Animal 15 (2021) 100266

Contents lists available at ScienceDirect

Animal

The international journal of animal biosciences

Interaction of CP levels in maternal and nursery diets, and its effect on performance, protein digestibility, and serum urea levels in piglets

K. Kroeske [a,b], N. Everaert [b], M. Heyndrickx [a,c], E. Arévalo Sureda [b], M. Schroyen [b], S. Millet [a,d,*]

[a] Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), 9090 Melle, Belgium
[b] Precision Livestock and Nutrition Unit, Gembloux Agro-Bio Tech, TERRA Teaching and Research Centre, University of Liège, 5030 Gembloux, Belgium
[c] Department of Pathology, Bacteriology and Poultry Diseases, Ghent University, Merelbeke, Belgium
[d] Department of Nutrition, Genetics and Ethology, Ghent University, Merelbeke, Belgium

ARTICLE INFO

Article history:
Received 9 October 2020
Revised 24 April 2021
Accepted 29 April 2021

Keywords:
Fecal consistency score
Feed conversion ratio
Growth
Pig feeding
Postweaning diarrhea

ABSTRACT

Reduced protein levels in nursery diets have been associated with a lower risk of postweaning diarrhea, but the interaction with CP levels in maternal diet on the performance of the offspring remains unclear. The objective of this study was to determine the effect of protein content in sow gestation and piglet nursery diets on the performance of the piglets until slaughter. This was studied in a 2 × 2 factorial trial (35 sows, 209 piglets), with higher or lower (**H** or **L**) dietary CP in sow diets (168 vs 122 g CP/kg) during late gestation. A standard lactation feed was provided for all sows (160 g CP/kg). For both sow treatments, half of the litters received a higher or lower CP in the piglet nursery diet (210 vs 166 g CP/kg). This resulted in four possible treatment combinations: HH, HL, LH and LL, with sow treatment as first and piglet treatment as second letter. For each phase, all diets were iso-energetic and had a similar level of essential amino acids. P_{s*p} is the p-value for the interaction effect between sow and piglet treatment. In the nursery phase (3.5–9 weeks of age), a tendency toward interaction between piglet and sow treatments with feed efficiency (P_{s*p} = 0.08) was observed with HH having the highest gain:feed ratio (**G:F**) (0.74 ± 0.01), LH the lowest (0.70 ± 0.01) and the other two groups intermediate. In the growing-finishing phase, an interaction was observed between the piglet and sow diets with decreased G:F for LH (P_{s*p} = 0.04) and a tendency toward interaction with increased daily feed intake for LH (P_{s*p} = 0.07). The sow diet showed a tendency toward a long-lasting effect on the dressing percentage and meat thickness of the offspring, which was higher for the progeny of H sows (P_s < 0.01 and P_s = 0.02, respectively). At 23 weeks, serum urea concentrations tended to be lower for the HH and LL groups (P_{s*p} = 0.07). Fecal consistency scores were higher at day 10–day 14 after weaning for piglets from L sows (P_s = 0.03 and P_s < 0.01, respectively). At day 7 after weaning, fecal consistency score was higher for piglets fed the higher protein diet (P_p < 0.01). At 8 weeks of age, the apparent total tract digestibility of CP (**$ATTD_{CP}$**) interacted between piglet and sow diet (P_{s*p} = 0.02), with HH showing the highest digestibility values. In conclusion, the protein levels in sow late-gestation and piglet nursery diets interacted with feed efficiency, $ATTD_{CP}$ and serum urea concentrations in the nursery phase.

Implications

We studied the interactions between the late-gestation diet and the nursery diet with the performance and health status of piglets until slaughter. The overall results show that sow late-gestation diet affected the offspring until slaughter. Reducing protein levels in the maternal late-gestation diet and increasing protein levels in the nursery piglet diet led to a higher fecal consistency score and feed intake after weaning without an increase in daily gain. Further research must be performed to confirm the results before they can become applicable in practice.

Introduction

Low protein levels in nursery diets have been associated with a lower risk of postweaning diarrhea (Ball and Aherne, 1987; Heo et al., 2009; Wan et al., 2020). The main reasons for antidiarrheal effects of low CP diets include decreased buffering capacity throughout the gastro-intestinal tract. The lower pH improves protein digestion and reduces the proliferation of pathogenic bacteria such as enterotoxigenic *Escherichia coli* (*E. coli*) (Heo et al., 2009; Ma et al., 2017). *E. coli* is often associated with

* Corresponding author at: Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Scheldeweg 68, 9090 Melle, Belgium.
E-mail address: sam.millet@ilvo.vlaanderen.be (S. Millet).

https://doi.org/10.1016/j.animal.2021.100266
1751-7311/

postweaning diarrhea and affects the piglets mostly during the first 14 days after weaning (Amezcua et al., 2002; Fairbrother et al., 2005). In addition, lower dietary CP levels decrease nitrogen excretion and consequently diminish the negative effects on the environment. To avoid negative effects on animal growth performance, amino acid profiles are commonly balanced using crystalline amino acids to achieve low protein diets (Kerr et al., 2003).

Maternal dietary CP during gestation has been shown to affect the phenotype of offspring until slaughter (Altmann et al., 2012a). This concept is known as 'maternal programming' or 'metabolic programming' and is defined as *a nutritional intrauterine and/or early postnatal stimulus or insult at a critical period of development with lasting or lifelong significance* (Kaske et al., 2010). According to this concept, providing both sow and fetus with optimal dietary amino acids during late gestation with optimal dietary amino acids will positively influence the development of the offspring (Seoane et al., 2020). However, what is optimal for the sow during gestation is different than what is optimal for the fetus (Gluckman and Hanson, 2004). Several studies have been conducted to explore the effect of late-gestation diets – either in combination or not with different lactation diets – on the performance of piglets until weaning (Altmann et al., 2012b; Luo et al., 2019; Rooney et al., 2020), but only few studies have examined the long-term effects of maternal dietary CP in pigs (Altmann et al., 2012a; Hines et al., 2019). Hines et al. (2019) supplemented L-arginine during different stages of gestation and found improved piglet performance until weaning, but no differences were found in finishers until slaughter. The mismatch theory states that when the fetal and postnatal environment do not match (e.g., poor in nutrients during fetal development, but rich in nutrients during their postnatal life), this may result in metabolic inefficiencies, suboptimal development and possibly even disease (van der Waaij et al., 2011; Lesuisse et al., 2018; Seoane et al., 2020). This trans-generational mismatch could also influence pig performance until slaughter. This has been addressed in very few studies with pigs and none in combination with CP levels. A study on broilers from van der Waaij et al. (2011) showed that the dietary CP imposed on parents directly affected how the offspring responded to their metabolized dietary CP levels (van der Waaij et al., 2011). Lesuisse et al. (2017) performed a multigenerational experiment with broiler breeders in which F0 (mother) and F1 (offspring) received either high or low (balanced) protein/amino acid diets. These studies showed that the diet imposed on F0 directly affected F1 until slaughter and seemed to influence how the offspring metabolized dietary CP levels. These observations may be similar for pigs; if so, this would suggest that the CP levels of the sow would need to be considered when formulating the diet of the piglets.

In the present trial, the objective was to study the interaction between CP levels in late gestation and nursery diet with the performance of piglets until slaughter. Therefore, we hypothesized that the CP level in the sow's diet during late gestation may affect how the piglets react to nursery diets with a different CP content, and that this difference may influence the piglet's health or resilience during the nursery phase and overall performance until slaughter. Health and resilience, which are related to postweaning diarrhea, were measured by immunoglobulins in milk and serum, fecal consistency in the nursery phase and fecal *E. coli* counts.

Material and methods

Experimental design

The study was a 2 × 2 factorial trial (35 sows, 209 piglets), with higher or lower CP levels in sow diets (168 vs 122 g CP/kg) during the last 5 weeks of gestation and high or low CP levels in piglet nursery diets (210 vs 166 g CP/kg). This resulted in four treatment groups: higher protein level in sow and piglet diets (**HH**), higher protein level in sow diet and lower protein level in piglet diet (**HL**), lower protein level in sow diet and higher protein level in piglet diet (**LH**), lower protein level in both piglet and sow diets (**LL**). The piglets were studied until slaughter.

All experimental procedures involving animals in this trial were approved by the Ethics Commission of Flanders Research Institute for Agriculture, Fisheries and Food (**ILVO**) (EC:2018/311). The experiments were performed from February 22nd, 2018 until October 17th, 2018.

Animals and management

The experiment was conducted at ILVO (Melle, Belgium). A total of 35 hybrid sows (Ra-Se; parity 1–8) were used for this experiment, divided over two batches (n = 18 sows in batch 1, n = 17 in batch 2) with a 3-week interval and an average parity of 3.7 ± 0.5 (batch 1 = 3.8 ± 0.6, batch 2 = 3.5 ± 0.7). All sows were inseminated with semen from either 1 or 2 Piétrain boars. During gestation, sows were group-housed in partly slatted concrete floor compartments of 19.00 m × 4.25 m and the temperature was set at 18 °C. Sows had access to a back-brush, several water drinking points, and separate compartments with mats. Natural daylight supplemented with artificial lighting was provided between 0730 and 1530 for the entire experimental period for sows and piglets.

At 107 ± 1 days after insemination and 7 ± 2 days before the expected farrowing date, sows were relocated from group housing to farrowing crates. The farrowing house consisted of four compartments with ten farrowing crates per compartment and two compartments per batch. Each farrowing crate consisted of a 1.90 m × 1.70 m PVC slatted floor with 1.75 m × 0.60 m metal slatted floor. Sows had access to an individual feeding trough and a water nipple. Two heat lamps per sow were provided for the piglets. The temperature in the farrowing compartments was kept constant at approximately 25 °C. At birth, the piglets were weighed and numbered with ear tags. All piglets stayed with their mother after birth (no cross fostering took place). After 1 week, the weak and small piglets from litters larger than 15 piglets were relocated and excluded from the experiment, resulting in an average of 12.1 ± 0.4 piglets per litter. As piglet intake of creep feed can vary and thus confound the experiment, no creep feed was provided.

The piglets used in the experiment were weaned at 3.5 weeks of age (24 ± 2 days after farrowing). At 28 ± 2 days after farrowing, the remaining non-experimental piglets were weaned and the sows were removed from the farrowing pens.

Each nursery pen in the experiment consisted of the six sibling piglets with the most average BW (three gilts and three barrows, n_{total} = 209, 35 pens). Due to a shortage of female piglets in one litter, the corresponding pen contained only two gilts and three barrows. Two additional pens contained four barrows and two gilts. Piglets were kept in pens of 1.8 m^2 with a slatted floor covered with PVC and enriched with a ball fastened to a chain. The feeding trough was 1 m wide and was located in the front of the pen and two water nipples on the back wall. The starting temperature was 26 °C; this was gradually decreased to 22 °C by the end of the nursery phase. In this phase, one piglet needed antibiotic treatment and was removed from the experiment. At 4.5 weeks of age, one barrow was removed from each pen (n_{pen} = 5) and euthanized for additional measurements (data not shown) (new n_{total} = 174).

After the nursery phase, at 9 weeks of age, the gilts (n = 101) from each pen were transferred to the fattening stables, housed per two or three (sisters) depending on the nursery composition of the pen, in half-slatted pens of 3.70 m^2 until slaughter. The feed-

ing trough, which measured 0.30 m × 0.39 m, was located in the front of the pen and two water nipples were found on the back wall. The pen was enriched with a ball on a chain and a chewing cotton chord. In this phase, one piglet needed antibiotic treatment and was removed from the experiment.

Diets

Sows were divided into two dietary treatment groups starting on d79 of gestation. This was 35 days before the expected farrowing date (114 ± 2 days after insemination). The diets contained two CP levels (122 vs 168 g CP/kg). These amounts are less than and greater than, respectively, the commercial CP content in feeds typically given in Belgium (140–160 g CP /kg). All feeds had similar essential amino acid and energy levels (6.5 g standardized ileal digestible (SID) lysine (LYS) g/kg, 9.1 MJ/kg net energy). Throughout the gestation period, before the treatments, all sows were fed a commercial gestation feed (Leievoeders N.V., Waregem, Belgium) in a feeding station (Nedap, Nedap Livestock Management, Groenlo, the Netherlands) set to 2.6 kg per sow per day. Directly after farrowing, all sows received the same lactation feed (162 g CP/kg, 8 g SID LYS/kg, 9.4 MJ/kg, Table 1). This was formulated according to the recommendations for commercial practices in Belgium (160–170 g CP/kg). Portions of sow lactation feed were increased gradually over 5 days up to 7.50 kg per day. As noted above, piglets did not receive creep feed during the lactation period. During the nursery phase, from 3.5 to 9 weeks of age, each pen was assigned to a lower (166 g/kg) or higher (210 g/kg) CP diet with similar levels of essential amino acids and energy levels (10.5 g SID LYS/kg, 9.8 MJ/kg, Table 1). These amounts are less than and greater than those recommended for commercial practices in Belgium (170–185 g CP/kg). (National Research Council (NRC), 2012; CVB, 2016). During the fattening period, all pigs received the same 3-phase diet (9–15 weeks of age, 15–20 weeks of age, and 20–24 weeks of age; Table 1). The diets contained 9.5, 8.5, 7.8 g SID lysine and 9.6, 9.5, 9.4 MJ/kg net energy for the first, second and third phases, respectively. The pigs had *ad libitum* access to water and feed. All feeds were pelleted and Acid Insoluble Ash (SiO2, AIA) was used as a digestibility marker in all experimental diets.

Measurements and analyses

Performance and carcass measurements

Sow BW and backfat thickness were measured on d78 and d107 of gestation, and again at 28 days after farrowing. Piglets' BW, daily feed intake (**DFI**), daily gain (**DG**) and gain-over-feed (**G:F**) were determined and calculated per phase and from birth until slaughter.

At 24 weeks of age, one gilt from each pen was euthanized for additional measurements (data not shown). This was the endpoint for the data collection on growth performance. The remaining gilt (s) were slaughtered at 25 weeks of age. Before slaughter, the gilts (n = 76) were fasted for 20 h and transported for 85 km to a commercial slaughterhouse. Carcass quality parameters (muscle thickness, fat thickness) were measured using the AutoFOM III (Carometec A/S, Denmark) from all animals. At the slaughterhouse, 15 gilts from batch 1 could not be identified, resulting in 61 animals with carcass measurements (n = 17 out of 25, 14 out of 27, 12 out of 22, 18 out of 26 resp. for HH, HL, LH, LL). Ham fat thickness = Average fat measurements at the Minimum Fat Thickness point, 7 cm from the backbone (R4P2). Ham fat max = maximal value of the ham cut. Dressing percentage was calculated by cold carcass weight divided by fasted live weight before transport to slaughter.

Lean meat percentage was calculated by the equation approved by the regulation 2012/416/EU (European Union (EU), 2012). Muscle growth per day was calculated based on the following equation:

$$\text{Muscle growth per day} = \frac{(\text{Slaughter weight} \times \text{LMP at slaughter}) - (\text{BW} \times 0.45)}{\text{days between start and slaughter}}$$

where BW at the end of the nursery period. The value of 0.45 is an estimate for the percentage of lean meat in pigs of 20 kg as a reference for the piglets at the end of the nursery phase according to the measurements of Susenbeth and Keitel (1988).

Euthanasia

The piglets were anesthetized using a Zoletil 100® (Virbac, Louvain la Neuve, Belgium) and Xyl-M® 2% (VMD, Arendonk, Belgium) intramuscular injection and euthanized using an intracardial injection of Release® 300 mg/ml (ECUPHAR NV/SA, Oostkamp, Belgium).

Blood analysis

Blood was collected intracardially from pigs that were euthanized for additional measurements (data not shown) at weaning (3.5 weeks of age, n = 12 (six gilts, and six barrows)), 4.5 weeks of age (n = 16 barrows), and 9 weeks of age after weaning (n = 16 barrows). At 13, 18 and 23 weeks of age, blood was sampled from one gilt per pen by puncturing the jugular vein using a PrecisionGlide needle; 9 mL blood was collected in BD Vacutainer™ SST™ II Advance Tubes (BD Life Sciences, San Antonio, TX, USA). After sampling, blood was kept on ice for approximately 1–4 h until serum was obtained by centrifuging at 1500*g* for 10 min at 4 °C; serum was frozen in Eppendorf tubes (2 ml) at −80 °C until further analysis. Urea concentrations in serum (mg/dL) were measured by UREAL v9 protocol by a commercial lab (Centrum voor Medische Analyse bv, Herentals, Belgium).

Colostrum/milk collection and analysis

Colostrum was collected from the posterior and medial teats within 3 h after farrowing for determination of CP, crude fat and concentration of immunoglobulins G and A. Milk was collected from the same teats 7 days after farrowing (all sows) and 14 days after farrowing (sows of the second batch). Colostrum and milk were hand-collected from sows within 20 minutes after administration of an intramuscular injection of 2 mL oxytocin to the neck. Before milking, all nipples were cleaned with ethanol wipes; sterile gloves were worn. Samples (between 10 and 30 mL) were collected in 50 mL plastic tubes and stored at −20 °C until further analysis.

Protein and fat content of colostrum and milk were determined using Fourier-transform infrared spectroscopy on a Standard Lactoscope FT-MIR automatic (Delta Instruments, Drachten, The Netherlands) (Leblois et al., 2017).

Immunoglobulin-G (IgG) and Immunoglobulin-A (IgA) concentrations were determined in colostrum and serum of sows by ELISA using goat-anti-pig antibodies, according to the manufacturer's recommendations (Bethyl Laboratories, Montgomery, AL, USA and R&D Systems, Oxon, UK, respectively) and as described in (Leblois et al., 2018). Samples in duplicate were quantified by interpolating their absorbance from the standard curves constructed with known concentrations of each pig immunoglobulin class and corrected for sample dilution according to the manufacturer protocols (E101-104): pig IgG ELISA Kit and (E101-102) Pig IgA ELISA Kit (Bethyl Laboratories, Montgomery, AL, USA and R&D Systems, Oxon, UK, respectively). The intra-assay coefficient of variability (%CV) was 3.49%, 4.06%, 3.19% and 4.14% for colostrum IgA and IgG and serum IgA and IgG, respectively. The detection range was 15.6–1 000 and 7.8–500 ng/ml for IgA and IgG assays, respectively. Colostrum was diluted 1:50 000 for IgA and

Table 1
Ingredient composition (%) and calculated[1] (analyzed) nutrient composition (g/kg, unless otherwise mentioned) of the eight feeds used during the experiment for all pig diets.

		Sow			Piglet				
		Gestation (last 5 weeks of gestation)			Postweaning (3.5–9 weeks of age)		Growing-finishing		
	Unit	Higher CP	Lower CP	Lactation	Higher CP	Lower CP	Phase 1 (9–14 weeks)	Phase 2 (15–20 weeks)	Phase 3 (21–25 weeks)
Ingredient composition									
Corn	%	12.3	26.0	20.0	5.85	15.00	25.00	20.0	21.00
Wheat	%	19.8	19.8	14.7	26.0	24.58	24.99	25.0	25.00
Beet pulp	%	9.40	11.7	7.16			5.00	5.00	6.00
Barley	%	10.0	10.0	20.0	24.0	30.0	17.4	25.0	25.0
Wheat middlings	%	13.3	9.24					1.47	1.06
Alfalfa meal	%	2.50	5.21	2.50			1.19	1.1	3.60
Palm kernels	%	2.25	3.94	5.00			2.47	1.62	
Beet molasses	%	3.00	3.00	3.50	3.00	3.00	3.00	3.00	3.00
Rapeseed meal	%	8.00	2.23	4.98	1.81	1.43			
Premix[2]	%	1.10	1.10	1.10	6.00	6.00	1.00	1.00	1.00
Toasted soybeans	%				12.32	9.00			
Soybean meal (47.2% CP)	%	13.0	1.95	14.14	12.0	1.15	13.6	10.8	8.97
Wheat gluten feed (77.6% CP)	%				2.26	2.00			
Potato protein	%				2.00	2.00			
Soy oil	%	3.00	1.72	2.95	1.73	1.22	2.09	1.98	1.71
Animal fat	%								
Celite	%	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	%	0.95	0.99	1.55	0.69	0.73	1.20	1.30	1.02
Phytase (Ronozyme, 1000 PHYT/g)[3]	%	0.10	0.10	0.15	0.15	0.15	0.10	0.10	0.10
Sodium bicarbonate	%		0.48			0.70	0.29	0.34	0.30
L-Lysine HCL	%		0.44	0.22	0.24	0.65	0.52	0.47	0.43
Monocalcium phosphate	%	0.05	0.36	0.43	0.39	0.51	0.27	0.10	0.11
Salt	%	0.36	0.24	0.43	0.41		0.37	0.33	0.35
L-threonine	%	0.01	0.22	0.09	0.06	0.25	0.22	0.19	0.18
Dl-methionine	%		0.15	0.05	0.08	0.19	0.18	0.14	0.13
L-Isoleucine	%		0.09	0.01		0.05			
L-Valine	%		0.08	0.08		0.15	0.07	0.05	0.03
L-tryptophan	%		0.04		0.02	0.09	0.05	0.03	0.03
L-histidine	%					0.04			
L-leucine	%					0.09			
Nutrient composition									
DM	g/kg	884 (897)	883 (900)	882 (899)	882 (904)	882 (899)	879 (888)	878 (894)	878 (895)
CP	g/kg	170 (168)	120 (122)	160 (162)	210 (210)	165 (166)	153 (152)	143 (141)	136 (136)
Crude fat	g/kg	54 (51)	43 (41)	54 (51)	58 (58)	48 (49)	43 (44)	40 (40)	36 (39)
Crude ash	g/kg	68 (62)	66 (64)	72 (70)	60 (58)	55 (52)	61 (58)	60 (58)	59 (55)
Crude fiber	g/kg	71 (65)	75 (67)	65 (59)	37 (36)	34 (31)	45 (39)	45 (40)	50 (47)
Net energy	MJ/kg	9.100	9.100	9.400	9.800	9.800	9.600	9.500	9.400
Starch	g/kg	259	331	300	302	377	379	389	395
Sugar	g/kg	59	45	53	86	74	46	45	44
NSP	g/kg	271	274	237	166	161	193	197	204
ADF	g/kg	92 (86)	93 (81)	80 (67)	44 (44)	41 (38)	56 (47)	56 (47)	59 (49)
ADL	g/kg	17 (18)	17 (14)	15 (12)	9 (6)	9 (6)	9 (7)	9 (6)	10 (6)
NDF	g/kg	208 (166)	208 (166)	164 (137)	105 (93)	108 (87)	134 (103)	140 (106)	143 (105)
Kalium	g/kg	9.8	7.5	8.7	10.0	7.5	7.7	7.3	7.5
Chlorine	g/kg	3.5	3.8	4.4	5.3	3.7	4.3	4.1	4.2
Calcium	g/kg	7.5	8.0	10.5	5.5	5.5	7.8	7.8	7.1
Phosphor	g/kg	5.0	4.5	4.6	4.8	4.6	3.8	3.5	3.3
SID lysine	g/kg	6.5	6.5	8	10.5	10.5	9.5	8.5	7.8
SID M + C	g/kg	4.3	4.3	4.6	6.3	6.3	5.7	5.2	4.8
SID methionine	g/kg	2.1	2.9	2.5	3.5	4.0	3.7	3.2	3.0
SID threonine	g/kg	4.7	4.7	5.6	6.8	6.8	6.3	5.7	5.3
SID tryptophan	g/kg	1.7	1.4	1.7	2.3	2.3	1.9	1.6	1.5
SID arginine	g/kg	8.8	4.8	8.1	10.8	6.9	7.6	6.8	6.2
SID leucine	g/kg	9.7	6.4	9.7	12.9	10.5	9.6	8.7	8.3
SID isoleucine	g/kg	5.3	3.8	5.2	7.2	5.4	4.8	4.4	4.1
SID valine	g/kg	6.2	4.6	6.8	8.1	7.4	6.3	5.7	5.2
SID histidine	g/kg	3.5	2.1	3.1	4.2	3.4	3.1	2.8	2.7
SID phenylalanine	g/kg	6.1	3.6	6.0	8.6	6.2	5.9	5.5	5.2

SID = Standardized ileal digestibility; NSP = non-starch polysaccharides; M + C = methionine and cysteine.

[1] According to CVB (2007).

[2] The sow premix provided the following quantities of vitamins and minerals per kilogram feed: vitamin A, 12 000 IU; vitamin D3, 2 000 IU; vitamin E, 75 mg; vitamin K3, 1 mg; vitamin B1, 1.5 mg; vitamin B2, 18 mg; calcium pantothenate 18 mg; vitamin B6, 4 mg; vitamin B12, 0.03 mg; vitamin PP, 25 mg; folic acid, 3 mg; biotin, 0.3 mg; choline, 432.5 mg; Ca, 888 mg; P, 54.6 mg; Mg, 165 mg; Na, 3 mg; Cl, 123 mg; K, 76 mg; S, 112 mg; Fe, 150 mg; Cu, 15 mg; Mn, 50 mg; Zn, 100 mg; I, 2 mg; Se, 0.4 mg. The piglet premix (6%) contained 80% dairy products and 20% vitamin and mineral premix, providing the following quantities of vitamins and minerals per kilogram of feed: vitamin A, 15 000 IU; vitamin D3, 2 000 IU; vitamin E, 100 mg; vitamin K, 2 mg; vitamin B1, 2.5 mg; vitamin B2, 7.5 mg; vitamin B5, 20 mg; vitamin B6, 5 mg; vitamin B12, 0.04 mg; vitamin C, 100 mg; vitamin PP, 30 mg; choline, 324 mg; folic acid, 3 mg; biotin, 0.15 mg; Ca, 516 mg; P, 419 mg; Mg, 165 mg; Na, 353 mg; Cl, 1 375 mg; K, 1 227 mg; S, 234 mg; Fe, 100 mg; Cu, 160 mg; Mn, 60 mg; Zn, 100 mg; I, 2 mg; Se, 0.4 mg.

[3] Ronozyme Hiphos, the concentration added provided 1–500, 500–1 000 or 1 000–1 500 phytase units/kg.

1:1 000 000 for IgG. Serum was diluted 1:10 000 for IgA and 1:200 000 for IgG analysis.

Feces collection and analysis of feed and feces

Fresh feces were sampled from the rectum of 35 sows at six and 1 week before and 3 weeks after farrowing, and from piglets at 8, 13, 18, and 23 weeks of age. Fecal sampling was performed once per day on five consecutive days and samples were stored at −20 °C. Sow feces were collected and analyzed per sow and piglet feces were collected once daily for five consecutive days from at least two piglets and pooled per pen. Feces samples were stored in the freezer until homogenization, then refrozen until freeze-drying. For the freeze-drying process, the drying chamber was set to −20 °C, the compensator to −55 °C and pressure was 0.7 mbar. The samples were dried until the sample temperature was 20 °C as measured with a probe. The dried samples were ground to pass through a sieve of 1 mm mesh size for further analysis of DM (European Commission (EC), 1971), AIA (McCarthy and Molloy, 1974), CP (ISO 5983–2), crude ash (ISO 5984), crude fat, crude fiber, acid detergent fiber, neutral detergent fiber, and acid detergent lignin (Van Soest et al., 1991). Amino acid composition was analyzed after acid hydrolysis (European Commission (EC), 1998) and tryptophan after alkaline hydrolysis (European Commission (EC), 2000). Apparent total tract digestibility of CP (**$ATTD_{CP}$**) was calculated using the formula:

$$ATTD_{cp} = 100 \times 1 - \frac{(NU_F \times AIA_D)}{NU_D \times AIA_F)}$$

where $ATTD_{cp}$ = apparent ATTD of CP, AIA_D = dietary concentration of AIA, NU_D = nutrient concentration of diet (Table 1), NU_F = nutrient concentration of feces, AIA_F = fecal concentrations of AIA (Kong and Adeola, 2014).

Fecal observations. After weaning, fecal consistency scores were determined during the nursery phase from 3.5 until 9 weeks of age. Scores were assigned twice a week per pen by the same person using a tagged visual analog scale (tVAS) (McCormack et al., 1988) from 0 to 100 mm (0 = normal, 20 = slightly less firm, 40 = yogurt-like consistency, 60 = diarrhea laying on the slats; 80 = diarrhea not laying on the slats; to 100 = watery diarrhea).

E. coli shedding. From each sow, fecal samples were taken from the rectum at d79 of gestation (before treatment), d107 of gestation, d108 (after moving into the farrowing crates) and 3 weeks after farrowing. Fecal samples from piglets were collected from two piglets per pen at 4 and 5 weeks. Fecal samples were diluted 1:10 with BIO-RAD maximum recovery diluent, homogenized by a Masticator (IUL Instruments, S.A., Barcelona, Spain) and incubated at 44 °C for 24 h on RAPID'E. coli 2 Medium (Bio-Rad Laboratories, Hercules, CA, USA). *E. coli* colony-forming units (CFUs) were counted using counting grids from Don Whitley Scientific Limited (Whitley Automated Spiral Plater, 1997).

Statistical analysis

Statistical analysis was performed using R version 3.6.1 (2019-07-05) – "Action of the Toes" ©2019 The R Foundation for Statistical Computing; platform i386-w64-mingw32/i386 (32-bit) with linear mixed-effect models (lme4 package) for single measurements. During the gestation and lactation periods, sow was the experimental unit. During the nursery and fattening phase, pen was the experimental unit. For performance parameters and $ATTD_{cp}$ of sows, the model included sow diet, pig diet and their interaction as a fixed factor and compartment as a random factor. For carcass quality measurements, carcass weight was included as the fixed factor and pen and slaughter date as a random effect. Sow weight and backfat thickness were considered as repeated measures and sampling date was used as a factor in the repeated measures ANOVA. Repeated measures ANOVA (lme4 package) was used to analyze performance parameters (sow and piglets), composition measures in colostrum and milk, $ATTD_{cp}$ of piglets, fecal consistency score and *E. coli* shedding. *E. coli* colony-forming unit per gram feces were calculated and logarithmically transformed before statistical analysis. During statistical analysis of *E. coli*, outliers were identified with the box plot function and were temporarily removed within RStudio to confirm that there were no effects on the results. The outliers were re-inserted. When an interaction was observed between sow and piglet treatments, a post hoc pairwise comparison with a Tukey correction between groups and estimated marginal means (emmeans package) was performed. For parameters measured during gestation and lactation in sows, the boar used for insemination was included as fixed factor. Parity of sows was included as fixed factor in relevant models related to sow reproduction parameters. For all parameters, differences were considered significant when $P < 0.05$ and a tendency was defined when $0.1 \geq P\text{-value} \geq 0.05$. Throughout the document, P_{s*p} denotes the p-value for the interaction between sow and piglet treatment, P_s for the effect of sow treatment and P_p for the effect of piglet treatment. All means reported in this paper are raw averaged numbers.

Results

At 11 days after weaning, one animal was excluded from the experiment as recommended by a veterinarian due to illness and antibiotic treatment.

Performance

Sow performance parameters did not differ significantly between treatments (Table 2). Progeny of sows receiving the H sow diet tended to have increased BW at birth (P_s = 0.10). At 9 weeks of age, piglets did not show significant differences in BW, DFI, and DG (Table 3). Sow and piglet diet tended to interact with feed efficiency (P_{s*p} = 0.08), with HH having the highest G:F (0.74 ± 0.01) and LH the lowest (0.70 ± 0.01) and the two other groups intermediate values. In phase 2 of the fattening period (15–20 weeks of age), pigs from the H sows tended to have a higher DG (P_s = 0.06) and higher G:F (P_s = 0.08). This effect was no longer visible in phase 3 (20–24 weeks of age). Over the total fattening phase, from 9 to 24 weeks of age, an interaction between sow and piglet feed was observed for G:F (P_{s*p} = 0.04) which was higher for HH and LL with a tendency toward higher DFI for LH compared to the other three treatment groups (P_{s*p} = 0.07). An increased dressing percentage and meat thickness was observed in progeny of sows that received a higher protein diet compared to those of L sows (P_s < 0.01 and P = 0.02 respectively, Table 4). No other significant differences in carcass composition nor daily muscle growth were observed.

Colostrum and milk composition

CP levels in colostrum and milk did not differ between treatment groups (P_s = 0.12, Table 5). Crude fat levels in colostrum did not differ between treatment groups (P_s = 0.98), but the fat content in milk was higher (P_s < 0.01) at d7 post-partum and tended to be higher (P_s = 0.06) at d14 post-partum in sows receiving the H diet.

Sows that received the L diet had higher IgG levels in colostrum (P_s = 0.05), while IgA levels did not differ. In the serum of sows, no significant differences in IgA or IgG levels were observed.

Table 2
Effect of CP level in late-gestation diet on reproduction parameters of sows.

Parameters	Measurement time[1]	Higher protein (H) (n = 18) Mean	SE	Lower protein (L) (n = 17) Mean	SE	P_{sow}
Parity		3.6	1	3.8	1	0.87
BW (kg)	−6 weeks	235	9	236	11	0.83
	−1 week	250	11	261	13	0.40
	Weaning	220	10	227	11	0.57
Backfat thickness (mm)	−6 weeks	15	1	15	1	0.79
	−1 week	15	1	17	1	0.35
	Weaning	11	1	12	1	0.44
Number of total piglets born per sow (#)		16.3	1.1	17.0	0.8	0.67
Live born piglets per sow (#)		14.9	0.7	14.8	0.6	0.33
Birth weight (of live-born piglets) (kg)		1.3	0.0	1.4	0.0	0.10
BW piglets at weaning (kg)		7.2	0.1	7.0	0.1	0.47
Number of weaned piglets per sow (#)		12.6	0.7	12.1	0.6	0.81
Feed intake sows during lactation (kg)		190	2	186	5	0.39

[1] 6 weeks before farrowing (−6 weeks) is just before the sows start receiving the treatment; −1 week = 1 week before farrowing; Weaning is at the time of weaning (3.5 weeks of age = 24 ± 2 days of age).

Table 3
Effect of CP levels in gestation and nursery diets on performance results of piglets from 3.5 to 24 weeks of age.

Sow feed:		Higher protein (H)		Lower protein (L)					
Piglet feed:	n[1]	Higher protein (HH) (n = 9) Mean	Lower protein (HL) (n = 9) Mean	Higher protein (LH) (n = 8) Mean	Lower protein (LL) (n = 9) Mean	SEM	P_{sow}	P_{piglet}	$P_{sow*piglet}$
BW (kg)									
9 weeks (gilts and barrows)	174	21.6	21.1	21.2	21.0	0.2	0.87	0.82	NS
9 weeks (gilts)[1]	101	21.2	21.6	21.0	20.9	0.3	0.69	0.89	NS
15 weeks	100	49	49	49	48	1	0.49	0.75	NS
20 weeks	100	79	78	77	75	1	0.09	0.55	NS
24 weeks	100	111	110	110	107	1	0.18	0.28	NS
Daily feed intake (g/d)									
3.5–9 weeks (nursery)	174	509	522	518	515	17	0.66	0.62	NS
9–15 weeks (phase 1)	101	1 275	1 325	1 351	1 212	26	0.82	0.60	NS
15–20 weeks (phase 2)	100	1 984	1 956	1 963	1 892	30	0.62	0.58	NS
20–24 weeks (phase 3)	100	2 533	2 526	2 725[a]	2 454[b]	49	0.44	0.09	0.11
9–24 weeks	100	1 884	1 892	1 964[a]	1 807[b]	30	0.22	**0.04**	*0.07*
Daily gain (g/d)									
3.5–9 weeks (nursery)	174	377	370	364	372	7	0.74	0.61	NS
9–15 weeks (phase 1)	100	660	652	660	636	11	0.62	0.49	NS
15–20 weeks (phase 2)	100	847[A]	829[A]	787[B]	781[B]	14	0.06	0.60	NS
20–24 weeks (phase 3)	100	947	943	984	938	16	0.52	0.34	NS
9–24 weeks	100	807	794	799	773	10	0.30	0.30	NS
Gain:Feed ratio (g/g)									
3.5–9 weeks (nursery)	174	0.74	0.71	0.70	0.72	0.01	0.45	0.29	*0.08*
9–15 weeks (phase 1)	100	0.52[A]	0.49[B]	0.49[B]	0.53[A]	0.01	0.78	0.97	**<0.01**
15–20 weeks (phase 2)	100	0.43	0.49	0.40	0.41	0.01	0.08	0.89	NS
20–24 weeks (phase 3)	100	0.30	0.29	0.29	0.30	0.01	0.81	0.46	NS
9–24 weeks	100	0.43	0.42	0.41	0.43	0.01	0.51	0.07	**0.04**

[A,B]Mean values sharing no common letter indicate a sow effect; [a,b]Mean values sharing no common letter indicate an interaction effect; NS = not significant.
[1] At 3.5 weeks of age, three gilt and three barrow siblings (n = 209) entered the nursery pens (n = 35). At 4.5 weeks of age, one barrow was removed from each pen. At 9 weeks of age, all remaining piglets were weighed (n = 174, five per pen). Only the gilts were used in the next phase (n = 101) and were weighed again.

Apparent total tract digestibility of CP

The $ATTD_{CP}$ in sows was higher for the H sow diet before farrowing (P_s < 0.01, Table 6). For the $ATTD_{CP}$ of piglets at 8 weeks of age, piglet and sow interacted (P_{s*p} = <0.01, Table 6), with HH showing the highest (86.3 ± 0.33), HL the lowest (83.9 ± 0.62) and LH and LL intermediate digestibility values.

Serum urea concentrations in sows and piglets

At weaning, offspring from H diet fed sows had lower urea concentrations in serum compared to offspring from L sows (P_s = 0.03, Table 7). At 4.5 weeks of age, urea concentrations were affected by both sow and piglet treatment, with no interaction between sow and piglet diet (P_s = 0.02, P_p = <0.01, Table 7). Starting at 9 weeks of age, the dietary protein level of the sow diet did not affect the serum urea levels in the piglets. In the fattening phase (at 9, 13, 18 and 23 weeks of age), no sow or piglet effect was observed, but a tendency toward an interaction (P_{s*p} = 0.07) was seen at 23 weeks of age, with the lowest serum urea levels in the LL and HH groups (P_{s*p} = 0.07).

Fecal consistency and E. coli shedding

All fecal consistency scores were below 25, indicating a low overall diarrhea score. No interactions were found between sow

Table 4
Effect of CP level in gestation and nursery diets on carcass measurements of pigs at slaughter.

Sow feed:	Higher protein (H)		Lower protein (L)				
Piglet feed:	Higher protein (HH) (n = 17)	Lower protein (HL) (n = 14)	Higher protein (LH) (n = 12)	Lower protein (LL) (n = 18)			
Carcass quality measures[1]	Mean	Mean	Mean	Mean	SEM	P_{sow}[2]	P_{piglet}[2]
Slaughter weight (kg)	119	117	117	115	1	0.38	0.57
Cold carcass weight (kg)	95	90	89	89	1	0.35	0.86
Dressing percentage (%)	78.9^{A}	78.9^{A}	78.0^{B}	77.6^{B}	0.6	**<0.01**	0.32
Fat thickness (mm)	8.9	7.9	8.3	8.4	1.0	0.40	0.86
Meat thickness (mm)	66^{A}	64^{A}	64^{A}	60^{B}	1	**0.02**	**0.06**
Ham fat thickness (mm)	7.2	6.5	7.2	6.7	0.6	0.40	0.78
Ham fat max (mm)	16.4	16.1	14.7	14.7	0.7	0.43	0.90
Lean Meat (%)	63	64	64	64	0	0.50	0.64
Muscle growth per day (weaning to slaughter) (g)	355	337	333	333	5	0.79	0.91
Muscle growth per day (fattening to slaughter) (g)	414	393	385	386	4	0.46	0.80

[A,B]Mean values without a common letter indicate a sow effect.

[1] Carcass quality parameters (muscle thickness, fat thickness) were measured using the AutoFOM III (Carometec A/S, Denmark). Ham fat thickness = Average fat measurements at the Minimum Fat Thickness (MFT) point, 7 cm from the backbone (R4P2); Ham fat max = maximal value of the ham cut. Dressing percentage was calculated by cold carcass weight divided by fasted live weight before transport to slaughter; Lean meat percentage was calculated by the equation approved by the regulation 2012/416/EU (European Commission, 2012). Muscle growth per day was calculated based on the equation: Muscle growth per day = ((Slaughter weight × Lean meat percentage at slaughter) − (W × 0.45))/(days between start and slaughter), where W = weight at the end of the nursery period. The value of 0.45 is an estimate for the percentage of lean meat in pigs of 20 kg according to the measurements of Susenbeth and Keitel (1988).

[2] $P_{sow*piglet}$ values are all >0.1, thus only the main effects of sow and piglet diet are presented in Table 4.

Table 5
Effect of CP level in sow diet on colostrum, milk and serum immunoglobulins G (IgG) and -A (IgA).

			Sow feed						
			Higher protein (H)			Lower protein (L)			
Measured	Matrix	Time	n[1]	Mean	SE	n[1]	Mean	SE	P_{sow}
CP (%)	Colostrum	Farrowing	16	16.42	0.43	16	17.12	0.43	0.12
	Milk	First week of lactation	14	5.37	0.06	13	5.49	0.14	0.44
	Milk	Second week of lactation	9	5.37	0.16	8	5.26	0.06	0.62
Crude fat (%)	Colostrum	Farrowing	16	7.01	0.40	16	6.91	0.44	0.98
	Milk	First week of lactation	14	8.56	0.27	13	8.01	0.31	**<0.01**
	Milk	Second week of lactation	9	9.03	0.61	8	7.97	0.34	0.06
IgG	Colostrum (mg/mL)	Farrowing	16	60.12	5.67	16	72.30	3.13	**0.04**
	Serum (μg/mL)	Farrowing	16	2.14	0.09	18	2.12	0.09	0.74
IgA	Colostrum (mg/mL)	Farrowing	16	0.83	0.07	16	0.84	0.1	0.97
	Serum (μg/mL)	Farrowing	16	13.14	1.41	18	14.53	2.61	0.67

[1] From the 35 sows divided in two batches, colostrum was successfully collected from only 32 sows. Milk was only successfully collected from 27 sows in week 1. For batch 2, milk was successfully collected at 2 weeks after farrowing (n = 17 sows).

Table 6
Effect of CP level in maternal and nursery diets on apparent total tract digestibility (ATTD) of CP of sows and their piglets.

Sow feed:			Higher protein (H) Mean		Lower protein (L) Mean				P_{sow}
$ATTD_{CP}$ SOWS									
1 week before farrowing			79.3		74.9				<0.001
Piglet feed:	Higher protein(HH) (n = 9) Mean	Lower protein(HL) (n = 9) Mean	Higher protein(LH) (n = 8) Mean	Lower protein(LL) (n = 9) Mean	SEM	P_{sow}	P_{piglet}	$P_{sow*piglet}$	
$ATTD_{CP}$ PIGLETS									
8 weeks	86.3^{a}	83.9^{b}	85.1^{ab}	85.3^{ab}	0.28	**0.03**	0.78	**<0.01**	
14 weeks	79.7	78.6	79.0	78.1	0.40	0.38	0.10	NS	
19 weeks	77.8	77.2	77.4	77.5	0.33	0.89	0.61	NS	
24 weeks	79.4	78.2	77.9	78.3	0.35	0.25	0.42	NS	

[a,b]Values with no superscript in common are significantly different according to Tukey's post hoc test; NS = not significant.

and piglet diets. In the nursery phase (3.5–9 weeks of age), lower fecal consistency scores were found in piglets from sows receiving the higher protein diet at d10 and d14 after weaning (P < 0.01). In addition, lower fecal consistency scores were found on d7 in the piglets fed a lower protein diet (P < 0.01, Fig. 1). Fecal consistency scores increased in H sow diets compared to the L sow diet on d10 (P_s = 0.03) and d14 (P_s < 0.01).

E. coli shedding from sows was not significantly different between treatment groups, but did show a difference over time (P < 0.001, Fig. 2A). *E. coli* shedding by piglets fed the H diet tended

Table 7
Effect of CP in maternal and nursery diets on serum urea concentrations (mg/dL) in sows and her piglets.

Sow treatment[1]	Higher protein (H)		Lower protein (L)				
	Mean	SE	Mean	SE			P_{sow}
Sows (6 weeks before farrowing)	21.36	0.27	21.34	1.27			0.90
Sows (1 week before farrowing)	24.59	1.27	15.73	0.69			**<0.01**
Piglets (at weaning)[2]	6.40	0.64	9.11	1.27			**0.03**

Piglet treatment[1]:	Higher protein(HH) Mean	Lower protein(HL) Mean	Higher protein(LH) Mean	Lower protein (LL)[2] Mean	SEM	P_{sow}	P_{piglet}	$P_{sow*piglet}$
4.5 weeks (nursery treatment)	23.91^{Ax}	13.84^{Ay}	20.24^{Bx}	8.84^{By}	1.45	**0.002**	**<0.001**	NS
9 weeks (nursery treatment)	21.30^{B}	5.68^{A}	23.95^{B}	4.58^{A}	1.72	0.57	**<0.001**	NS
Week 13 (phase 1)	8.38	7.52	9.75	7.42	0.44	0.62	0.22	NS
Week 18 (phase 2)	8.86	10.46	9.06	9.75	0.45	0.85	0.38	NS
Week 23 (phase 3)	10.37	12.91	12.92	10.66	0.68	0.91	0.92	0.07

^{A,B}Mean values sharing no common letter indicate a sow effect; $^{x,\ y}$Mean values sharing no common letter indicate a piglet effect; NS = not significant.
[1] For sow treatment, the individual sows were the experimental units, thereof n_{total} = 35, n_H = 18 and n_L = 17. For piglets at weaning (3.5 weeks of age), n = 24 (12 per sow treatment). At 4.5 and 9 weeks, eight samples of serum per treatment were available. For piglet treatment at 13, 18 and 23 weeks of age, the pen was the experimental unit; and n_{total} = 35, n_{HH} = 9, n_{HL} = 9, n_{LH} = 8 and n_{LL} = 9.
[2] Weaning is at 3.5 weeks of age.

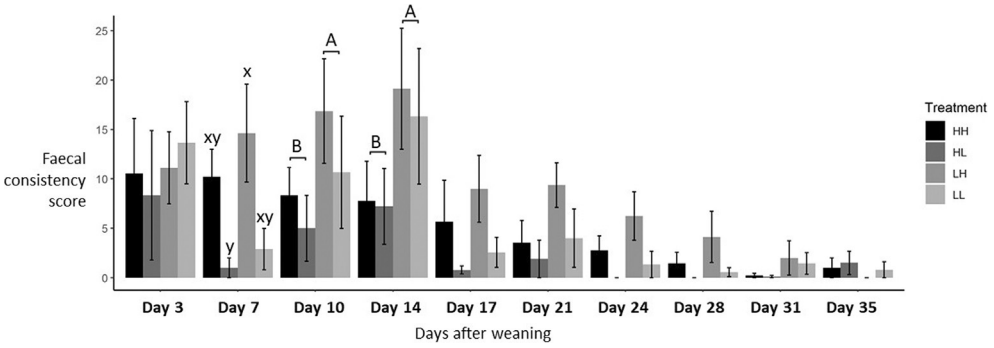

Fig. 1. Effect of divergent CP in maternal and nursery diets on fecal consistency of piglets during the nursery phase (n = 35, pen). Bar chart with ± SE whiskers. The significant differences between treatments (P < 0.05) are indicated with different letters within the same measurement day (AB for sow treatment, xy for piglet treatment). The original scale is from 0 (no diarrhea) to 100 (watery diarrhea). HH = High sow, High piglet dietary CP, HL = High sow, Low piglet dietary CP, LH = Low sow, High piglet dietary CP, LL = Low sow, Low piglet dietary CP.

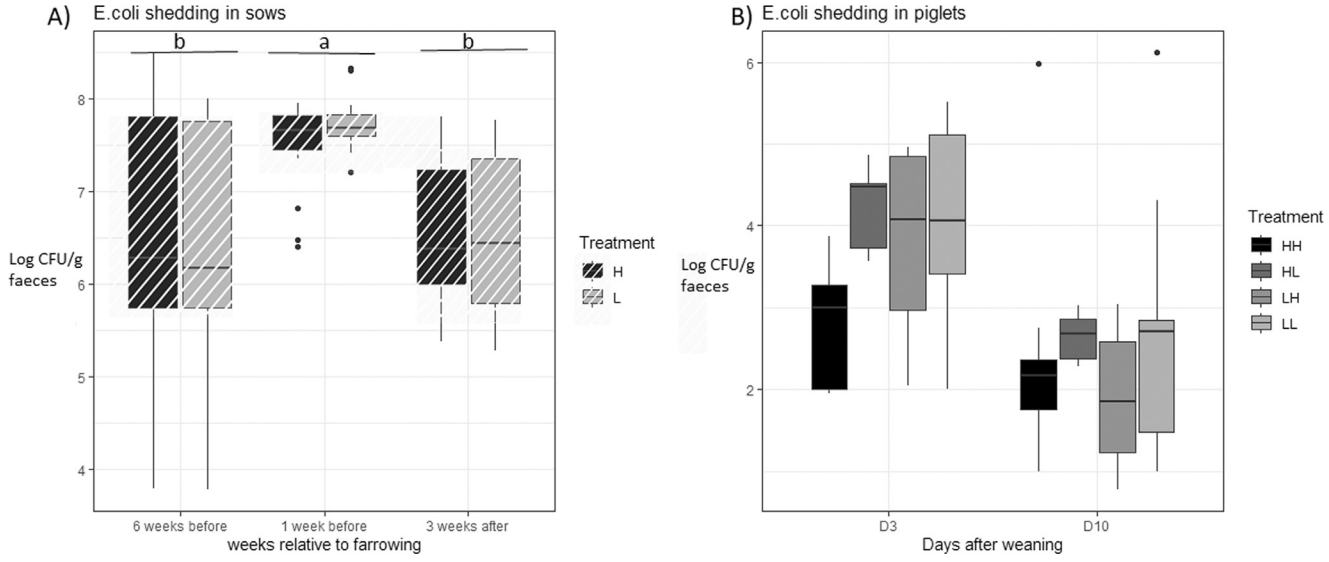

Fig. 2. Effect of divergent CP in maternal and nursery diets on *E. coli* shedding in sows (left) and piglets (right) feces expressed as log colony-forming unit (CFU)/g feces. A) Sow (n = 35) feces were collected at two time points during gestation (before and at the end of treatment) and at week 3 of the lactation phase. The error bars indicate SEM and the line within the box the median. No significant differences were observed between treatments, but time had a statistically significant effect between days where d78 of gestation and d21 after farrowing were compared to 1 week before and 1 day in the farrowing crates (P < 0.001). H = high sow dietary CP, L = low sow dietary CP. B) Piglets feces collected during the nursery phase at 3 days after weaning (d3, n = 26) and 10 days after weaning (d10, n = 32). Piglets receiving the H diet showed a tendency to lower *E. coli* shedding on day 3 (P_p = 0.067). HH = High sow, High piglet dietary CP, HL = High sow, Low piglet dietary CP, LH = Low sow, High piglet dietary CP, LL = Low sow, Low piglet dietary CP.

to be lower at d3 after weaning (P_p = 0.067, Fig. 2B). From d3 to d10, a decrease was observed in average *E. coli* shedding of piglets ($P < 0.001$).

Discussion

It is increasingly recognized that periparturient events may have long-term effects on offspring (Kennedy et al., 2019; Shang et al., 2019; Seoane et al., 2020). To our knowledge, few studies have been performed on possible interactions between the late-gestation diet and the nursery diet with the performance and health status of piglets until slaughter. In the present study, we hypothesized that the response of piglets to a low or high CP nursery diet may be affected by the maternal diet received during late gestation.

Differences between groups were generally small, but a few parameters supported the maternal programming hypothesis. One such parameter was feed efficiency (G:F), with the highest efficiency for HH animals and the lowest for HL animals. Similarly, the HH piglets showed higher $ATTP_{CP}$ values than the HL piglets, while no difference was observed between LL and LH. Thus, when sows were fed a higher protein and their piglets a lower protein diet (mismatched), these piglets showed a decrease in the $ATTD_{CP}$ compared to the piglets fed a higher protein diet, supporting our initial hypothesis. This measured apparent digestibility is the result of digestion and absorption processes occurring in the small intestine, but an effect of the fermentation processes occurring in the large intestine cannot be excluded.

The other mismatch group (LH) did not show different $ATTD_{CP}$ compared to LL. In addition, the LH group had the highest feed intake between 9 and 24 weeks, but this was not reflected in either increased DG or G:F and was not visible at other time intervals. Several studies on the topic of prenatal protein and offspring mismatching have been documented, but mainly in rats with a shortage of dietary amino acids. Brenseke et al. (2013) reviewed many factors that enable maternal programming as well as 'environmental factors' (Brenseke et al., 2013). These 'environmental factors' in pigs include the gut and feed, thus dietary CP could support piglet programming. This was also observed in chickens: Lower CP diets fed to breeders during laying could improve the feed conversion ratio of the F1, but also had an indirect influence on the N efficiency in the F2 animals (Lesuisse et al., 2018). The programming was reflected in the serum urea levels at 23 weeks of age, which tended to be lower in the matched compared to the corresponding mismatched group (LL vs LH, and HH vs HL), but this must be interpreted with caution. The exact reason for the decreased performance of mismatched groups is unclear and the experiment should be repeated to confirm observed effects.

The sow diet had direct effects on certain sow parameters, which then in turn could affect the piglets. The $ATTD_{CP}$ in sows 1 week before farrowing was higher for the H diet compared to the L diet. The difference between $ATTD_{CP}$ was unexpected as the SID of amino acid had a similar formulation between the sow diets. Fermentation processes in the large intestines according to available nutrients may explain the difference. In addition, H sows showed higher serum urea concentrations while they did not have any difference in performance results; this indicates an excess amount of protein (Brown and Cline, 1974).

At slaughter, a tendency for a sow-diet effect was noted for the dressing percentage and meat thickness, i.e., higher values for offspring of H fed sows. Similarly, Maresca et al. (2019) observed a greater dressing percentage in the offspring of Angus cows when the mothers were fed high levels of CP during late gestation (Maresca et al., 2019).

Interestingly, H sows had lower colostrum IgG levels, which is the most abundant immunoglobulin in colostrum and milk (Hurley and Theil, 2013), while protein concentration was unaffected. Protein levels in colostrum and milk are generally assumed to be stable and independent of protein intake (Dourmad et al., 1998; Mahan, 1998). Conversely, fat content in colostrum and milk is considered the most variable fraction. We observed a higher fat content in milk of H sows at week 1 and week 2. During the suckling phase, the higher protein maternal diet resulted in significantly lower serum urea levels in piglets, while the opposite was found 1 week after weaning. Although we cannot readily explain this, it does suggest that the maternal diet influenced the nitrogen metabolism of the progeny. In the nursery phase, offspring of the H sows had higher serum urea levels compared to offspring of the L sows, independent of the diet they were given. In addition, these piglets had lower fecal consistency scores on d10 and d14 after weaning, while sow diet did not affect *E. coli* counts.

Piglets fed a lower protein nursery diet showed slightly lower (better) fecal consistency scores in comparison to piglets fed a nursery diet richer in protein; this is in agreement with other studies (Nyachoti et al., 2006; Heo et al., 2009; Opapeju et al., 2009). Despite the statistical differences observed in fecal consistency, the clinical relevance is probably low, as most piglets on both diets showed relatively firm feces. The hygiene status on the experimental farm was probably better than that of most commercial farms with less strict biosecurity protocols, where postweaning diarrhea is more frequently reported. Previous studies with enterotoxigenic *E. coli* challenges observed good fecal consistency for piglets fed lower protein diets (Opapeju et al., 2009; Bhandari et al., 2010; Pollock et al., 2019). Similarly, Heo et al. (2008) found a decreasing incidence of postweaning diarrhea at 8 days after weaning in piglets fed lower protein diets without a challenge (Heo et al., 2008). We found a tendency toward higher *E. coli* counts in feces on d3 after weaning for piglets receiving the lower protein diet, irrespective of the maternal treatment. Overall, the abundance of *E. coli* present per gram feces was low, but was nonetheless higher at 3 vs 10 days after weaning. At 3 days post weaning, piglets had not yet eaten large portions of their diet, thus the diet may not yet have influenced microbial composition and excretion (Metz and Gonyou, 1990).

The rather limited effects of the nursery diet may be explained by the similar amino acid profiles in all piglet diets. It is well known that balanced amino acid profiles are more important for performance than the CP levels (Yue and Qiao, 2008; Millet et al., 2018; Jansman et al., 2019). Millet et al. (2018) determined the breaking point of the limiting amino acid lysine at a SID Lys to CP ratio of 0.064. In the present trial, a ratio of SID LYS:CP of 0.060 (higher protein piglet diet) and 0.064 (lower protein piglet diet) was used for the nursing diets. In the present experiment, only average piglets were used, thus it cannot be excluded that results would have differed when including the smallest or the largest piglets.

Conclusion

This study showed that the protein level in sow late-gestation and piglet nursery diets interacted with feed efficiency, $ATTD_{CP}$ and urea levels in the nursery phase. Independent of the pig diet, the maternal dietary protein level in late gestation affected serum urea level, fecal consistency scores and performance of pigs up until slaughter.

Ethics approval

The research and pig-rearing were carried out in accordance with the local ethics committee guidelines on animal testing and

care of animals. All procedures were done according to the recommendations of animal rearing and their welfare (Belgium).

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available upon request.

Authors ORCIDs

Sam Millet: https://orcid.org/0000-0003-2311-9381;
Kikianne Kroeske: https://orcid.org/0000-0002-6675-2913;
Nadia Everaert: https://orcid.org/0000-0001-9282-6288;
Martine Schroyen: https://orcid.org/0000-0003-3855-8141;
Marc Heyndrickx: https://orcid.org/0000-0002-2913-2686;
Ester Arévalo Sureda: https://orcid.org/0000-0002-3299-1510.

Author contributions

K. Kroeske performed the experiments, analyzed the data and wrote the manuscript as part of her PhD doctoral research. N. Everaert, M. Heyndrickx, M. Schroyen, and S. Millet designed the experiment. E.A. Sureda helped with ELISA measurements. All authors provided critical feedback on the manuscript and helped out during the experiments.

Declaration of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

The authors acknowledge B. Ampe, M. Aluwé, E. Beeckman, B. De Bock, C. De Cuyper, S. De Smet, L. De Wilde, B. De Witte, S. Decubber, J. Devos, K. Dierkens, L. Douidah, A. Van Havermaet, E. Kowalski, A. Lechevestrier, R. Limpens, L. Paternostre, J. Uerlings, H. Uitterhaegen, A. Van Den Broeke, E. Van Rymenant, Ann Van De Walle and R. Vanderhaute for their much appreciated assistance and support throughout the experiment.

Financial support statement

This study was supported and funded by Flanders Research Institute for Agriculture, Fisheries and Food (ILVO) and University of Liège – Gembloux Agro-Bio Tech (Precision Livestock and Nutrition Unit).

References

Altmann, S., Murani, E., Metges, C.C., Schwerin, M., Wimmers, K., Ponsuksili, S., 2012a. Effect of gestational protein deficiency and excess on hepatic expression of genes related to cell cycle and proliferation in offspring from late gestation to finishing phase in pig. Molecular Biology Reports 39, 7095–7104.

Altmann, S., Murani, E., Schwerin, M., Metges, C.C., Wimmers, K., Ponsuksili, S., 2012b. Maternal dietary protein restriction and excess affects offspring gene expression and methylation of non-SMC subunits of condensin I in liver and skeletal muscle. Epigenetics 7, 239–252.

Amezcua, R., Friendship, R.M., Dewey, C.E., Gyles, C., Fairbrother, J.M., 2002. Presentation of postweaning Escherichia coli diarrhea in southern Ontario, prevalence of hemolytic *E. coli* serogroups involved, and their antimicrobial resistance patterns. Canadian Journal of Veterinary Research 66, 73–78.

Ball, R., Aherne, F., 1987. Influence of dietary nutrient density, level of feed intake and weaning age on young pigs. II. Apparent nutrient digestibility and incidence and severity of diarrhea. Canadian Journal of Animal Science 67, 1105–1115.

Bhandari, S., Opapeju, F., Krause, D., Nyachoti, C., 2010. Dietary protein level and probiotic supplementation effects on piglet response to Escherichia coli K88 challenge: Performance and gut microbial population. Livestock Science 133, 185–188.

Brenseke, B., Prater, M.R., Bahamonde, J., Gutierrez, J.C., 2013. Current Thoughts on Maternal Nutrition and Fetal Programming of the Metabolic Syndrome. Journal of Pregnancy 2013, 1–13.

CVB 2016. Feed Table for pigs. Voedernormen varkens en voederwaarden voedermiddelen voor varkens. Federatie Nederlandse Diervoederketen and Wageningen Livestock Research, Rijswijk and Wageningen, the Netherlands.

Brown, J.A., Cline, T.R., 1974. Urea Excretion in the Pig: an Indicator of Protein Quality and Amino Acid Requirements. The Journal of Nutrition 104 (5), 542–545. https://doi.org/10.1093/jn/104.5.542.

Dourmad, J., Noblet, J., Etienne, M., 1998. Effect of protein and lysine supply on performance, nitrogen balance, and body composition changes of sows during lactation. Journal of Animal Science 76, 542–550.

European Commission (EC), 1971. Determination of moisture. Commission Directive 71/393/EEC establishing Community methods of analysis for the official control of feedingstuffs. Official Journal of the European Communities L279, 7–18.

European Commission (EC), 1998. Establishing Community methods of analysis for the determination of amino-acids, crude oils and fats, and olaquindox in feedingstuffs and amending Directive 71/393/EEC. Commission Directive 98/64/EC. Official Journal of the European Communities L257, 14–23.

European Commission (EC), 2000. Establishing Community methods of analysis for determination of vitamin A, vitamin E and tryptophan in feeding stuffs. Commission Directive 2000/45/EC. Official Journal of the European Communities L174, 32–50.

European Union (EU), 2012. Commission Implementing Decision of 19 July 2012 authorising methods for grading pig carcasses in Belgium (notified under document C(2012) 4933). Commission Directive 2012/416/EU. Official Journal of the European Union L194, 33–38

Fairbrother, J.M., Nadeau, E., Gyles, C.L., 2005. Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Animal Health Research Reviews 6, 17–39.

Gluckman, P.D., Hanson, M.A., 2004. Developmental origins of disease paradigm: a mechanistic and evolutionary perspective. Pediatric Research 56, 311–317.

Heo, J.M., Kim, J.C., Hansen, C.F., Mullan, B.P., Hampson, D.J., Pluske, J.R., 2008. Effects of feeding low protein diets to piglets on plasma urea nitrogen, faecal ammonia nitrogen, the incidence of diarrhoea and performance after weaning. Archives of Animal Nutrition 62, 343–358.

Heo, J.M., Kim, J.C., Hansen, C.F., Mullan, B.P., Hampson, D.J., Pluske, J.R., 2009. Feeding a diet with decreased protein content reduces indices of protein fermentation and the incidence of postweaning diarrhea in weaned pigs challenged with an enterotoxigenic strain of Escherichia coli. Journal of Animal Science 87, 2833–2843.

Hines, E.A., Romoser, M.R., Kiefer, Z.E., Keating, A.F., Baumgard, L.H., Niemi, J., Haberl, B., Williams, N.H., Kerr, B.J., Touchette, K.J., Ross, J.W., 2019. The impact of dietary supplementation of arginine during gestation in a commercial swine herd: II. Offspring performance. Journal of Animal Science 97, 3626–3635.

Hurley, W.L., Theil, P.K., 2013. Immunoglobulins in mammary secretions. In: McSweeney, P.L.H., Fox, P.F. (Eds.), Advanced Dairy Chemistry: Volume 1A: Proteins: Basic Aspects. 4th ed. Springer, US, Boston, MA, USA, pp. 275–294.

Jansman, A., Cirot, O., Corrent, E., Lambert, W., Ensink, J., van Diepen, J.T.M., 2019. Interaction and imbalance between indispensable amino acids in young piglets. Animal 13, 941–949.

Kaske, M., Wiedemann, S., Kunz, H., 2010. Metabolic programming: background and potential impact for dairy cattle. Vlaams Diergeneeskundig Tijdschrift 79, 445–451.

Kennedy, V.C., Gaspers, J.J., Mordhorst, B.R., Stokka, G.L., Swanson, K.C., Bauer, M.L., Vonnahme, K.A., 2019. Late gestation supplementation of corn dried distiller's grains plus solubles to beef cows fed a low-quality forage: III. effects on mammary gland blood flow, colostrum and milk production, and calf body weights. Journal of Animal Science 97, 3337–3347.

Kerr, B.J., Southern, L.L., Bidner, T.D., Friesen, K.G., Easter, R.A., 2003. Influence of dietary protein level, amino acid supplementation, and dietary energy levels on growing-finishing pig performance and carcass composition. Journal of Animal Science 81, 3075–3087.

Kong, C., Adeola, O., 2014. Evaluation of amino Acid and energy utilization in feedstuff for Swine and poultry diets. Asian-Australasian Journal of Animal Sciences 27, 917–925.

Leblois, J., Massart, S., Li, B., Wavreille, J., Bindelle, J., Everaert, N., 2017. Modulation of piglets' microbiota: differential effects by a high wheat bran maternal diet during gestation and lactation. Scientific Reports 7, e7426.

Leblois, Julie, Massart, Sébastien, Soyeurt, Hélène, Grelet, Clément, Dehareng, Frédéric, Schroyen, Martine, Li, Bing, Wavreille, José, Bindelle, Jérôme, Everaert, Nadia, 2018. Feeding sows resistant starch during gestation and lactation impacts their faecal microbiota and milk composition but shows limited effects on their progeny. Plos one 13 (7), e0199568. https://doi.org/10.1371/journal.pone.0199568.

Lesuisse, J., Li, C., Schallier, S., Leblois, J., Everaert, N., Buyse, J., 2017. Feeding broiler breeders a reduced balanced protein diet during the rearing and laying period impairs reproductive performance but enhances broiler offspring performance. Poultry Science 96, 3949–3959.

Lesuisse, J., Schallier, S., Li, C., Bautil, A., Li, B., Leblois, J., Buyse, J., Everaert, N., 2018. Multigenerational effects of a reduced balanced protein diet during the rearing and laying period of broiler breeders. 2. Zootechnical performance of the F1 broiler offspring. Poultry Science 97, 1666–1676.

Luo, W., Xu, X., Luo, Z., Yao, J., Zhang, J., Xu, W., Xu, J., 2019. Effect of fish oil supplementation in sow diet during late gestation and lactation period on litter

characteristics, milk composition and fatty acid profile of sows and their offspring. Italian Journal of Animal Science 19, 8–17.
Ma, N., Tian, Y., Wu, Y., Ma, X., 2017. Contributions of the Interaction Between Dietary Protein and Gut Microbiota to Intestinal Health. Current Protein & Peptide Science 18, 795–808.
Mahan, D., 1998. Relationship of gestation protein and feed intake level over a five-parity period using a high-producing sow genotype. Journal of Animal Science 76, 533–541.
Maresca, S., Valiente, S.L., Rodriguez, A.M., Testa, L.M., Long, N.M., Quintans, G.I., Pavan, E., 2019. The influence of protein restriction during mid- to late gestation on beef offspring growth, carcass characteristic and meat quality. Meat Science 153, 103–108.
McCarthy, M., Molloy, N., 1974. Review of stability of liquid jets and the influence of nozzle design. The Chemical Engineering Journal 7, 1–20.
McCormack, H.M., Horne, D.J., Sheather, S., 1988. Clinical applications of visual analogue scales: a critical review. Psychological Medicine 18, 1007–1019.
Metz, J., Gonyou, H., 1990. Effect of age and housing conditions on the behavioural and haemolytic reaction of piglets to weaning. Applied Animal Behaviour Science 27, 299–309.
Millet, S., Aluwe, M., De Boever, J., De Witte, B., Douidah, L., Van den Broeke, A., Leen, F., De Cuyper, C., Ampe, B., De Campeneere, S., 2018. The effect of crude protein reduction on performance and nitrogen metabolism in piglets (four to nine weeks of age) fed two dietary lysine levels1. Journal of Animal Science 96, 3824–3836.
National Research Council (NRC), 2012. Nutrient Requirements of Swine. National Academies Press, Washington, DC, USA.
Nyachoti, C.M., Omogbenigun, F.O., Rademacher, M., Blank, G., 2006. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. Journal of Animal Science 84, 125–134.
Opapeju, F.O., Krause, D.O., Payne, R.L., Rademacher, M., Nyachoti, C.M., 2009. Effect of dietary protein level on growth performance, indicators of enteric health, and gastrointestinal microbial ecology of weaned pigs induced with postweaning colibacillosis. Journal of Animal Science 87, 2635–2643.
Pollock, J., Hutchings, M.R., Hutchings, K.E.K., Gally, D.L., Houdijk, J.G.M., 2019. Changes in the Ileal, but Not Fecal, Microbiome in Response to Increased Dietary Protein Level and Enterotoxigenic Escherichia coli Exposure in Pigs. Applied and Environmental Microbiology 85, 1–12.
Rooney, H.B., O'Driscoll, K., O'Doherty, J.V., Lawlor, P.G., 2020. Effect of increasing dietary energy density during late gestation and lactation on sow performance, piglet vitality, and lifetime growth of offspring. Journal of Animal Science 98, 1–14.
Seoane, S., De Palo, P., Lorenzo, J.M., Maggiolino, A., Gonzalez, P., Perez-Ciria, L., Latorre, M.A., 2020. Effect of increasing dietary amino acid concentration in late gestation on body condition and reproductive performance of hyperprolific sows. Animals 10, 99–108.
Shang, Q., Liu, H., Liu, S., He, T., Piao, X., 2019. Effects of dietary fiber sources during late gestation and lactation on sow performance, milk quality, and intestinal health in piglets. Journal of Animal Science 97, 4922–4933.
Susenbeth, A., Keitel, K., 1988. Partition of whole body protein in different body fractions and some constants in body composition in pigs. Livestock Production Science 20, 37–52.
van der Waaij, E.H., van den Brand, H., van Arendonk, J.A., Kemp, B., 2011. Effect of match or mismatch of maternal-offspring nutritional environment on the development of offspring in broiler chickens. Animal 5, 741–748.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583–3597.
Wan, K., Li, Y., Sun, W., An, R., Tang, Z., Wu, L., Chen, H., Sun, Z., 2020. Effects of dietary calcium pyruvate on gastrointestinal tract development, intestinal health and growth performance of newly weaned piglets fed low-protein diets. Journal of Applied Microbiology 128, 355–365.
Yue, L., Qiao, S., 2008. Effects of low-protein diets supplemented with crystalline amino acids on performance and intestinal development in piglets over the first 2 weeks after weaning. Livestock Science 115, 144–152.