake; growth rate; cortisol / ratio; behavior
4	End of lactation (weaning d28)	Feed intake; BF loss	Feed intake; growth rate; cortisol / ratio; behavior; feces
5	Postweaning—5 wk		Growth rate; feed intake, conversion; cortisol / ratio weaning/birth; behavior; feces
6	Postweaning—9 wk		Feces

Fecal microbiota

Sow fecal samples were collected at the end of gestation, i.e., approximately 1 day after giving birth. Piglet fecal samples were collected at 4 timepoints (T): T1 is shortly after birth (d0), T2 is at weaning (d28), T3 is 1 wk after weaning (d35), and T4 is 4 wk after weaning (d56, Table 2). For both sows and piglets, individual fecal grab samples were collected immediately at defecation and immediately frozen in liquid nitrogen and stored at −80 °C. Note, that sometimes stimulation was needed for the animal to defecate. The microbiome compositions were studied at the bacterial phylum and genus levels. The microbiome composition was studied as described previously (Schokker et al., 2015a). Stored samples (−20 °C) were thawed and mixed 1:1 with PBS, vortexed, and spun. After filtering steps, the QIAamp DNA Stool Mini Kit protocol was used as described by the manufacturer (Qiagen, Venlo, Hilden, Germany). Samples were eluted in the elute buffer and thereafter DNA quality was measured on the Nanodrop (Agilent Technologies, Cheadle, UK). Samples were sequenced by targeted-amplicon 16S sequencing, V3 and V4, (V3_F: 5'CCTACGGGAGGCAGCAG-3'; and V4_R: 5'GGACTACHVGGGTWTCT3') on the MiSeq and analyzed for taxonomy profile per sample with clustering by profile by using QIIME (Caporaso et al., 2010). The QIIME pipeline has been described before (Schokker et al., 2015a). After sequencing, we performed analyses of the summarized taxa at the genus level and used the relative values enabling comparison of the different samples. The diversity as described by the Shannon index was calculated by the vegan package (vegan_2.6-4) within the R environment. The Shannon diversity index (H) is an index that is commonly used to characterize species diversity in a community. The Shannon index accounts for both abundance and evenness of the microbiota species present.

Subsequently, we performed a redundancy analysis on the genus level of the microbiota data using the model: y = treatment_gestation (R / A) * switching_piglets (RG_AL / RG_RL / AG_AL / AG_RL) + room + error. Statistical significance testing for over-, and underrepresentation of the bacterial groups was made at the genus level by performing the Wilcoxon signed-rank test, and *P*-values were converted to false discovery rate values to correct for multiple testing. The principal coordinates analysis (**PCoA**), a method to explore and visualize similarities or dissimilarities of data, was used to analyze the differences among the 4 piglet groups. The sow data were expressed for the feeding group and for individual sows.

Statistical analyses

Performance traits were statistically analyzed with ANOVA in Genstat (VSN International, Hemel Hempstead, UK), with the data on pen level and 8 pens within a room. To minimize confounding factors related to sow differences, piglets from the same litter were evenly distributed across replicate rearing pens receiving the same treatment. This ensured that any observed effects could be attributed to the treatments themselves, and therefore sow effects were not included in the statistical model. The model was y = treatment_gestation * switching_piglets + room + pen (within room).

Behavior scan sampling data of piglets (on pen scale) were statistically analyzed with a generalized linear mixed model (GLMM in Genstat) with binomial distribution, with the data on piglet-age-day-period level. Main effects and 2-way interactions of treatment_gestation, switching_piglets, gender, weeknumber_after_weaning, and day_period were tested in a model, where random effects for room, pen, week (within pen), and period (within week) were included. Differences are discussed at a $P \leq 0.05$ for all statistical analyses.

Results

Performance, stress testing, and behavior of sows from insemination to farrowing

To monitor the effect of the different feeding strategies, the performance of the sows was measured. In ad lib-fed sows significantly higher average feed intake ($P < 0.001$), weight gain ($P = 0.04$), and back fat thickness ($P = 0.01$) were observed (Table 3), showing the effect of the feeding regimes, but the number of piglets and total litter birth weight were not significantly different. Hair cortisol levels in the gestating sows showed higher values in the restricted-fed sows ($P = 0.02$, Table 3). Eating-related behavior, i.e., chewing air, showed significantly higher values in restricted-fed sows ($P = 0.002$), 19.8 in restricted-fed sows compared to 2.3 in ad lib-fed sows (Table 3). While eating ($P < 0.001$) and no activity ($P = 0.03$) were significantly higher in ad lib-fed sows (Table 3). Searching for food-related behavior was not different between the 2 feeding regimes.

Performance, cortisol, and behavior of sows and piglets during lactation

The focus of these investigations was from the sow, both the sow and the switched sow. The average feed intake of the

Table 3. Performance and behavior of sows from insemination to farrowing

Measured trait	Restricted-fed sows	Ad libitum-fed sows	SEM[1]	*P*-value
Average feed intake (kg/d)	2.93	4.58	0.13	<0.001
Weight gain (kg)	61.50	96.10	8.12	0.039
Back fat gain (mm)	1.90	7.40	0.86	0.011
Hair cortisol (ng/g)	172.4	93.7	1.2	0.021
Behavior[2]				
Nosing, rooting, or scraping ground	7.80	2.00		NS[3]
Chewing air	19.8	2.30		0.002
Eating	0	7.80		<0.001
No activity	59.8	75.7		0.030
Farrowing information				
Number of piglets				NS[3]
Total birth weight				NS[3]

[1] Standard error of the mean.
[2] Percentage of scans, means ± s.e.m.
[3] Not significant.

sows did not differ between the groups, but the loss of the backfat of the sows during lactation showed significantly higher values ($P < 0.01$, Table 4) in the sows fed ad lib during gestation in both groups of nursing own piglets and piglets from restricted-fed sows.

The AG-AL and AG-RL piglets showed higher growth rate as compared with the other groups of piglets, but the switch of the piglets between sows did not affect the growth rate ($P = 0.63$), although there was an effect of starting weight among the groups. The feed intake of the piglets was similar in all groups ($P = 0.42$, Table 4). The playing time of the piglets showed a tendency ($P = 0.09$, Table 4) for the treatment effect and a significant effect for switch ($P = 0.04$, Table 4), with especially shortly after birth a higher activity of piglets, i.e., playing of AG-AL was 0.075%, AG-RL was 0.063%, RG-RL was 0.05%, and RG-AL was 0.045%.

Piglets' performance postweaning

Postweaning piglet performance was measured for the 4 groups of piglets (Table 5). Although the starting weights of the groups were similar, switching the piglets to sows with different feeding strategies resulted in a significant interaction effect ($P = 0.006$, Table 5). Neither growth rate nor feed intake of the piglets differed among the groups, but switching the piglets to sows with different feeding strategies resulted in a significant interaction effect for feed conversion ($P = 0.02$, Table 5), with the RG-AL piglets having a lower feed conversion than the other groups.

The hair cortisol levels showed a tendency ($P = 0.08$, Table 6) of effect of the feeding level of the sow during gestation with the highest cortisol levels found in RG-RL and RG-AL piglets, while the novel object test was similar among the 4 groups of piglets (Table 6). However, the novel object test gave different effects of switching piglets for female and male piglets, an interaction effect of switch by gender was observed, Table 7). Activity levels (i.e., active, lying, and exploring) showed a specific effect of switching by week number in the newborn piglets (Tables 8 and 9), for all 3 interaction terms $P = 0.01$. Making physical contact differed between Treatment by gender ($P = 0.04$, Table 8), as well as Time of day by treatment ($P = 0.04$, Table 8). The percentage of physical contact of males from restricted-fed sows was 4.8 and 2.8 for males from adlib fed sows, whereas for females from restricted-fed sows this was 3.0 and 3.8 for females from adlib fed sows. For Time of day by treatment effect we observed the percentage of physical contact for piglets from restricted-fed sows; period 1 to 6, 2.5, 4.4, 3.1, 3.1, 6.0, and 4.8, whereas for piglets from ad lib-fed sows; period 1 to 6, 3.6, 1.7, 5.4, 1.8, 4.7, and 4.0, respectively.

Fecal microbiota composition and diversity of the sows

The fecal microbiota was studied at the bacterial phylum level and the bacterial genus level. The fecal microbiota diversity was studied with the Shannon index and the PCoA. All Figures with detailed information of the results can be found in Supplementary material 2.

At the phylum level, the fecal microbiome of the sows (Fig. S1) showed a high abundance of Firmicutes and Bacteroidetes, which together make up about 85% of the microbiome. This is high compared to other phyla present in the microbiome. *Spirochaetes* and *Proteobacteria* follow at distance, approximately 10% and 5%, respectively. The diet treatment groups of sows showed no major differences in their overall composition. Comparing individual sows some differences may be noted, mainly for the abundances of the *Spirochaetes*.

At the genus level (Fig. S2), when focusing on the top 10 genera, all genera that were not in the top 10 are grouped in "Other", which was most abundant with about 40%. This group represents all bacterial phylae or genera not belonging to the top 10 abundance of phylae or genera, respectively. So, although each group is not in the top 10, together they were more abundant than any phylum or genus, respectively. The diet treatment groups of sows showed high similarity. Only the least abundant *Megasphaera* varied between the groups (based on the average) being highest in the restricted-fed sows, lower in the ad lib-fed sows, and about deficient in the ad lib-fed sows giving lactation to piglets from the restricted-fed sows (i.e., AG-RL). This difference was more pronounced in the individual sow data.

The Shannon index showed no differences, although the mean value of the ad lib-fed sows was smaller than for the

Table 4. Performance, cortisol, and behavior of sows and piglets during lactation

Traits	RG-RL[1]	AG-AL[1]	RG-AL[1]	AG-RL[1]	SEM[2]	*P*-value treatment	*P*-value switch	*P*-value interaction
Number of sows	5	6	4	4				
Average parity	4.8	3.7	4.3	4.0				
Sows feed intake (kg/d)	7.42	7.37	7.42	6.86	0.26			
Sows loss of backfat until weaning (mm)	3.1	4.2	2.4	7.0	0.97	<0.01		
Piglet growth (g/d)[3]	236	286	235	271	14.13	0.01	0.63	0.67
Feed intake piglets (g/d)[3]	25.6	22.2	18.4	22.7		1.00	0.42	0.32
Hair cortisol								
Cortisol piglets weaning (nmol/L)	158	224	183	222		0.30	0.72	0.65
Ratio of cortisol weaning-birth[4]	0.41	0.30	0.33	0.21		0.08	0.16	0.73
Behavior								
% playing period = time of day[5]						0.09	0.04	
Period 1	0.050	0.075	0.045	0.063			Lower	
Period 2	0.041	0.033	0.048	0.018			Partly lower	
Period 3	0.034	0.014	0.016	0.017			Partly Lower	
Period 4	0.025	0.035	0.025	0.030			equal	
Period 5	0.032	0.012	0.082	0.050			Higher	
Period 6	0.021	0.066	0.103	0.042			Partly higher	
Postweaning								
Starting weight (kg)	9.3	8.9	7.6	8.2		0.89	0.01	0.19
Feed conversion (kg feed/kg weight gain)	1.54	1.50	1.45	1.52		0.57	0.11	0.02

[1] RG-RL: piglets born from sows restricted-fed during gestation and fostered from their own sow during lactation, AG-AL: piglets born from sows ad libitum fed during gestation and fostered from their own sow during lactation, RG-AL: piglets born from sows restricted-fed during gestation and after birth switched to sows ad libitum fed during gestation, AG-RL: piglets born from sows ad libitum fed during gestation and after birth switched to sows restricted-fed during gestation.

[2] Standard error of the mean.

[3] Gram per day.

[4] Hair samples collected at birth and 2 days after weaning.

[5] 6 periods of 1 h starting at 0900, 1000, 1100, 1300, 1400, and 1500 hours.

restricted-fed sows (Fig. S3). The PCoA analysis showed no differences, indicating major similarities in the fecal microbiota of the sows fed the 2 different feeding level regimes (Fig. S4).

Fecal microbiota composition and diversity of the piglets at the phylum level

The 4 groups of piglets (AG-AL, AG-RL, RG-AL, RG-RL) at 4 time points (T1 to T4) were analyzed and results were compared with the sows, and among the groups of piglets. The fecal microbiome of the piglets at T1 (shortly after birth) differed from the sows, although the *Firmicutes* were also the most abundant group of bacteria here. The second group were the *Proteobacteria*, which were almost similar in abundance, and in the RG-RL group even higher abundant than the *Firmicutes*. Together with the *Fusobacteria* and the *Bacteroidetes* this almost makes up 100% of the microbiome composition (Fig. S5).

The fecal microbiome of the piglets at T2 (weaning) is more diverse than the microbiome at T1 (Fig. S6). The *Firmicutes* were about 50% of the microbiome and the *Bacteroides* make up about 25% to 30% of the microbiome. The order of the Proteobacteria and the *Spirochates* is reversed as compared to the sow microbiome. Several of the minor groups found in the piglets were not found in the sows, including *Elusimicrobiota*, *Fusobacteria*, *Lentisphaerae*, and *Actinobacteria*, together making up about 10% of the microbiome. At T2 the AG-AL and RG-RL piglets showed a little higher abundance of the *Bacteroides* than the other groups.

The fecal microbiome of the piglets at T3 (i.e., 1-wk postweaning) were only slightly different from the fecal microbiome at T2 (Fig. S7). Only the order of minor bacterial groups differs. The comparison with the sows is similar with the comparison at T2. The groups at T3 were more similar than at T2, with only very minor differences among the 4 groups. No differences in bacterial phylae among the 4 piglet groups were visualized.

The fecal microbiome of the piglets at T4 (i.e., 4 wk postweaning) showed high similarity to the fecal microbiome at T3 (Fig. S8). Overall, the reversed abundance of the *Proteobacteria* and the *Spirochaetes* as compared with the sow microbiota remains. The RG-RL piglet group showed a higher abundance of the *Proteobacteria* than the other piglet groups.

Fecal microbiota composition and diversity of the piglets at the genus level

The fecal microbiome on the genus level of the piglets at T1 is different from sows (Fig. S9). The most abundant bacterial groups, *Clostridium sensu stricto*, *Fusobacterium*, *Escherichia/Shigella*, *Bacteroides*, *Actinobacillus*, *Veillonella*, *Pasteurellaceae*, *Clostridioides*, and *Epulopiscium* were not found in the top 10 of the sows at all. In the RG-RL group the *Fusobacterium* abundance is reduced and the *Escherichia/Shigella* abundance is increased as compared to the other groups.

Table 5. Piglet performance postweaning

	RG-RL[1]	AG-AL[1]	RG-AL[1]	AG-RL[1]	Main effect of sow feeding during gestation	Main effect of switching piglets to other treatment	Interaction effect
Number of pens	4	4	4	4			
Total number of piglets	48	48	48	48			
Starting weight (kg)	8.16	9.29	8.86	7.62	0.88	0.19	0.01
End weight (kg)	22.32	23.71	23.14	22.20	0.79	0.18	0.68
Growth (g/d)	404	412	412	416	0.63	0.97	0.81
Average feed intake (kg/d)	0.61	0.63	0.61	0.60	0.76	0.54	0.47
Feed conversion (kg/kg)	1.52	1.54	1.50	1.45	0.57	0.11	0.02

[1] RG-RL: piglets born from sows restricted-fed during gestation and fostered from their own sow during lactation, AG-AL: piglets born from sows ad libitum fed during gestation and fostered from their own sow during lactation, RG-AL: piglets born from sows restricted-fed during gestation and after birth switched to sows ad libitum fed during gestation, AG-RL: piglets born from sows ad libitum fed during gestation and after birth switched to sows restricted-fed during gestation.

Table 6. Cortisol and novel object test of piglets, *P*-values

	RG-RL[1]	AG-AL[1]	RG-AL[1]	AG-RL[1]	Main effect of sow feeding during gestation	Main effect of switching piglets to other treatment	Interaction effect
Number of pens	4	4	4	4			
Ratio of cortisol weaning/birth[2]	0.41	0.30	0.33	0.21	0.08	0.16	0.73
Novel object test results (s)	177	259	205	257	0.37	0.79	0.95

[1] RG-RL: piglets born from sows restricted-fed during gestation and fostered from their own sow during lactation, AG-AL: piglets born from sows ad libitum fed during gestation and fostered from their own sow during lactation, RG-AL: piglets born from sows restricted-fed during gestation and after birth switched to sows ad libitum fed during gestation, AG-RL: piglets born from sows ad libitum fed during gestation and after birth switched to sows restricted-fed during gestation.

[2] Hair samples collected at birth and 2 days after weaning, ratio = cortisol level at weaning / cortisol level at birth.

Table 7. Novel object test gender by switching results of piglets

Gender	Own sow	Switched sow
Boar	264.1[1]	207.6
Gilt	166.1	255.4

[1] Time (in seconds) spent on exploration of novel object.

The fecal microbiome on the genus level of the piglets at T2 (i.e., at weaning) differs from the fecal microbiome at T1 (Fig. S10). At T2 the top 10 groups of genera only represent about 40% of the gut microbiome, with the "Other" group composed of all other bacterial genera being about 60% of the total bacterial abundance. The highest abundant group of bacteria, the Christensenellaceae_R-7 was not found in the sow among the top 10 genera. Except for *Escherichia/Shigella* and *Bacteroides* all other groups were also observed in the fecal microbiome of the sows, albeit with different abundances. The bacterial abundance at the genera level showed high similarity among the 4 piglet groups.

The fecal microbiome of the piglets at T3 (i.e., 1-wk postweaning) made a shift towards the fecal microbiome of the sow as compared to the gut microbiome of the piglets at T2 (Fig. S11). Bacteria with low abundance and not found in the sow include *Agathobacter*, and *Anaerovibrio*. These 2 bacterial genera also differed in abundance among the 4 piglet treatment groups with lowest expression in the RG-AL group and highest expression in the AG-RL and the RG-RL groups. Although with a low abundance, the Prevotella_9 was 2 - 3 times higher in the RG-RL group of piglets than in the other groups of piglets. Note that the group "Other", composed of many bacterial groups, comprised 45% to 55% of the total bacterial abundance.

The fecal microbiome of the piglets at T4 has approximately the same top 10 bacterial genera as the fecal microbiome of the piglets at T3, but abundances have changed remarkably (Fig. S12), but the group "Other" still shows about 50% of the total bacterial abundance. Compared with the fecal microbiome of the sow the *Megasphaera* group is remarkable, being the lowest abundant group in the sow it is highest abundant in the fecal microbiome of the piglets at T4. The *Phascolarctobacterium* group showed the highest variability being highest abundant in the AG-RL and the RG-AL groups, and lowest in the AG-AL and the RG-RL groups. The *Ruminococcaceae* bacteria has disappeared from the top 10

Table 8. Effects of treatment, switching, gender, and time of day on postweaning behavioral traits[1] in piglets

	Active	Lying	Exploring	Physical contact	Aggression	Walking and playing
Fixed term	F pr	F pr	F pr	F pr	F pr	F pr
Treatment[2]	0.62	0.64	0.89	0.68	0.96	0.67
Switching[3]	0.65	0.79	0.85	0.96	0.38	0.24
Treatment by switching	0.90	0.93	0.94	0.84	0.65	0.98
Treatment by gender	0.26	0.25	0.34	0.04	0.74	0.84
Switching by gender	0.13	0.21	0.38	0.60	0.10	0.38
Week number[4] by treatment	0.81	0.80	0.17	0.15	0.73	0.81
Week number by switching	0.01	0.01	0.01	0.36	0.49	0.27
Time of day[5] by treatment	0.65	0.75	0.99	0.04	0.58	0.23
Time of day by switching	0.63	0.76	0.86	0.64	0.22	0.69

Significant *P*-values are indicated by underlining.
[1] Focusing on the fixed terms relevant to the hypothesis.
[2] Treatment; restricted or ad libitum-fed sow.
[3] Switching; switching the piglets to sows with different feeding strategies.
[4] Week number; week after weaning.
[5] Time of day; refers to the period of the day.

Table 9. Effect of week number by switching the piglets to sows with different feeding strategies piglets on activity, lying, and exploring levels of

	Week after weaning	Own sow	Switched sow
% Activity	1	24.6	34.1
	2	33.3	27.1
	3	35.2	29.3
% Lying	1	74.5	63.2
	2	66.1	71.9
	3	63.6	69.8
% Exploring	1	8.8	14.7
	2	15.0	11.4
	3	16.5	13.7

panel of genera at T4. The RG-RL and AG-AL piglets groups showed lower abundance of the Phascolarctobacterium genus than the other piglet groups.

Fecal microbiota diversity analysis and exploration and visualization of similarities or dissimilarities of data

The Shannon index of the fecal microbiota of the piglets at weaning were investigated for the 4 different piglet management groups (Fig. S13). The results indicated only minor differences exists among the 4 groups of piglets. The PCoA analysis of the fecal microbiota of the piglets at weaning (Fig. S14) also did not indicate major differences among the 4 groups of piglets.

Discussion

Effectiveness of the experimental design for the sows

Events in early life, both fetal and newborn, influence processes in later life, e.g., related to health and performance. Feeding of the sows during gestation may be such an event in early life, affecting prenatal development of the piglets. Potentially, several mechanisms can be involved, such as gut microbiome composition changes, and stress, which are all related to each other and may cooperate to respond to the dietary constraints. To study these events of feeding during gestation in an animal model an experiment was designed with gestating sows fed a high (ad lib) feeding level and gestating sows with a restricted feeding level. Ad lib and restricted feed intake differ a lot in kilograms of weight gain of the sows, but a bit less regarding total energy intake, due to the different feed compositions. Different performance parameters of the sows, including feed intake, weight gain, and back fat gain, showed that the experimental design differentiated the sows into 2 groups, which has also been observed in other studies (Carrión-López et al., 2022; Gaillard and Dourmad, 2022; Jung et al., 2024). Hair cortisol showed that the sows seemed to be stressed by the applied feed management, with restricted sows showing higher cortisol levels as expected. This may not only be caused by the restricted feeding (Bahnsen et al., 2021; Jung et al., 2024) but could also be by being housed in the same compartment and hearing the ad lib-fed sows eating during the day. Behavioral tests showed feed level related behavior, such as searching for feed, chewing air, eating, and inactivity. These results underline the effectiveness of the experimental design for the sows (Lawrence and Terlouw, 1993; Meunier-Salaün et al., 2001). Importantly, the contrast of the effect of the feed management of the sows' during gestation is large, suggesting the effects in the offspring may be expected. If energy intake is similar, the effect is mainly caused by satiety (Douglas et al., 1998; De Leeuw et al., 2008), which may not seem such a large contrast but for the sows apparently is. The number of piglets born, and the total birth weight of the litter were not significantly different for the 2 sow groups indicating that the feed management of the sows did not affect in utero survival and growth. It was shown that, proper feeding strategies during gestation and lactation need to be applied and combined between sows and their litters (Solà-Oriol and Gasa, 2017).

Sows and piglets during lactation

During lactation, the sows of both gestational feeding groups received the same commercial feed, while the piglets were either left with their own sow or switched to a sow of the other feeding group. Consequently, any differences in performance or microbiome composition between piglets of the 4 groups can be related to the feed management of the sows during the gestation. Sow feeding regimes did not significantly alter the core, a relatively stable and consistent group of microorganisms that are found in a significant proportion of individuals, (fecal) microbiome of sows, indicating a robust and stable microbial community, as observed by previous studies (Miura et al., 2024).

Maternal body condition, including backfat thickness, influences the daily weight gain of offspring up to weaning, as well as the milk fat composition (Amdi et al., 2013). The loss of backfat of sows was highest with sows fed ad lib during gestation nursing piglets born from restricted-fed sows (i.e., AG-RL piglets) ($P < 0.01$). Whereas the loss of backfat was lowest for restricted-fed sows with piglets from ad lib-fed sows (i.e., RG-AL piglets). While this may indicate that these piglets needed less feed from the sow, it may also be the result of a lower backfat thickness in these sows. An alternative explanation could be that the amount of fat components in the milk differed between the sows of different feed management during gestation. Daily milk production has been shown in restricted-fed sows, but these sows were restricted fed during lactation (De Bettio et al., 2016). On the other hand, restricted-fed lactating sows showed higher milk fat content as compared to higher energy fed sows (Noblet and Etienne, 1986). Generally, there is an inverse relationship between milk fat percentage and milk yield, because the energy required to produce higher volumes of milk can dilute the fat content (Zhe et al., 2023a). Furthermore, milk fat production was linked to the reduction in backfat depth, whereas milk protein production was linked with the decrease in loin muscle depth during lactation (Costermans et al., 2020). Unfortunately, for this study data on milk fat composition of sows fed restricted during gestation were not available. The growth of the piglets during lactation, measured by body weight at birth and at weaning, was highest in piglets born from ad lib-fed sows during gestation of both ad lib and restricted-fed sows during lactation. The growth of the piglets from restricted-fed sows was significantly lower of sows of both ad lib and restricted-fed sows during lactation. Thus, there seems to be an influence of the feeding levels of the sows during gestation on the performance of the piglets during lactation. An explanation for this relationship is that restricted feeding during gestation could limit the availability of essential nutrients (including protein, carbohydrates, fats, minerals, vitamins, and choline) for fetal development, potentially affecting the piglets' vitality and / or ability to thrive postnatally. Piglets with a higher vitality absorb (more) essential nutrients and antibodies better, leading to faster growth, fewer diseases, and increased performance. The cortisol levels in RG-AL piglets decreased, which may indicate a lower stress level in the piglets related to the feeding level of the sow during gestation. Since the feeding level of the sow during gestation is related to the cortisol level of the sow, there seems to be a relation between the cortisol level of the sow and the cortisol levels of the offspring in later life. This may indicate an epigenetic effect of stress and / or cortisol levels, already shown before (Moonat and Pandey, 2012; Stankiewicz et al., 2013).

Postweaning performance

The effect of the switch of the piglets between sows differently fed during gestation was significant for the weaning weight of the piglets indicating that the piglets responded to the experimental treatments. This result may be of importance for commercial farms where switching of piglets may be practiced for sows with large litter sizes, where it has been observed that suckling decreases after switching (Guzmán-Pino et al., 2021). The feed conversion of the AG-RL piglets after weaning was lowest suggesting that these piglets were more efficient and that the piglets adapted to the gestational feeding level of the sow.

Behavioral traits such as playing, aggression, physical contact, and exploring / activity were affected by the sex of the piglets and are therefore difficult to relate to the effect of feeding level of the sow during gestation. This behavioral difference/effect may have implications in later life, i.e., for a sow in group housing. Because social interactions and hierarchies formed by piglets often persist into adulthood. Sows that were dominant as piglets may maintain their dominance in group housing, which can disrupt the social structure and cause stress for less dominant sows (Nieuwamerongen et al., 2014). However, since the performance and behavior of the piglets differed among the groups of piglets even after weaning the feeding level of the sows during gestation affected behavior in later life. However, the behavioral traits may also be related to stress, i.e., cortisol levels (Moonat and Pandey, 2012; Stankiewicz et al., 2013; Hullar and Fu, 2014)..

Microbiota development and composition

The gut microbiota composition and diversity have been related to performance in pigs (Rist et al., 2013; Ramayo-Caldas et al., 2016; Wang et al., 2019; Bergamaschi et al., 2020). The gut microbiota shifts in composition during the later stages of gestation or early during or after birth related to colostrum and milk composition (Frese et al., 2015; Trevisi et al., 2021). Furthermore, while the gut microbiota composition and diversity remain flexible during life, the initial gut microbiota relates to the changes of the microbiota during later life (Mach et al., 2015). Thus, the gut microbiota, often referring to fecal microbiota, composition and diversity of either the sows or the piglets may explain the observed effects on piglet performance (Arsenault et al., 2024) and behavior (Clavell-Sansalvador et al., 2024).

In this experiment the feed provided to the sows during gestation differed between a restricted-fed commercial diet and a less concentrated and more saturated diet fed ad lib. In general, despite the differences in feed composition and amount of feed between the groups of sows, we found surprising similarities between the groups of animals, both sows and piglets. This may indicate that feed differences during gestation have less effect on changing the fecal microbiome than during other periods in life. Also other feed components, such as the water applied, can affect microbiome acquisition (Baumann-Dudenhoeffer et al., 2018). This suggests that future studies should monitor water intake of sows and piglets as well when applying different feeding strategies to determine if this is a potential determinant of the microbiome.

The fecal microbiota composition and diversity of the 4 piglet groups differed especially for the AG-AL piglets. Thus, the Shannon index of the AG-AL piglets indicated the lowest diversity as compared to the other piglet groups.

At the phylum level the fecal microbiomes of the different piglet groups were highly similar with only some minor changes: comparing individual sows revealed that the abundances of the *Spirochaetes* differed among the groups. *Spirochaetes* are a group of bacteria that cause prevalent diseases such as leptospirosis, Lyme, relapsing fever and intestinal spirochaetosis

(Amat Villegas et al., 2004). Most spirochaetes are free-living and anaerobic, but there are numerous exceptions. Spirochaete bacteria are diverse in their pathogenic capacity and the ecological niches that they inhabit, as well as molecular characteristics including guanine-cytosine content and genome size (Gupta et al., 2013).

At the genus level the *Megasphaera* varies between the groups being highest in the RG-AL and the RG-RL groups, lower in the AG-AL group, and about deficient in the AG-RL group. *Megasphaera* are a genus of the *Firmicutes* bacteria, which are a member of the *Clostridia*. They are Gram-negative bacteria isolated from the female genital tract (Srinivasan et al., 2019) and these are short-chain fatty acid producing bacteria and linked to different growth stages (Luo et al., 2022). Where high average daily gain piglets had increased relative abundance of *Megasphaera* (Mahmud et al., 2023).

Comparing the changes of the microbiota within each pig groups composition over time we investigated 4 points in time: T1 to T4, shortly after birth, at weaning, and weaning + 1 wk and 4 wk, respectively. During gestation, the piglets received dietary components from the restricted or *ad lib*-fed sows. Therefore, it was expected that major differences could be found. At T1 the influence of the sow diets during gestation will be part of the diet, and the start of the lactation period will also affect the fecal microbiome. T1 is an early stage of the development of the postnatal fecal microbiome. Therefore, it is not surprising that the fecal microbiome is quite different from the fecal microbiome of the sow. At T1 the *Proteobacteria* at the phylum level, and the *Escherichia/Shigella* and “other group” at the genus level were higher in the RG-RL group of piglets. The *Proteobacteria* are a major phylum of Gram-negative bacteria. They include a wide variety of pathogenic genera, such as Escherichia, Salmonella, Vibrio, Helicobacter, Yersinia, Legionellales, and many others (Madigan, Brock Biology of Microorganisms, Global Edition, 15/E). Most members are facultatively or obligately anaerobic, and thus uniquely prepared to the gut environment. *Proteobacteria* associated with the imbalance of microbiota of the lower reproductive tract of women are associated with inflammation (Bennett et al., 2015). Furthermore, a wide variety in the types of metabolism exists. This makes it difficult to determine specific traits of the *Proteobacteria* related to epigenetic characteristics in our experiment. The *Escherichia/Shigella* are a combined group of *Escherichia* and *Shigella* because the bacteria of the genus *Shigella* and *Escherichia coli* are phenotypically similar (Escherichia, Shigella, and Salmonella—Manual of Clinical Microbiology—Wiley Online Library) (Nataro et al., 2011) and the genetic materials are highly related and use the same invasive mechanism to cause diarrheal diseases (van den Beld et al., 2019).

The changes in the fecal microbiome from T1 to T2 suggest that T1 is still significantly influenced by gestation, as dietary milk intake considerably altered the fecal microbiome. The fecal microbiome of T2 piglets has more similarities with the sow fecal microbiome. So, the sows feeding the piglets had similar fecal microbiome with the piglets. This may suggest that during lactation the feed provided to the sow is a major determinant of the piglet fecal microbiome composition and development. Whether there is a direct relation affecting the fecal microbiome, or that the fecal microbiome is maturing is presently unknown. This observation may also be associated with more explorative behavior with increase of age. Several new top 10 bacterial groups were found in the fecal microbiome at T2, which may be the effect of the milk. Minor groups of the fecal microbiome of the T2 piglets were not found in the sows, i.e., *Elusimicrobiota*, *Fusobacteria*, *Lentisphaerae*, and *Actinobacteria*. Although minor, these groups make up together about 10% of the microbiome. Thus, the influence could be interesting. *Elusimicrobiota*, *Fusobacteria*, *Lentisphaerae*, and *Actinobacteria* are groups of bacteria are bacteria that are widespread, although their roles in the gut are not always clear. *Fusobacteriota* are obligately anaerobic nonspore-forming Gram-negative bacilli, which are believed to be either pathogenic or commensal or both. They are involved in a wide spectrum of human infections causing tissue necrosis and septicemia and they may cause or be related to human colon cancer (Bullman et al., 2017). *Lentisphaerota* is a phylum of bacteria closely related to *Chlamydiae* and *Verrucomicrobia* (Cho et al., 2004; Limam et al., 2010). One phenotype, *L. marina*, consists of terrestrial gut microbiota from mammals and birds. *Actinobacteria*, which share the characteristics of both bacteria and fungi, are widely distributed in both terrestrial and aquatic ecosystems. Some Actinobacteria have its own negative effect against plants, animals, and humans (Dhanasekaran et al., 2016). The *Proteobacteria* showed reverse abundance comparing the T1 and T2 groups of piglets (see above for information on *Proteobacteria*).

Comparing the piglet groups showed higher abundance of the *Bacteroides* in the AG_AL and RG-RL piglet groups. *Bacteroides* are present in the feces and are the predominant bacteria of the gut and *Bacteroides* spp. are one of the major groups of commensals of the body, which are also found in the upper respiratory tract, mouth, and urogenital tract of humans and animals (Patrick, 2015). *Bacteroides* are known for their ability to break down complex carbohydrates and proteins, which can enhance nutrient absorption and utilization in piglets. This improved nutrient utilization can lead to better growth rates and overall performance (Luo et al., 2022). A higher abundance of *Bacteroides* in pig fecal microbiota is associated with improved nutrient utilization, enhanced gut health, better immune function, positive behavioral traits, and smoother adaptation to dietary changes (Guevarra et al., 2018; Luo et al., 2022; Arsenault et al., 2024).

After weaning (T2) the dietary composition changes considerably as well as exposure to sow feces. Therefore, major changes in the fecal microbiomes at T3 and T4 were expected. Indeed, the gut microbiome of the piglets at T3 made a shift towards the fecal microbiome of the sow as compared to the fecal microbiome of the piglets at T2. The fecal microbiome of the piglets at T4 has approximately the same bacterial genera as the fecal microbiome of the piglets at T3, but abundances have changed remarkably. Thus, while the weaning-related change appeared fast in the gut microbiome, the microbiome composition remained changing for a longer period even though the piglets received the same diet during this period. In the fecal microbiome of the piglets at T3 and T4 the *Agathobacter*, and *Anaerovibrio* bacteria were unique.

Agathobacter is a bacterial genus from the family of *Lachnospiraceae*. *Agathobacter rectalis* is a Gram-positive, butyrate-producing, anaerobic, rod-shaped and non-spore-forming bacterium from the genus of *Agathobacter* with a single flagellate which occur in the rumen content of sheep and cows (Rosero et al., 2016). *Agathobacter* could be a potential clinical biomarker of antitumor activity (Martini et al., 2022). These 2 bacterial genera also differed in abundance among the 4 piglet groups with lowest abundance in the RG-AL group and highest expression in the AG-RL and the RG-CL groups. This could indicate that there is an effect of the composition of the milk related to the feeding of the sow. Feeding of the sow has a profound impact on milk composition and yield (Zhe et al., 2023b), which in turn affects the microbiome and health of the piglets. Thus, ensuring adequate nutrition for the sow is crucial for the health and performance of their offspring. The highest abundance was found in the piglets of the restricted-fed sows. Although the relative abundance is low, the *Prevotella_9* genus was higher in the RG-CL group as compared with the other piglet groups. The abundance of *Prevotella_9* was in negative correlation with spleen index (Liang et al., 2023). The gut microbiome of MS patients was characterized by a decrease of *Prevotella_9* and the abundance of *Prevotella_9* was negatively correlated with the Th17 cell frequency (Zeng et al., 2019). Thus, this result may be related to a difference in immune function of the RG-RL piglet group.

The fecal microbiome of the piglets at T4 showed high abundance of the *Megasphaera* genus, which has been described above. The *Ruminococcaceae* bacteria has disappeared from the top 10 panel of genera at T4. *Ruminococcaceae* is a family of bacteria in the class Clostridia. All Ruminococcaceae are obligate anaerobes. Within the family, *Faecalibacterium prausnitzii* is notable as an abundant commensal bacterium of the gut microbiota. In addition, several members of Ruminococcus are found in the gut (Rajilić-Stojanović and de Vos, 2014).

The RG-RL group of piglets showed a higher abundance of the *Proteobacteria* than the other piglet groups (see above for a discussion on the *Proteobacteria*). The *Phascolarctobacterium* genus showed the highest variability being highest abundant in the AG-RL and the RG-AL groups, and lowest in the AG-AL and the RG-RL groups. Thus, the highest abundance was found in the piglets that switched between sows during lactation differently fed during gestation. Piglets that remained with their own sow showed the lowest abundance of this bacterial genus. The *Phascolarctobacterium* genus are a group of bacteria that are abundantly colonized in the gut and can produce short-chain fatty acids, including acetate and propionate, and can be associated with the metabolic state and mood of the host. In humans the colonization rate varied between 43.3% and 93.3%, and the abundance of Phascolarctobacterium faecium ranged between 3.22 and 5.76 log cells/g (<1 yr old) and 3.06 and 9.33 log cells/g (>1 yr old). There was presence of Phascolarctobacterium faecium-like bacteria in younger individuals with a gradual increase in the number of bacteria maintained at a high level with increasing ages (between 1 and 60 yr old), but with a decrease in elderly individuals (>60 yr old) (Wu et al., 2017). Thus, abundance variations related to age can be expected in pigs too.

Of all 4 piglet groups the RG-RL group showed the most divergent composition as compared to the other 3 piglet groups. The piglets of this group were born from restricted-fed sows and received milk from the same sows. As a results of the restricted gestational feeding the piglets could have higher stress levels than the other piglet groups. Alternatively, the gestational restricted feeding of the sow could have affected the metabolism of the piglets, for example to use nutrients highly efficient. The effects on the metabolism could have lasted until weeks after weaning, although the piglets of all groups were eating the same feed after weaning. Thus, this may suggest that an epigenetic mechanism is active because of the gestational feed differences of the sows.

Summarizing, we found several changes in the fecal microbiome during the development of piglets, some of which may be related to the diet of the sow during gestation. Since the diet given to the sows differed only during gestation, differences among the piglet groups during lactation and after weaning must relate to the gestational period. The biological mechanism behind this memory effect is potentially epigenetic (D’Urso and Brickner, 2014). Epigenetic genome modifications persist during life (Heijmans et al., 2008) and affect the physiology of the animal in later life (Lillycrop and Burdge, 2011; Murgatroyd and Spengler, 2011). Furthermore, fecal microbiota composition differences can be related to differential microbiota metabolic capacity, for example on short-chain fatty acid synthesis. The short-chain fatty acids synthesized by the gut microbiota have major effects on the host immune system and brain functioning (Silva et al., 2020; Yoo et al., 2020; Doifode et al., 2021). This could explain the observed behavior differences of the piglets. Concluding, the observed gut microbiome differences among the 4 groups of piglets could at least partially explain the observed performance and behavior differences.

Conclusions

We conducted a pig study demonstrating that feed management strategies for gestating sows significantly impacted sow related aspects, such as that ad lib-fed sows had higher feed intake, weight gain, and backfat gain compared to restricted-fed sows. Additionally, these ad lib-fed sows also had lower cortisol levels during gestation and lactation, and were more active and engaged in eating. Their offspring’s development was also impacted. Specifically, sow feeding regimes influenced piglet performance, piglets from ad lib-fed sows performed better overall. Furthermore, early behavioral adjustments following switching of the piglets between sows were ultimately followed by increased activity levels, and shifts in the gut microbiome suggest a critical role for maternal diet in shaping offspring health.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

Acknowledgement

This work was funded by the WUR internal program KB34 Towards a Circular and Climate Neutral Society (2019-2022), project KB-34-2A-3 Epigenetics.

Conflict of interest statement. The authors declare no conflicts of interest.

Data Availability

All data are available for cooperation research.

Disclaimer

We did not use AI in any part of the research. We did not use AI during writing of the manuscript.

Author Contributions

Marinus F.W. te Pas (Conceptualization, Writing—original draft, Writing—review & editing), Dirkjan Schokker (Investigation, Methodology, Writing—review & editing), Marion Kluivers-Poodt (Conceptualization, Investigation, Methodology, Supervision, Writing—review & editing), Johan van Riel (Investigation, Methodology, Visualization), and Johanna Rebel (Project administration, Supervision, Writing—review & editing)

References

Amat Villegas, I., E. Borobio Aguilar, R. Beloqui Perez, P. de Llano Varela, S. Oquiñena Legaz, and J. M. Martínez-Peñuela Virseda. 2004. [Colonic spirochetes: an infrequent cause of adult diarrhea]. Gastroenterol. Hepatol. 27:21–23. doi:10.1016/s0210-5705(03)70440-3

Amdi, C., L. Giblin, A. A. Hennessy, T. Ryan, C. Stanton, N. C. Stickland, and P. G. Lawlor. 2013. Feed allowance and maternal backfat levels during gestation influence maternal cortisol levels, milk fat composition and offspring growth. J. Nutr. Sci. 2:e1. doi:10.1017/jns.2012.20

Arsenault, M., B. Lillie, K. Nadeem, E. Khafipour, and A. Farzan. 2024. Progression of swine fecal microbiota during early stages of life and its association with performance: a longitudinal study. BMC Microbiol. 24:182. doi:10.1186/s12866-024-03336-y

Bahnsen, I., K. V. Riddersholm, L. V. de Knegt, T. S. Bruun, and C. Amdi. 2021. The effect of different feeding systems on salivary cortisol levels during gestation in sows on herd level. Animals (Basel). 11:1074. doi:10.3390/ani11041074

Baumann-Dudenhoeffer, A. M., A. W. D'Souza, P. I. Tarr, B. B. Warner, and G. Dantas. 2018. Infant diet and maternal gestational weight gain predict early metabolic maturation of gut microbiomes. Nat. Med. 24:1822–1829. doi:10.1038/s41591-018-0216-2

Bennett, J. E., R. Dolin, and M. J. Blaser, editors. 2015. Copyright. In: Mandell, Douglas, and Bennett's principles and practice of infectious diseases (eighth edition). Philadelphia: W.B. Saunders; p. iv. https://www.sciencedirect.com/science/article/pii/B978145574801300326X

Bergamaschi, M., F. Tiezzi, J. Howard, Y. J. Huang, K. A. Gray, C. Schillebeeckx, N. P. McNulty, and C. Maltecca. 2020. Gut microbiome composition differences among breeds impact feed efficiency in swine. Microbiome. 8:110. doi:10.1186/s40168-020-00888-9

Bracke, M. B. M., and H. A. M. Spoolder. 2008. Novel object test can detect marginal differences in environmental enrichment in pigs. Appl. Anim. Behav. Sci. 109:39–48. doi:10.1016/j.applanim.2007.01.014

Bullman, S., C. S. Pedamallu, E. Sicinska, T. E. Clancy, X. Zhang, D. Cai, D. Neuberg, K. Huang, F. Guevara, T. Nelson, et al. 2017. Analysis of Fusobacterium persistence and antibiotic response in colorectal cancer. Science. 358:1443–1448. doi:10.1126/science.aal5240

Campos, P. H. R. F., B. N. Silva, J. L. Donzele, R. F. M. Oliveira, and E. F. Knol. 2012. Effects of sow nutrition during gestation on within-litter birth weight variation: a review. Animal. 6:797–806. doi:10.1017/s1751731111002242

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, et al. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods. 7:335–336. doi:10.1038/nmeth.f.303

Carrión-López, M. J., J. Madrid, S. Martínez, F. Hernández, and J. Orengo. 2022. Effects of the feeding level in early gestation on body reserves and the productive and reproductive performance of primiparous and multiparous sows. Res. Vet. Sci. 148:42–51. doi:10.1016/j.rvsc.2022.05.002

Cho, J.-C., K. L. Vergin, R. M. Morris, and S. J. Giovannoni. 2004. Lentisphaera araneosa gen. nov., sp. nov, a transparent exopolymer producing marine bacterium, and the description of a novel bacterial phylum, Lentisphaerae. Environ. Microbiol. 6:611–621. doi:10.1111/j.1462-2920.2004.00614.x

Clavell-Sansalvador, A., R. Río-López, O. González-Rodríguez, L. J. García-Gil, X. Xifró, G. Zigovski, J. Ochoteco-Asensio, M. Ballester, A. Dalmau, and Y. Ramayo-Caldas. 2024. Effect of group mixing and available space on performance, feeding behavior, and fecal microbiota composition during the growth period of pigs. Animals (Basel). 14:2704. doi:10.3390/ani14182704

Clouard, C., B. Kemp, D. Val-Laillet, W. J. J. Gerrits, A. C. Bartels, and J. E. Bolhuis. 2016. Prenatal, but not early postnatal, exposure to a Western diet improves spatial memory of pigs later in life and is paired with changes in maternal prepartum blood lipid levels. FASEB J. 30:2466–2475. doi:10.1096/fj.201500208R

Costermans, N. G. J., N. M. Soede, A. Middelkoop, B. F. A. Laurenssen, R. E. Koopmanschap, L. J. Zak, E. F. Knol, J. Keijer, K. J. Teerds, and B. Kemp. 2020. Influence of the metabolic state during lactation on milk production in modern sows. Animal. 14:2543–2553. doi:10.1017/S1751731120001536

D'Urso, A., and J. H. Brickner. 2014. Mechanisms of epigenetic memory. Trends Genet. 30:230–236. doi:10.1016/j.tig.2014.04.004

De Bettio, S., A. Maiorka, L. N. E. Barrilli, R. Bergsma, and B. A. N. Silva. 2016. Impact of feed restriction on the performance of highly prolific lactating sows and its effect on the subsequent lactation. Animal. 10:396–402. doi:10.1017/S1751731115002001

De Leeuw, J. A., J. E. Bolhuis, G. Bosch, and W. J. J. Gerrits. 2008. Effects of dietary fibre on behaviour and satiety in pigs: symposium on 'Behavioural nutrition and energy balance in the young.'. Proc. Nutr. Soc. 67:334–342. doi:10.1017/S002966510800863X

Dhanasekaran, D., Y. Jiang, D. Dhanasekaran, and Y. Jiang. 2016. Actinobacteria - basics and biotechnological applications. https://www.intechopen.com/books/5056

Doifode, T., V. V. Giridharan, J. S. Generoso, G. Bhatti, A. Collodel, P. E. Schulz, O. V. Forlenza, and T. Barichello. 2021. The impact of the microbiota-gut-brain axis on Alzheimer's disease pathophysiology. Pharmacol. Res. 164:105314. doi:10.1016/j.phrs.2020.105314

Douglas, M. W., J. E. Cunnick, J. C. Pekas, D. R. Zimmerman, and E. H. von Borell. 1998. Impact of feeding regimen on behavioral and physiological indicators for feeding motivation and satiety, immune function, and performance of gestating sows. J. Anim. Sci. 76:2589–2595. doi:10.2527/1998.76102589x

Flint, H. J. 2012. The impact of nutrition on the human microbiome. Nutr. Rev. 70:S10–S13. doi:10.1111/j.1753-4887.2012.00499.x

Foster, J. A., L. Rinaman, and J. F. Cryan. 2017. Stress & the gut-brain axis: regulation by the microbiome. Neurobiol. Stress. 7:124–136. doi:10.1016/j.ynstr.2017.03.001

Fouhse, J. M., R. T. Zijlstra, and B. P. Willing. 2016. The role of gut microbiota in the health and disease of pigs. Anim. Front. 6:30–36. doi:10.2527/af.2016-0031

Foxcroft, G. R., W. T. Dixon, M. K. Dyck, S. Novak, J. C. S. Harding, and F. C. R. L. Almeida. 2009. Prenatal programming of postnatal development in the pig. Soc. Reprod. Fertil. Suppl. 66:213–231. doi:10.1530/biosciprocs.18.0028

Foxcroft, G. R., W. T. Dixon, S. Novak, C. T. Putman, S. C. Town, and M. D. A. Vinsky. 2006. The biological basis for prenatal programming of postnatal performance in pigs. J. Anim. Sci. 84:E105–E112. doi:10.2527/2006.8413_supplE105x

Frese, S. A., K. Parker, C. C. Calvert, and D. A. Mills. 2015. Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome. 3:28. doi:10.1186/s40168-015-0091-8.

Gaillard, C., and J.-Y. Dourmad. 2022. Application of a precision feeding strategy for gestating sows. Anim. Feed Sci. Technol. 287:115280. doi:10.1016/j.anifeedsci.2022.115280

Gardiner, G. E., B. U. Metzler-Zebeli, and P. G. Lawlor. 2020. Impact of intestinal microbiota on growth and feed efficiency in pigs: a review. Microorganisms. 8:1886. doi:10.3390/microorganisms8121886

Greff, M. J. E., J. M. Levine, A. M. Abuzgaia, A. A. Elzagallaai, M. J. Rieder, and S. H. M. van Uum. 2019. Hair cortisol analysis: an update on methodological considerations and clinical applications. Clin. Biochem. 63:1–9. doi:10.1016/j.clinbiochem.2018.09.010

Guevarra, R. B., S. H. Hong, J. H. Cho, B. -R. Kim, J. Shin, J. H. Lee, B. N. Kang, Y. H. Kim, S. Wattanaphansak, R. E. Isaacson, et al. 2018. The dynamics of the piglet gut microbiome during the weaning transition in association with health and nutrition. J. Anim. Sci. Biotechnol. 9:54. doi:10.1186/s40104-018-0269-6

Gupta, R. S., S. Mahmood, and M. Adeolu. 2013. A phylogenomic and molecular signature based approach for characterization of the phylum Spirochaetes and its major clades: proposal for a taxonomic revision of the phylum. Front. Microbiol. 4:217. doi:10.3389/fmicb.2013.00217

Guzmán-Pino, S. A., D. Luna, L. C. Salazar, P. Poblete-Grez, and J. Figueroa. 2021. Effect of switching sows' positions during lactation on suckling behaviour and performance of co-mingling piglets. Appl. Anim. Behav. Sci. 241:105391. doi:10.1016/j.applanim.2021.105391

Heijmans, B. T., E. W. Tobi, A. D. Stein, H. Putter, G. J. Blauw, E. S. Susser, P. E. Slagboom, and L. H. Lumey. 2008. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc. Natl. Acad. Sci. U. S. A. 105:17046–17049. doi:10.1073/pnas.0806560105

Hullar, M. A. J., and B. C. Fu. 2014. Diet, the gut microbiome, and epigenetics. Cancer J. 20:170–175. doi:10.1097/ppo.0000000000000053

Indrio, F., S. Martini, R. Francavilla, L. Corvaglia, F. Cristofori, S. A. Mastrolia, J. Neu, S. Rautava, G. Russo Spena, F. Raimondi, et al. 2017. Epigenetic matters: the link between early nutrition, microbiome, and long-term health development. Front. Pediatr. 5:178. doi:10.3389/fped.2017.00178

Jung, S. -W., S. Do, J.-C. Jang, J. Hong, G. Lee, and Y. Y. Kim. 2024. Effect of feeding frequency on reproductive performances and stress responses in gestating sows. J. Anim. Sci. Technol. 66:135–144. doi:10.5187/jast.2023.e42

Lawrence, A. B., and E. M. C. Terlouw. 1993. A review of behavioral factors involved in the development and continued performance of stereotypic behaviors in pigs. J. Anim. Sci. 71:2815–2825. doi:10.2527/1993.71102815x

Liang, L., H. Lin, Y. Cai, S. Lin, J. Tan, G. Ma, and L. Yuan. 2023. Effect of Danggui Niantongtang on intestinal flora in adjuvant-induced arthritis rats of wind-dampness-heat Arthralgia: based on 16S rDNA sequencing. Chin. J. Exp. Tradit. Med. Formulae 24:18–27. https://pesquisa.bvsalud.org/portal/resource/pt/wpr-965644

Lillycrop, K. A., and G. C. Burdge. 2011. Epigenetic changes in early life and future risk of obesity. Int. J. Obes. 35:72–83. doi:10.1038/ijo.2010.122

Limam, R. D., T. Bouchez, R. Chouari, T. Li, I. Barkallah, A. Landoulsi, and A. Sghir. 2010. Detection of WWE2-related Lentisphaerae by 16S rRNA gene sequencing and fluorescence in situ hybridization in landfill leachate. Can. J. Microbiol. 56:846–852. doi:10.1139/w10-065

Liu, R. T. 2017. The microbiome as a novel paradigm in studying stress and mental health. Am. Psychol. 72:655–667. doi:10.1037/amp0000058

Luo, Y., W. Ren, H. Smidt, A. -D. G. Wright, B. Yu, G. Schyns, U. M. McCormack, A. J. Cowieson, J. Yu, J. He, et al. 2022. Dynamic distribution of gut microbiota in pigs at different growth stages: composition and contribution. Microbiol. Spectr. 10:e0068821. doi:10.1128/spectrum.00688-21

Mach, N., M. Berri, J. Estellé, F. Levenez, G. Lemonnier, C. Denis, J. -J. Leplat, C. Chevaleyre, Y. Billon, J. Doré, et al. 2015. Early-life establishment of the swine gut microbiome and impact on host phenotypes. Environ. Microbiol. Rep. 7:554–569. doi:10.1111/1758-2229.12285

Mahmud, M. R., C. Jian, M. K. Uddin, M. Huhtinen, A. Salonen, O. Peltoniemi, H. Venhoranta, and C. Oliviero. 2023. Impact of intestinal microbiota on growth performance of suckling and weaned piglets. Microbiol. Spectr. 11:e0374422. doi:10.1128/spectrum.03744-22

Martini, G., D. Ciardiello, M. Dallio, V. Famiglietti, L. Esposito, C. M. D. Corte, S. Napolitano, M. Fasano, A. G. Gravina, M. Romano, et al. 2022. Gut microbiota correlates with antitumor activity in patients with mCRC and NSCLC treated with cetuximab plus avelumab. Int. J. Cancer 151:473–480. doi:10.1002/ijc.34033

Meunier-Salaün, M. C., S. A. Edwards, and S. Robert. 2001. Effect of dietary fibre on the behaviour and health of the restricted fed sow. Anim. Feed Sci. Technol. 90:53–69. doi:10.1016/s0377-8401(01)00196-1

Miura, H., T. Tsukahara, and R. Inoue. 2024. Whole-genome metagenomic analysis of functional profiles in the fecal microbiome of farmed sows with different reproductive performances. Microorganisms. 12:2180. doi:10.3390/microorganisms12112180

Moonat, S., and S. C. Pandey. 2012. Stress, epigenetics, and alcoholism. Alcohol Res. 34:495–505. https://pmc.ncbi.nlm.nih.gov/articles/PMC3860391/pdf/arcr-34-4-495.pdf

Murgatroyd, C., and D. Spengler. 2011. Epigenetic programming of the HPA axis: early life decides. Stress. 14:581–589. doi:10.3109/10253890.2011.602146

Nataro, J. P., C. A. Bopp, P. I. Fields, J. B. Kaper, and N. A. Strockbine. 2011. Chapter 35. *Escherichia*, *Shigella*, and *Salmonella*. In: Versalovic, J., K. C. Carroll, G. Funke, J. H. Jorgensen, M. L. Landry, D. W. Warnock, editors. doi:10.1128/9781555816728.ch35

Nieuwamerongen, S. E. van, J. E. Bolhuis, C. M. C. van der Peet-Schwering, and N. M. Soede. 2014. A review of sow and piglet behaviour and performance in group housing systems for lactating sows. Animal. 8:448–460. doi:10.1017/S1751731113002280

Noblet, J., and M. Etienne. 1986. Effect of energy level in lactating sows on yield and composition of milk and nutrient balance of piglets. J. Anim. Sci. 63:1888–1896. doi:10.2527/jas1986.6361888x

Pan, X., D. Gong, D. N. Nguyen, X. Zhang, Q. Hu, H. Lu, M. Fredholm, P. T. Sangild, and F. Gao. 2018. Early microbial colonization affects DNA methylation of genes related to intestinal immunity and metabolism in preterm pigs. DNA Res. 25:287–296. doi:10.1093/dnares/dsy001

Patrick, S. 2015. Chapter 51 - bacteroides. In: Tang Y.-W., M. Sussman, D. Liu, I. Poxton, and J. Schwartzman, editors. Molecular medical microbiology (second edition). Boston: Academic Press. p. 917–944. https://www.sciencedirect.com/science/article/pii/B9780123971692000512

Pond, W. G. 1973. Influence of maternal protein and energy nutrition during gestation on progeny performance in swine. J. Anim. Sci. 36:175–182. doi:10.2527/jas1973.361175x

Rajilić-Stojanović, M., and W. M. de Vos. 2014. The first 1000 cultured species of the human gastrointestinal microbiota. FEMS Microbiol. Rev. 38:996–1047. doi:10.1111/1574-6976.12075

Ramayo-Caldas, Y., N. Mach, P. Lepage, F. Levenez, C. Denis, G. Lemonnier, J. -J. Leplat, Y. Billon, M. Berri, J. Doré, et al. 2016. Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. ISME J. 10:2973–2977. doi:10.1038/ismej.2016.77

Rehfeldt, C., and G. Kuhn. 2006. Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. J. Anim. Sci. 84:E113–E123. doi:10.2527/2006.8413_supple113x

Rehfeldt, C., M. F. W. Te Pas, K. Wimmers, J. M. Brameld, P. M. Nissen, C. Berri, L. M. P. Valente, D. M. Power, B. Picard, N. C. Stickland, et al. 2011. Advances in research on the prenatal development of skeletal muscle in animals in relation to the quality of muscle-based food. I. Regulation of myogenesis and environmental impact. Animal. 5:703–717. doi:10.1017/S1751731110002089

Rist, V. T. S., E. Weiss, M. Eklund, and R. Mosenthin. 2013. Impact of dietary protein on microbiota composition and activity in the gastrointestinal tract of piglets in relation to gut health: a review. Animal. 7:1067–1078. doi:10.1017/S1751731113000062

Roseboom, T. J., J. H. van der Meulen, A. C. Ravelli, C. Osmond, D. J. Barker, and O. P. Bleker. 2001. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. Mol. Cell. Endocrinol. 185:93–98. doi:10.1016/s0303-7207(01)00721-3

Rosero, J. A., J. Killer, H. Sechovcová, J. Mrázek, O. Benada, K. Fliegerová, J. Havlík, and J. Kopečný. 2016. Reclassification of

Eubacterium rectale (Hauduroy *et al*. 1937) Prévot 1938 in a new genus Agathobacter gen. nov. as Agathobacter rectalis comb. nov., and description of Agathobacter ruminis sp. nov., isolated from the rumen contents of sheep and cows. Int. J. Syst. Evol. Microbiol. 66:768–773. doi:10.1099/ijsem.0.000788

Schokker, D., G. Veninga, S. A. Vastenhouw, A. Bossers, F. M. de Bree, L. M. T. E. Kaal-Lansbergen, J. M. J. Rebel, and M. A. Smits. 2015a. Early life microbial colonization of the gut and intestinal development differ between genetically divergent broiler lines. BMC Genomics. 16:418. doi:10.1186/s12864-015-1646-6

Schokker, D., J. Zhang, S. A. Vastenhouw, H. G. H. J. Heilig, H. Smidt, J. M. J. Rebel, and M. A. Smits. 2015b. Long-lasting effects of early-life antibiotic treatment and routine animal handling on gut microbiota composition and immune system in pigs. PLoS One. 10:e0116523. doi:10.1371/journal.pone.0116523

Silva, Y. P., A. Bernardi, and R. L. Frozza. 2020. The role of short-chain fatty acids from gut microbiota in gut-brain communication. Front. Endocrinol. 11:25. doi:10.3389/fendo.2020.00025

Solà-Oriol, D., and J. Gasa. 2017. Feeding strategies in pig production: sows and their piglets. Anim. Feed Sci. Technol. 233:34–52. doi:10.1016/j.anifeedsci.2016.07.018

Srinivasan, S., M. A. Beamer, T. L. Fiedler, M. N. Austin, M. V. Sizova, S. M. Strenk, K. J. Agnew, G. A. N. Gowda, D. Raftery, S. S. Epstein, et al. 2019. Megasphaera lornae sp. nov., Megasphaera hutchinsoni sp. nov., and Megasphaera vaginalis sp. nov.: novel bacteria isolated from the female genital tract. Int. J. Syst. Evol. Microbiol. 71:004702. doi:10.1099/ijsem.0.004702

Stankiewicz, A. M., A. H. Swiergiel, and P. Lisowski. 2013. Epigenetics of stress adaptations in the brain. Brain Res. Bull. 98:76–92. doi:10.1016/j.brainresbull.2013.07.003

Toth, L. A., and T. W. Gardiner. 2000. Food and water restriction protocols: physiological and behavioral considerations. Contemp. Top. Lab. Anim. Sci. 39:9–17. https://scispace.com/pdf/food-and-water-restriction-protocols-physiological-and-2ak02lzbxd.pdf

Trevisi, P., D. Luise, F. Correa, and P. Bosi. 2021. Timely control of gastrointestinal eubiosis: a strategic pillar of pig health. Microorganisms. 9:313. doi:10.3390/microorganisms9020313

van den Beld, M. J. C., E. Warmelink, A. W. Friedrich, F. A. G. Reubsaet, M. Schipper, R. F. de Boer, D. W. Notermans, M. W. F. Petrignani, E. van Zanten, J. W. A. Rossen, et al; on behalf of the IBESS working group. 2019. Incidence, clinical implications and impact on public health of infections with Shigella spp. and entero-invasive Escherichia coli (EIEC): results of a multicenter cross-sectional study in the Netherlands during 2016–2017. BMC Infect. Dis. 19:1037. doi:10.1186/s12879-019-4659-y

Voreades, N., A. Kozil, and T. L. Weir. 2014. Diet and the development of the human intestinal microbiome. Front. Microbiol. 5:494. doi:10.3389/fmicb.2014.00494

Wang, X., T. Tsai, F. Deng, X. Wei, J. Chai, J. Knapp, J. Apple, C. V. Maxwell, J. A. Lee, Y. Li, et al. 2019. Longitudinal investigation of the swine gut microbiome from birth to market reveals stage and growth performance associated bacteria. Microbiome. 7:109. doi:10.1186/s40168-019-0721-7

Wilson, A. S., K. R. Koller, M. C. Ramaboli, L. T. Nesengani, S. Ocvirk, C. Chen, C. A. Flanagan, F. R. Sapp, Z. T. Merritt, F. Bhatti, et al. 2020. Diet and the human gut microbiome: an international review. Dig. Dis. Sci. 65:723–740. doi:10.1007/s10620-020-06112-w

Wu, F., X. Guo, J. Zhang, M. Zhang, Z. Ou, and Y. Peng. 2017. Phascolarctobacterium faecium abundant colonization in human gastrointestinal tract. Exp Ther Med 14:3122–3126. doi:10.3892/etm.2017.4878

Xue, X., and T. C. Henderson. 2006. Video-based animal behavior analysis from multiple cameras. In: 2006 IEEE International Conference on Multisensor Fusion and Integration for Intelligent Systems. p. 335–340. https://ieeexplore.ieee.org/document/4042077

Yoo, J. Y., M. Groer, S. V. O. Dutra, A. Sarkar, and D. I. McSkimming. 2020. Gut microbiota and immune system interactions. Microorganisms. 8:1587. doi:10.3390/microorganisms8101587

Zeng, Q., J. Gong, X. Liu, C. Chen, X. Sun, H. Li, Y. Zhou, C. Cui, Y. Wang, Y. Yang, et al. 2019. Gut dysbiosis and lack of short chain fatty acids in a Chinese cohort of patients with multiple sclerosis. Neurochem. Int. 129:104468. doi:10.1016/j.neuint.2019.104468

Zhe, L., U. Krogh, C. Lauridsen, M. O. Nielsen, Z. Fang, and P. K. Theil. 2023a. Impact of dietary fat levels and fatty acid composition on milk fat synthesis in sows at peak lactation. J. Anim. Sci. Biotechnol. 14:42. doi:10.1186/s40104-022-00815-y

Zhe, L., U. Krogh, C. Lauridsen, M. O. Nielsen, Z. Fang, and P. K. Theil. 2023b. Impact of dietary fat levels and fatty acid composition on milk fat synthesis in sows at peak lactation. J. Anim. Sci. Biotechnol. 14:42. doi:10.1186/s40104-022-00815-y