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1 ORIGINAL ARTICLE

2 **Nutritional strategies to cope with reduced litter weight gain and** 3 **total tract digestibility in lactating sows**

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6 **Summary**

7 Twelve lactating sows were used to evaluate the effects of reducing dietary crude protein (CP) (14% vs. 12%)
8 and increasing neutral detergent fibre (NDF) levels (18% vs. 22%) on litter performance, total tract apparent
9 digestibility and manure composition in a 4 × 4 latin square arrangement during a 36-day lactation period. Diets
10 were isoenergetic (2.9 Mcal ME/kg) and had similar total lysine content (0.9%). In addition, a second aim was
11 to compare a reference external marker method (Cr₂O₃) with an internal feed marker [acid-insoluble ash (AIA)]
12 for the calculation of apparent total tract digestibility of nutrients in lactating sows. The reduction of dietary CP
13 level in lactating sows had no effect on either live-weight or backfat thickness or apparent total tract digestibility
14 of nutrients. However, the piglets' average daily gain (ADG) was reduced in low dietary CP diets, which suggests
15 that sows reduced milk production due to an underestimation of certain essential amino acid requirements (e.g.
16 valine). The increase of dietary NDF level did not affect sow and litter performance. Nevertheless, the total tract
17 apparent digestibility of organic matter, CP and carbohydrates was reduced, and ether extract digestion was
18 increased in high NDF compared to normal NDF diets equally balanced for ME and lysine content. The coeffi-
19 cients of total tract apparent digestibility of nutrients in lactating sows were greater when using AIA compared to
20 Cr₂O₃ marker, regardless of dietary CP or NDF level, but their coefficients of variation were lower in the former
21 than in the latter. In lactating sows, a trade-off between litter performance and nutrient digestion is established
22 when reducing dietary CP or increasing NDF levels while maintaining similar lysine content through synthetic
23 amino acids and balancing metabolizable energy through dietary fat sources.

24 **Keywords** swine, feed formulation, nutrient requirements, nutrient excretion, faecal digestibility, indigestible markers

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28 **Introduction**

29 In some European countries, for example Spain, the
30 sow diets are normally lower in energy than in other
31 world areas because high-fibre (and lower energy)
32 ingredients are more economical relative to maize
33 than in USA (Tokach and Golçalves, 2015). Therefore,
34 ingredients with higher fibre content, such as sugar-
35 beet pulp, wheat bran or sunflower meal, are often
36 used at low levels in the lactation diet, which is nor-
37 mally supplied on an *ad libitum* pattern to meet nutri-
38 ent requirements.

39 In addition, the current environmental concern
40 leads to avoid nitrogen excretion, which can be
41 achieved by increasing the quality of dietary protein
42 (e.g. adding synthetic amino acids) to reduce feed
43 crude protein (CP), together with an increase of diet-
44 ary neutral detergent fibre (NDF) (as a measure of

45 fermentable fibre). This combination may reduce the
46 digesta transit time and shifts the nitrogen excretion
47 balance from urine to faeces (Morgan and Whitte-
48 more, 1988), although its use has been mostly studied
49 in growing-finishing pigs (Zervas and Zijlstra, 2002;
50 Morazán et al., 2015). Results obtained in growing
51 pigs cannot be fully extrapolated to lactating sows
52 because adult intestine has a greater digestion capacity
53 (Noblet and Shi, 1993). Moreover, digestibility data
54 from dry sows have scarce value to apply to nursing
55 sows, as feed composition and digestive traits (e.g.
56 intake level) differ from non-lactating animals.

57 This experiment hypothesized that the reduction of
58 dietary CP and increase of NDF would allow improved
59 apparent digestibility coefficients of nutrients without
60 detrimental effects on productive performance of lean
61 lactating sows. In addition, a secondary aim was to
62 compare a reference external marker method (Cr₂O₃)

with an internal feed marker [acid-insoluble ash (AIA)] for the calculation of apparent total tract digestibility of nutrients in lactating sows.

Materials and methods

Animals, diets and experimental design

This experiment was conducted in the research facilities of the *Centre d'Estudis Porcins* (Torrelameu, Lleida, North-eastern Spain, 41°42'24"N, 0°42'11"E; 201 m above sea level) between June 2012 and January 2013. All procedures were carried out under Project Licence CEEA 03/01-10 and approved by the in-house Ethics Committee for Animal Experiments at the University of Lleida. The care and use of animals were in accordance with the Spanish Policy for Animal Protection RD53/2013, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

One week before farrowing, twelve pregnant sows (Large White × Landrace) equally managed during gestation were allocated in individual farrowing crates (2 × 2.1 m²) in a controlled environment room equipped with a forced ventilation system which drove an airflow (78.4 ± 4.4 m³/h) that allowed maintaining mean daily temperature (21.1 ± 1.8 °C) and mean relative humidity (59.6 ± 5.1%).

The experiment was designed as a repeated 4 × 4 Latin square arrangement, with 12 sows fed four different diets during four periods per block. Immediately after farrowing [nine primiparous (live-weight 209 ± 11 kg) and three multiparous sows (live-weight 285 ± 7 kg)], litters were standardized (10.2 ± 1.0 piglets) and dams were randomly assigned to 1 of 4 diets that were formulated to provide normal crude protein and normal NDF (14% CP and 18% NDF), normal crude protein and high NDF (14% CP and 22% NDF), low crude protein and normal NDF (12% CP and 18% NDF) or low crude protein and high NDF (12% CP and 22% NDF) (Table 1).

The diets were formulated to be isoenergetic [2.90 Mcal of metabolizable energy (ME)/kg of feed], and they were supplemented with synthetic amino acids to achieve an ideal amino acid ratio for lysine, methionine, threonine and tryptophan, on the basis that Lys was the first-limiting amino acid. In addition, diets were fortified to meet vitamin and mineral requirements (NRC (National Research Council), 2012) and they were added phytase to improve phosphorus digestibility.

The diets (milled-ground through a 6-mm screen, which yielded 1- to 2-mm-sized feed meal) mainly comprise cereals (maize and wheat), alfalfa meal and

Table 1 Feedstuff and nutrient composition of the experimental diets differing in crude protein (CP) content (low, LP vs. normal, NP) and neutral detergent fibre (NDF) content (normal, NF vs. high, HF)

Feedstuff, %	LP		NP	
	NF	HF	NF	HF
Maize	50.2	41.6	40.4	30
Wheat (CP, 112 g/kg)	20	9.7	20	14.3
Gluten feed (CP, 210 g/kg)	4.4	3.2	10	10
Soybean meal (CP, 470 g/kg)	5	–	9.2	2.1
Sunflower meal (CP, 360 g/kg)	1.3	15	1.5	15
Alfalfa meal (CP, 152 g/kg)	14.8	11.2	14.8	10.8
Sugar-beet pulp	–	8	–	8
Cereal straw	–	3	–	3
Sunflower oil	–	4.7	–	3.4
Calcium carbonate	1.4	1	1.4	1
Sodium chloride	0.5	0.5	0.5	0.5
L-Lysine	0.57	0.52	0.44	0.4
L-Threonine	0.13	0.06	0.03	–
L-Tryptophan	0.04	0.01	0.03	–
Bicalcium phosphate dihydrate	1.4	1.2	1.4	1.2
Vitamin–mineral premix*	0.3	0.3	0.3	0.3
Calculated composition†				
ME, Mcal/kg	2.90	2.90	2.90	2.90
NE, Mcal/kg	2.13	2.21	2.14	2.26
Lys, %	0.90	0.86	0.94	0.86
SID Lys, %	0.71	0.79	0.81	0.73
SID Lys/ME, g/Mcal	2.45	2.72	2.79	2.52
Met, %	0.21	0.26	0.24	0.28
Met+Cys, %	0.42	0.48	0.50	0.55
Thr, %	0.56	0.53	0.56	0.54
Trp, %	0.17	0.15	0.19	0.17
Val, %	0.54	0.62	0.64	0.70
Analysed composition				
DM, %	88	89.5	89	89.7
NDF, %	17.5	22.2	18.7	23
CP, %	11.8	11.5	14.1	13.7
Total Lys, %	0.90	1.11	1.00	0.94
Starch, %	43.7	34.1	37.8	33.8
Ash, %	7.5	7.1	7.6	7.5
Ether extract, %	2.3	6.9	2.3	5.4
Phosphorus, %	0.69	0.65	0.73	0.79
Potassium, %	0.53	0.59	0.71	0.69

*Provided by kilogram of completed diet: 12 000 IU of vitamin A, 1600 IU of vitamin D3, 37.5 mg of vitamin E (dl-alpha-tocopheryl acetate), 2 mg of vitamin K3; 0.2 mg of biotin, 172 mg of choline (choline chloride), 2.5 mg of folic acid, 21.3 mg of niacin, 11.5 mg of pantothenic acid, 3.9 mg vitamin B2, 1.2 mg of vitamin B1, 2.7 mg of vitamin B6, 0.02 mg of vitamin B12; 0.9 mg of sepiolite, 0.1 mg of ethoxyquin, 0.02 mg of propyl gallate; 250 phytase units (FTU/kg) (EC 3. 1. 3. 26); 0.05 mg of cobalt (cobalt carbonate monohydrate), 10 mg of copper sulphate, 0.7 mg of iodine (anhydrous calcium iodate), 90 mg of iron (ferrous carbonate), 39.3 mg of manganese oxide, 0.2 mg of sodium selenite, 105.5 mg of zinc oxide.

†Based on FEDNA (2010).

gluten feed as a source of CP, and/or oilseed meals, sugar-beet pulp and cereal straw as a source of NDF. The composition (based on FEDNA 2010 composition

tables) and analysed nutrient value (AOAC International 2000) of the diets are shown in Table 1. Chromic oxide (in feed incorporated at a dose of 2 g/kg of feed) and AIA were used as external and internal indigestible digestibility markers respectively.

Each experiment consisted of four phases (9 ± 1 day each) and lasted 36 ± 2 days. Every phase included an adaptation period of 6 days and an excreta collection period of 3 days. At the first day of diet adaptation, the sows received 3.5 kg of feed and it was gradually increased ($+0.5$ kg/day) until a maximum of 7 kg or 9 kg in primiparous and multiparous sows respectively. The sows were fed three times a day (09:00, 13:00 and 18:00 h) in mash form (2:1, water-to-feed ratio). Fresh water (pH = 8.15, electric conductivity = $490 \mu\text{S}/\text{cm}$, sodium concentration = $20.0 \text{ mg}/\text{l}$; chloride concentration = $24.1 \text{ mg}/\text{l}$) was offered *ad libitum* through additional nipple drinkers.

The piglets were provided dextran iron supplement intramuscular (Dextrafer-200, Laboratorios Syva, León, Spain) and coccidiostatic (Tratol, Pfizer Salud Animal, Alcobendas, Spain) on the 5th day of age. During the period 3 and 4 of the experiment (from day 19 of age onwards), the litters were creep fed with a pre-starter diet (19.5% CP, 1.4% total lysine and 6.2% crude fat) at a fixed amount of 75 g/piglet and 200 g/piglet, during period 3 and 4 respectively.

Measurements

Feed and water use were recorded daily. Every morning between 08:00 and 09:00 h, feed refusals were weighed and removed and diets were supplied according to the amount refused the previous day. Feed intake was calculated daily as the difference between feed allowance and refusals collected on the next morning on a dry matter basis. The water intake of each farrowing crate was measured by water flow meters within the delivery lines and recorded every morning. Extreme values that deviated more than ± 4 SD from the mean of the measured data were excluded from the data sets.

A drawer was located beneath each lactation crate to allow the collection of slurry (faeces and urine). During the excreta collection (days 6 to 9 of each period), the excreta was collected once daily from the drawers below lactation crates and weighed to calculate the manure yield. In addition, the pH and electrical conductivity (EC) of slurry were measured *in situ* with a hand-held electrode (MM40; Crison Instruments S.A., Alella, Spain). Thereafter, 1 kg homogeneous samples were collected for subsequent

analysis of physical and chemical composition and stored at -20°C .

Faecal samples (approximately 50 g) were collected using rectal stimulation at 09:00 h the last 3 days of each dietary period. Faecal samples were stored at -20°C until chromium and proximate chemical analysis. After thawing, faecal samples from each sow were pooled to produce one grab sample per collection period.

The total tract apparent digestibility of nutrients was calculated using the nutrient-to-marker ratio in the diet and faeces, as follows:

$$\text{Apparent digestibility coefficient (\%)} = 100 \times \left[100 \times \left(\frac{\text{marker}_{\text{diet}}}{\text{marker}_{\text{faeces}}} \right) \times \left(\frac{Z_{\text{faeces}}}{Z_{\text{diet}}} \right) \right]$$

where Z_{faeces} and Z_{diet} are the nutrient concentrations (%) in the faeces and in the diet respectively; $\text{marker}_{\text{faeces}}$ and $\text{marker}_{\text{diet}}$ are the concentrations (%) of chromium oxide or AIA in the faeces and in the diet respectively.

Individual piglet live-weight was recorded within 24 h after birth. The sows and their litters were individually weighed at the beginning and at the end of each excreta collection period (days 6 and 9; days 15 and 18; days 24 and 27; and days 33 and 36 of lactation for phase I to IV respectively). Sows' backfat thickness was measured at the same time at 6.5 cm from the midline on both sides of the last rib (P2 site) by A-mode ultrasonography (Lean-meater, Renco Corporation, Minneapolis, MN, USA).

Milk samples of approximately 40 ml of four sows were manually extracted at days 26 (phase III) and 35 (phase IV) of lactation. After suckling on these days, the piglets were separated from the dam, and sows were injected with 10 IU oxytocin i.m. and 10 min later all functional glands were hand milked. The samples were stored at -20°C until composition analysis.

During the phases I and II, before creep feeding was offered, milk production was estimated from initial piglet live-weights (LW) and subsequent ADG at each phase according the equation developed by Noblet and Etienne (1989):

$$\text{Milk production (g/day)} = (2.50 \times \text{piglet ADG}) + (80.2 \times \text{initial piglet LW}) + 7$$

Chemical analyses

Feed and faecal dry matter (DM) (index no. 934.01), ash (index no. 942.05), acid hydrolysed ether extract (EE) (index no. 920.39) contents were determined according to the AOAC methods (AOAC, 2000). Neutral detergent fibre analyses were carried out

following the sequential procedure of Van Soest et al. (1991), with the Ankom200/220 fibre analyzer (Ankom Technology, 1998). The NDF assay was conducted with a heat stable amylase and expressed exclusive of residual ash (aNDFom). Feed samples were also analysed for total lysine (HPLC–fluorescence), crude fibre (Weende method), starch (polarimetry), phosphorus (ultraviolet–visible spectroscopy) and potassium (atomic absorption spectrometry). All the samples were analysed in duplicate.

The concentration of carbohydrates (CHO) in diets and faeces was calculated as follows (Urriola and Stein, 2012):

$$\text{CHO} = \text{DM} - (\text{CP} + \text{EE} + \text{Ash})$$

Feed and faecal samples were analysed for Cr concentration after nitro-perchloric acid (ratio 5:1) digestion (De Vega and Poppi, 1997) using inductively coupled plasma optical emission spectroscopy (HORIBA Jobin Yvon, Activa family, with AS-500 Autosampler; HORIBA Scientific, Madrid, Spain).

Acid-insoluble ashes were analysed according to a standard procedure (BOE, 1995) based on the method of Shrivastava and Talapatra (1962). Briefly, 5 g of ground samples of feed or excreta was hydrolysed in a beaker with 75 ml of 3 N HCl and 25 ml of distilled water and boiled for 30 min. The sample was then filtered through ash-free filter paper and washed the residue with 50 ml of hot distilled water. The filter with residue was put in a tared crucible, dried at 103 °C for 24 h and ashed at 550 °C for 3 h for crude ash determination. Afterwards, ashes were transferred again to a beaker with 75 ml of 3 N HCl and gently boiled for 15 min. The dilution was filtered again through an ash-free filter and washed with hot distilled water until disappearance of acid reaction. Finally, the filter with residue was dried and ashed on a tared crucible at 550 °C for 3 h. The crucible and its content were cooled in a desiccator to room temperature and weighed to calculate the AIA content.

The excreta samples were analysed for dry matter (forced air oven at 60° C), bulk density (densimetry), electrical conductivity (EC) (conductometry), pH (electrometry), ammonium-N (volumetric titration), phosphorus (ultraviolet-visible spectroscopy) and potassium (atomic absorption spectrometry), according to the standard methods for the examination of water and wastewater (APHA, 1995). Organic N content of manure was calculated as the difference between total N and ammonium-N content on DM basis.

The milk samples were allowed to thaw at room temperature and agitated to obtain a homogeneous

mixture. An aliquot of milk was freeze-dried for the analysis of gross energy (GE) using a bomb calorimeter (Gallenkamp auto bomb CBA-305-010M; Sussex, UK) and CP (total N × 6.25) according to AOAC (2000) (Table 2).

Statistical analysis

The data were analysed using the SAS statistical software (SAS Institute, Cary, NC, USA) by means of a repeated-measures mixed model (PROC MIXED):

$$Y_{ijklm} = \mu + \text{PHASE}_i + A_j + \text{CP}_k + \text{NDF}_l + \text{BATCH}_m + (\text{PHASE}_i \times \text{CP}_j) + (\text{PHASE}_i \times \text{NDF}_l) + (\text{CP}_k \times \text{NDF}_l) + E_{ijklmn}$$

where Y_{ijklm} = dependent variable, μ = overall mean, PHASE_i = phase of lactation effect ($i = 1, 2, 3, 4$), A_j = sow random effect j , CP_k = dietary CP level effect ($k = \text{LP, NP}$), NDF_l = dietary NDF level effect ($l = \text{NF, HF}$), BATCH_m = batch effect (1, 2, 3) and E_{ijklmn} = residual error.

The models analysing the sources of variation of the coefficients of apparent total tract digestibility of nutrients in the sows considered the marker (AIA, Cr_2O_3) and its second degree interactions with dietary CP and dietary NDF effects. In this case, the phase of lactation effect was omitted to simplify these models as it did not reduce the residual variance. The experimental unit for the parameters included in the study was the individual sow. Variances were unequal; therefore, calculations of the standard error (SE) and degrees of freedom were based on the Kenward–Roger method. Differences between least square means were assessed using the Tukey test. Values are presented as least square means ± SE. The level of significance was set at 0.05, but tendencies were commented if the level of significance was below 0.10. Second-degree interaction effects were retained in the models, and they are specifically commented in the text if they reached statistical significance.

Table 2 Milk composition in lactating sows at days 26 and 35 (phase 3 and 4 respectively) when fed diets differing in crude protein (CP) content (low, LP vs. normal, NP) and neutral detergent fibre (NDF) content (normal, NF vs. high, HF)

Item	CP		NDF	
	LP	NP	NF	HF
Gross energy, Mcal/kg of milk	1.24	1.22	1.22	1.24
CP, g/100 g of milk	5.25	5.54	5.41	5.38

Results

Sow and litter productive performance

The productive performance of sows is presented in Table 3. Feed intake was similar between CP dietary levels ($p > 0.05$) and between dietary NDF levels ($p > 0.05$). As expected, the lactation phase did affect sow consumption. Average daily feed intake was lower during the first lactation period than subsequently (4.8 vs. 6.5 ± 0.14 kg DM/day for lactation phase 1 vs. respectively).

The reduction of dietary CP and/or the increase of dietary NDF did not affect either water intake or the water-to-dry matter feed ratio disappearance (Table 3, $p > 0.05$). The proportion of sows LW and backfat thickness variation were not different among treatments ($p > 0.05$). However, the estimated milk production between sows fed low and normal CP dietary levels was significantly different ($p < 0.001$). Milk yield was reduced by decreasing CP dietary level.

There were no differences in the coefficient of variation (CV) of litter LW at the end of each phase, while the piglets' ADG was affected by the interaction between CP level and lactation phase (Fig. 1; $p < 0.05$); and between NDF level and lactation phase (Fig. 1; $p < 0.05$). The normal dietary CP levels produced greater piglet ADG than low CP dietary levels at phase II and IV ($p < 0.05$). Moreover, piglets from dams fed high dietary NDF level had higher ADG than those from normal dietary NDF level at phase 4 of the study (28 to 36 days of lactation) ($p < 0.05$).

Faecal dry matter content and total tract digestibility of nutrients

The reduction of dietary CP level triggered an increase of faecal DM content during the first lactation phase (from parturition until day 9 of lactation) (27.1 vs. $23.5 \pm 0.7\%$ of DM, in LP and NP diets respectively; $p < 0.05$). Subsequently, these differences between diets were not significant (phases 2, 3 and 4, or from day 10 until day 36 of lactation) (overall 24.8 vs. $24.6 \pm 0.6\%$ of DM, in LP and NP diets respectively). The increase of dietary NDF did not affect faecal DM content throughout the experimental period (overall 24.6 vs. $25.1 \pm 0.35\%$, in NF and HF diets respectively; $p > 0.05$).

The apparent total tract digestibility of nutrients in sows fed different dietary CP and NDF levels and according to each marker methodology is presented in Table 4. There were no interactions ($p > 0.05$) between dietary CP level, NDF level and marker method for any of the analysed variables.

The reduction of dietary CP did not alter significantly the coefficient of apparent total tract digestibility of the analysed nutrients ($p > 0.05$). However, there was a tendency ($p = 0.08$) for reduced total tract CP digestibility in LP compared to NP diets. Likewise, dietary NDF level affected organic matter, DM, CP, CHO and EE coefficients of apparent total tract digestibility. In all of them, except for EE, a reduction of total tract digestibility coefficient was observed when increasing dietary NDF levels.

Table 3 Productive performance of lactating sows fed diets varying crude protein (CP) content (low, LP vs. normal, NP) and neutral detergent fibre (NDF) content (normal, NF vs. high, HF)

Item	CP		NDF		SE	p-Value		
	LP	NP	NF	HF		CP	NDF	CP × NDF
Sows parameters								
Feed intake, kg DM/day	6.14	6.04	6.18	6.00	0.10	0.452	0.195	0.420
Water intake, litres/day	30.0	27.7	30.31	27.31	3.20	0.512	0.380	0.301
Water-to-feed ratio, litres/kg	4.58	4.29	4.62	4.26	0.479	0.613	0.521	0.194
Initial LW, kg	202.0	201.2	201.9	201.3	4.00	0.759	0.819	0.221
Individual LW variation, %	-2.94	-2.55	-2.85	-2.65	0.76	0.732	0.867	0.643
Initial backfat thickness, mm	8.81	9.05	9.13	8.74	0.59	0.434	0.208	0.060
Back fat thickness variation, mm*	-0.54	-0.72	-0.77	-0.50	0.20	0.549	0.375	0.158
Estimated milk yield, kg/day	7.70 ^b	9.70 ^a	8.87	8.53	5.27	<0.001	0.603	0.893
Litter parameters								
Piglet ADG, g	217 ^b	252 ^a	233	236	9.9	0.031	0.802	0.223
Coefficient of variation of litter LW at the end of phase, %	16.93	16.43	16.99	16.37	1.73	0.577	0.464	0.625

Within rows, least square means with different superscript letters (a,b) are different ($p < 0.05$).

*Backfat thickness measured at the P2 position (65 mm from the midline).

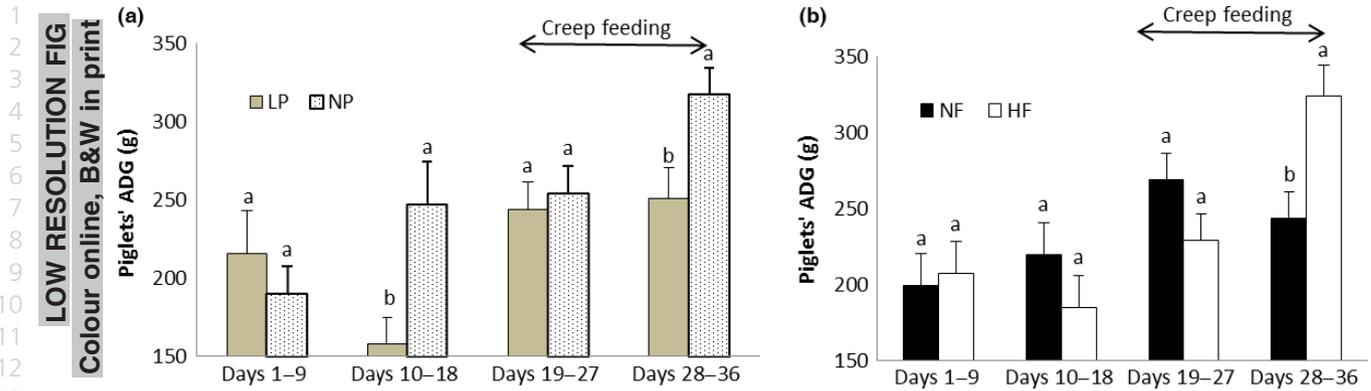


Fig. 1 Effect of dietary crude protein (CP) level (low, LP vs. normal, NP) (a) and neutral detergent fibre (NDF) (b) according to lactation phase on piglets' average daily gain (ADG, g). Different letters above each bar indicate significant differences between dietary CP and/or NDF level ($p < 0.05$).

Table 4 Coefficients of apparent total tract digestibility of nutrients in lactating sows fed diets varying crude protein (CP) content (low, LP vs. normal, NP) and neutral detergent fibre (NDF) content (normal, NF vs. high, HF)

Nutrient (%)	CP		NDF		Marker		SE	p-Value*		
	LP	NP	NF	HF	AIA	Cr ₂ O ₃		CP	FND	Marker
Organic matter, OM	92.94	93.11	94.05 ^x	92.00 ^y	93.87 ^a	92.18 ^b	0.40	0.750	<0.001	0.002
Dry matter, DM	91.86	92.14	93.08 ^x	90.92 ^y	92.99 ^a	91.02 ^b	0.43	0.652	0.001	0.002
Neutral detergent fibre, NDF	35.89	38.99	39.32	35.56	43.32 ^a	31.57 ^b	3.38	0.376	0.281	0.001
Crude Protein, CP	64.14	68.41	69.36 ^x	63.19 ^y	70.32 ^a	62.24 ^b	1.88	0.079	0.012	0.001
Carbohydrates, CHO	78.76	77.44	82.19 ^x	74.01 ^y	80.73 ^a	75.47 ^b	1.27	0.425	<0.001	0.002
Ether extract, EE	35.46	33.96	15.41 ^y	54.00 ^x	41.09 ^a	28.33 ^b	3.30	0.691	<0.001	0.002
Phosphorus, P	35.72	38.28	37.09	36.87	39.73	34.59	1.95	0.445	0.947	0.158

Within rows, least square means with different superscript letters (a,b) are different ($p < 0.05$).

*The interactions between CP levels. FND level and marker were not significant in any parameter ($p > 0.05$).

In addition, marker methodology affected total tract digestibility results. All nutrients except phosphorus showed higher digestibility values ($p < 0.05$) when the inert marker (AIA) was used as a reference substance compared to the external marker (Cr₂O₃). The coefficients of variation of the total tract digestibility of nutrients were approximately twice in Cr₂O₃ compared to AIA (12.2% and 7.5% for carbohydrates, 19.9% and 11.8% for CP and 84.8% and 38.5% for NDF respectively).

Manure composition

The daily amount of manure produced per sow differed statistically between dietary CP levels ($p = 0.05$, Table 5). Sows fed low dietary CP levels produced less manure per day. There were no statistical differences between dietary NDF levels or among lactation phases in the daily amount of fresh manure produced per sow ($p > 0.05$). Nevertheless, manure output (on a DM basis) was affected by dietary NDF level

($p < 0.05$). The increase of dietary NDF increased manure output (kg DM/day).

Manure composition was not affected by CP dietary levels ($p > 0.05$), but pH of manure was lower in sows fed low CP compared to normal CP diets ($p < 0.01$). The increase of the dietary NDF level reduced the density ($p < 0.01$), the EC ($p < 0.05$), and the phosphorus (P₂O₅) content of slurry ($p < 0.05$). In addition, there was a tendency for greater OM content ($p = 0.09$) and lower ammonium-N content ($p = 0.07$) in manure from sows fed high NDF compared to normal NDF diets.

Discussion

Effect of reducing dietary CP on sow and litter performance, apparent total tract digestibility and manure composition

The reduction of dietary CP did not affect significantly sow feed intake, LW change or backfat losses, in agreement with earlier studies (Dourmad et al., 1998;

Table 5 Manure yield and composition from lactating sows housed with their litters and fed diets varying crude protein (CP) content (low, LP vs. normal, NP) and neutral detergent fibre (NDF) content (normal, NF vs. high, HF)

Item	CP		NDF		SE	p-Value*	
	LP	NP	NF	HF		CP	NDF
Manure production, kg/day	13.43 ^a	17.33 ^b	15.55	15.20	1.66	0.050	0.118
Manure output, kg DM/day	2.29	2.62	2.25 ^b	2.66 ^a	0.18	0.118	0.050
Manure composition†							
pH	6.45 ^a	6.76 ^b	6.65	6.55	0.07	0.001	0.246
EC (dS/m)	12.04	13.02	13.33 ^b	11.72 ^a	0.72	0.230	0.038
Density, g/l	1029.64	1034.63	1036.82 ^b	1027.45 ^a	2.801	0.144	0.007
Dry matter, %	16.46	15.38	13.72 ^a	18.12 ^b	1.32	0.336	<0.001
Organic matter, % on a DM basis	83.27	85.08	82.50	85.85	1.52	0.347	0.089
Total N, g/kg DM	34.91	35.06	38.84	31.13	3.18	0.971	0.106
Ammonium-N, g/kg DM	8.09	8.85	10.33	6.59	1.39	0.664	0.073
Organic N, g/kg DM	26.83	26.21	28.50	24.54	1.90	0.797	0.161
Phosphorus, g/kg DM	14.10	12.89	15.74 ^b	11.25 ^a	1.32	0.504	0.022
Potassium, g/kg DM	23.32	20.31	24.12	19.52	2.23	0.294	0.166

*The interactions between CP level and FND level were not significant in any parameter ($p > 0.05$).

†Manure elements are expressed in the form they may be uptaken by plants: $\text{NH}_4\text{-N}$ (ammonium-N), P_2O_5 (phosphorus) and K_2O (potassium).

Yang et al., 2009; Huber et al., 2015). A positive relationship between dietary CP level (from 5% to 23% of CP) and feed intake was observed in gilts during a 4-week lactation (Shields et al., 1985; King et al., 1993). In the present study, the maintenance of similar dietary lysine levels in all diets attenuated the potential differences in sow feed intake. Before creep feeding was applied (phases 1 and 2, days 1–18), the estimated energy balance (Noblet and Etienne, 1989) would have been lower in the sows fed NP diets compared to their counterparts fed LP diets. However, the reduction in litter growth rate (and subsequently milk yield) in the LP group was not counterbalanced by lower body reserves mobilization.

The piglets' ADG from sows fed LP diets was 36% less than piglets' ADG from sows fed NP diets during phase 2 (days 10–18) of lactation. Because litter growth rate and milk production are closely related (Noblet and Etienne, 1989), the reduction of litter litter growth rate suggests that dietary CP may be the main limiting factor for piglets' growth. Therefore, milk yield, that peaks at 3rd week post-farrowing (Etienne et al., 2000), may be impaired by low dietary CP (around 12% of CP). Similar results were reported in primiparous sows by King et al. (1993) and Manjarin et al. (2012), who found a decreased litter growth rate and milk production in response to a deficient dietary CP supply (below 13.5%). Accordingly, Kusina et al. (1999) found a positive relationship between dietary CP level (graded values from 5.5% to 15.5% of dietary CP) and milk production, and Huber et al. (2015) reported increased efficiency of using retained N for milk protein production in response to improvement

in dietary amino acid balance with crystalline amino acid supplementation.

Applying creep feeding at the third phase (days 19–27) of lactation compensated the differences between piglets' ADG of sows fed diets with different dietary CP level. However, such effect was transient as at the fourth phase (days 28–36), the piglets raised by sows fed normal dietary CP level had greater ADG than those from sows fed low dietary CP level. Probably, these results were due to the fixed equal amount of creep feed supplement in all dietary treatments. After all, the reduction of piglets' ADG from sows fed low CP diets did not have deleterious effects on the coefficient of variation of litter LW at the end of each lactation phase.

Moreover, Laspiur et al. (2009) suggested that the imbalances due to excesses or deficiencies in dietary amino acids reduce the efficiency of the dietary nitrogen utilization by the animal, limiting milk protein synthesis. The order of limiting amino acids for lactating sows varies according to body tissue mobilization level (Kim et al., 2009). In this study, the *ad libitum* feed supply may support a low level of sow LW losses. In this situation, sows without body protein loss need more dietary valine relative to lysine, and thereby, the most limiting amino acids are lysine, valine and threonine. Although an attempt was made to attain an ideal amino acid pattern on the basis of lysine, methionine, threonine and tryptophan requirements, the effective valine supply did not met the actual estimated requirements according to NRC (2012) and it would have been necessary to supplement the diet with crystalline valine, especially to the LP diets.

1 The reduction of dietary CP had no impact on the
2 sow apparent total tract digestibility of most nutrients
3 but only a slightly lower total tract digestibility of diet-
4 ary CP, as previously found in growing pigs when
5 reducing dietary CP from 18% to 12.3% (Le Bellego
6 et al., 2001).

7 Faecal dry matter content was only increased dur-
8 ing phase 1 (days 1–9) of lactation in sows fed low
9 dietary CP levels. Tabeling et al. (2003) show that par-
10 turation leads to a higher DM content in faeces and
11 reduced defecation frequency. In our study, greater
12 DM content in faeces and lower manure production
13 was noticeable in the first days post-partum of sows
14 fed low dietary CP, although it could not be associated
15 with lower water disappearance.

16 In the present study, the reduction of dietary CP did
17 not produce significant differences in manure compo-
18 sition but only a lower pH. Some previous reports
19 found that low CP diets consistently decreased man-
20 ure ammonium content (Kerr et al., 2006; Ziemer
21 et al., 2009). This discrepancy may be probably due to
22 the fact that both dietary CP levels evaluated in the
23 current study were rather low (12% vs. 14% of CP),
24 and thus, the potential to decrease the nitrogen
25 excreted may have reached a threshold.

26 27 28 **Effect of increasing dietary NDF on sow and litter** 29 **performance, apparent total tract digestibility and** 30 **manure composition**

31 The increase of dietary NDF did not affect negatively
32 either sow feed intake, LW or backfat thickness. High-
33 fibre diets are normally used during pregnancy to
34 induce satiety and prevent constipation, and they are
35 also recommended during, at least, the days around
36 farrowing to improve colostrum composition and
37 therefore to reduce piglet mortality (Loisel et al.,
38 2013). However, their use is restricted during lactation
39 so that feed energy density is not impaired. In this
40 study, vegetable fat sources (sunflower oil) were used
41 to balance the energy content of high NDF diets,
42 which were consumed at a similar amount to normal
43 NDF diets.

44 Feed intake decreases as the dietary energy concen-
45 tration increases, and as a result, total lysine intake
46 may decline when formulating high-fibre diets for lac-
47 tating sows at a certain lysine percentage of the diet.
48 Hence, it is recommended to use the SID lysine to ME
49 ratio as the optimum method of expressing the lysine
50 requirement (Xue et al., 2012). Although NRC (2012)
51 requirements for lactation sows are based on greater
52 ME and SID lysine content than in the present study
53 (3.3 Mcal/kg vs. 7.2–8.7 g/kg respectively), the

ad libitum feeding pattern allowed meeting most of the
estimated nutrient requirements of sows on a daily
basis. However, according to the above-mentioned
order or limiting amino acids (lysine, valine and thre-
onine) when sow LW losses are reduced (Kim et al.,
2009), the increase of dietary NDF (22% vs. 18%
NDF) allowed greater valine-to-lysine ratio (59% vs.
49%) and greater threonine-to-lysine ratio (60% vs.
54%) compared to normal NDF diets.

The dietary NDF levels of sows had only mild
effects on litter performance. The piglets raised by
sows fed dietary high NDF only showed greater ADG
at the end of lactation (phase 4, days 28–36) com-
pared to their counterparts raised by dams fed normal
NDF. This outcome is in agreement with Renaudeau
et al. (2003), who found that litter LW gain was
greater in lactating sows fed high NDF diets (22%
NDF vs. 19% NDF) during a 28-day lactation.
Although the estimated milk yield during phases 1
and 2 (days 1 to 18 of lactation) based on piglets'
ADG (Noblet and Etienne, 1989) did not differ
between sow dietary NDF levels, there might be sub-
sequent differences in milk yield, its nutrient compo-
sition or lactation curve persistency between sows fed
high and normal NDF diets.

Despite the lack of differences in sow productive
performance, the nutrient digestion was highly
affected by increasing dietary NDF. Even though diets
were formulated to be isoenergetic, the apparent total
tract digestibility coefficients of OM, DM, CHO and CP
were decreased while EE digestibility was increased by
using high NDF diets. This may be due to the
increased flow of OM, DM and CHO to the terminal
ileum as a consequence of a lower digestibility of non-
starch polysaccharides (Schulze et al., 1994; Serena
et al., 2008). The reduction of the apparent total tract
digestibility of CP may be due to increased endoge-
nous amino acid losses when feeding high-fibre diets
because of increased production of mucin (Mosenthin
et al., 1994; Schulze et al., 1994).

In contrast, the apparent total tract digestibility of
EE was increased by using high NDF diets. Noblet and
Shi (1993) reported a curvilinear relationship between
dietary EE and the apparent total tract digestibility of
EE, which is reduced when the fibre content of the
diet rises above 200 g NDF/kg. In this study, the feed
NDF content surpassed that threshold but the fat
digestibility was not impaired. The current results are
in agreement with Le Goff et al. (2002), who found
that moderate increases of dietary fibre (inclusion of
235 g/kg of maize bran that yielded 197 g NDF/kg
of diet) reduced the apparent total tract digestibility of
nutrients in growing pigs but did not affect heat

1 production when data were adjusted for a similar ME
2 intake.

3 The faecal DM content of sows was not affected by
4 dietary NDF. These results are not in agreement with
5 Tabeling *et al.* (2003), who observed that faeces
6 became markedly softer with higher contents of crude
7 fibre in sow diets. This difference could be attributed
8 to the dietary fibre sources used, as in the present
9 study, they were mainly sugar-beet pulp and a mini-
10 mum of cereal straw, whereas in that case they were
11 provided through oat and soybean hulls. The increase
12 of dietary NDF showed a concomitant reduction of
13 manure density together with an increase manure
14 produced by sows on a dry matter basis (kg DM/day),
15 as evidenced in growing pigs (Moeser and van Kem-
16 pen, 2002; Morazán *et al.*, 2015).

17 The organic nitrogen content of manure did not dif-
18 fer between dietary NDF treatments, but there was
19 tendency for lower ammonium nitrogen content in
20 manure from lactating sows fed high NDF diets. It has
21 been hypothesized that increasing dietary NDF level
22 by sugar-beet pulp addition may alter N excretion by
23 reducing urinary N excretion in favour of faecal N
24 excretion (Canh *et al.*, 1997), but this response may
25 only be noticeable at high dietary CP levels (Lynch
26 *et al.*, 2008). This could trigger lower ammonia emis-
27 sions from manure because N excreted as urine is
28 mainly in the form of urea, which is converted into
29 ammonia and carbonate by the urease enzyme pre-
30 sent in faeces (Van der Peet-Schwering *et al.*, 1999).

31 Although no differences were found in the coeffi-
32 cient of total tract apparent digestibility of phospho-
33 rus, the content of this mineral in manure was
34 reduced when increasing dietary NDF level. Phospho-
35 rus is essential for gastrointestinal bacteria due to its
36 function as a constituent of primary cell metabolites
37 (Metzler and Mosenthin, 2008). Thus, we hypothe-
38 sized that certain microbiota present in manure might
39 have partially consumed phosphorus after excretion.
40 Accordingly, these authors suggested that high fibre
41 feed benefits the growth of certain microbial popula-
42 tion that can consume undigested phosphorus.

43 44 45 **Effect of marker used to estimate apparent total tract** 46 **digestibility**

47 The coefficients of apparent total tract digestibility of
48 the present study were in line with results reported
49 by Noblet and Shi (1993) for adult sows. However,
50 the recorded values may differ between external
51 markers (as chromic oxide, Cr₂O₃) and internal feed
52 marker (as AIA). The greater total tract digestibility
53 measurements when using AIA compared to Cr₂O₃

were independent of diet, as there were no interac-
tions between the marker and dietary CP or NDF
level. The main differences arose in the coefficient
of apparent total tract digestibility of the least digesti-
ble nutrients (NDF, 37.2% higher estimate in AIA
than in Cr₂O₃; and EE, 45.0% higher estimate in AIA
than in Cr₂O₃). Similar results were described by
Moughan *et al.* (1991) in growing pigs when com-
paring the nutrient digestibility calculated by these
two methods.

The present results could be due to the fact that
recovery rates of AIA are higher than that of Cr₂O₃.
A review from Sales and Janssens (2003) revealed a
recovery range of AIA (as an indigestible marker)
from 84 to 192%. On the contrary, Cr₂O₃ has shown
an incomplete recovery range in faeces from 75 to
87% (Moore, 1957; McCarthy *et al.*, 1974). Further-
more, Clawson *et al.* (1955) suggested that the recov-
ery of Cr₂O₃ is affected by a diurnal excretion pattern.
Therefore, its recovery rate may be incomplete, and it
would underestimate the digestibility coefficients of
nutrients.

Even though Cr₂O₃ has been widely used as a refer-
ence marker to determine total tract digestibility in
several species, including pigs (Ishikawa, 1966; Ishi-
kawa and Sugimura, 1973), it has several drawbacks.
Apart from incomplete recovery, it has major disad-
vantages due to carcinogenicity associated to analyti-
cal procedures for its determination (Delagarde *et al.*,
2010), and it involves difficulties to handle and mix
accurately in feed at low dosage levels. In lactating
sows, AIA may be an effective marker to estimate the
total tract apparent digestibility of those nutrients that
are greatly digested throughout the gastrointestinal
tract (e.g. carbohydrates).

In conclusion, the reduction of dietary CP level in
lactating sows had no effect on either LW or backfat
thickness or apparent total tract digestibility of nutri-
ents. However, the piglets' ADG was reduced in low
dietary CP diets, which suggests that sows reduced
milk production due to an underestimation of certain
essential amino acid requirements (e.g. valine). The
increase of dietary NDF level did not affect sow and lit-
ter performance, but the total tract apparent digestibil-
ity of OM, CP and carbohydrates was reduced and EE
digestion was increased in high NDF compared to nor-
mal NDF diets equally balanced for ME and lysine
content. Finally, The coefficients of total tract appar-
ent digestibility of nutrients in lactating sows were
greater when using AIA compared to Cr₂O₃ marker,
regardless of dietary CP or NDF level, but their coeffi-
cients of variation were lower in the former than in
the latter.

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