Blood Serum Metabolite and Volatile Fatty Acid Profiles of Finishing Pigs Fed Diets with High and Low Levels of Energy and Crude Protein

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Abstract

Background: Studying how dietary imbalances affect blood serum metabolite (**BSM**) and faecal volatile fatty acids (**VFA**) profiles may help to understand whether pigs are fed adequate diets in commercial farms. The present study aimed to evaluate the effect of high and low net energy (**NE**) and crude protein (**CP**) diets on performance, BSM and VFA profiles on finishing pigs.

Results: Twenty pens of 11 pigs (87.0 ± 4.10 kg; 18 weeks old) were assigned to 1 out of 5 dietary treatments (**DT**): control (**C**; 10.03 MJ/kg NE, 160.0 g/kg CP, and 9.5 g/kg SID Lys), low CP (**LCP**; 10.03 MJ/kg NE, 132.0 g/kg CP, 7.5 g/kg SID Lys), high CP (**HCP**; 10.03 MJ/kg NE, 188.0 g/kg CP, 11.5 g/kg SID Lys), low NE (**LNE**; 9.61 MJ/kg NE, 160.0 g/kg CP, 9.5 g/kg SID Lys) and high NE (**HNE**; 10.45 MJ/kg NE, 160.0 g/kg CP, 9.5 g/kg SID Lys). Pigs were followed for 10 days and blood and faecal samples were collected at the end of the trial. Performance was not affected by DT ($P > 0.05$). Albumin and glucose did not differ between DT ($P > 0.05$). HNE pigs had higher triglycerides (0.42 ± 0.03 mmol/L) and creatinine (133.8 ± 3.97 µmol/L) than LNE pigs (0.28 ± 0.03; 117.2 ± 3.97; $P < 0.05$); however, HNE pigs had lower total protein (60.9 ± 1.51 g/L) than C pigs (67.4 ± 1.51; $P = 0.033$). LCP pigs had higher cholesterol (2.4 ± 0.08 mmol/L) than LNE pigs (2.0 ± 0.08; $P = 0.015$); while HCP pigs had higher serum urea nitrogen (13.6 ± 0.95 mg/dL) than the other DT (7.5 ± 0.95; $P < 0.001$). Total VFA (mmol/kg) did not differ among DT ($P > 0.05$), but C and HNE pigs had higher branched-chain fatty acids (6.3 ± 0.38% of total VFA) than LNE pigs (4.4 ± 0.38; $P < 0.05$).

Conclusions: Dietary imbalances in energy and protein affect BSM and VFA profile. BSM and VFA analysis may be good indicators to detect unbalanced diets in pig farms, especially serum urea nitrogen, to detect an excess of protein.

Background

The actual nutritional value of a diet for a particular pig farm is affected by different factors such as feed manufacturing (1), physicochemical characteristics of feed ingredients (2), feed form and delivery method (3), management and body weight (**BW**) variability (4, 5), and health status (6, 7), among others. With so many factors affecting nutritional value of feed, dietary imbalances are not rare and can result in extra cost for the farmers and potential health problems for the pigs. Methods accounting for the effect of these factors on nutritional value of feeds and the nutrient requirements of pigs at farm level are of great interest to improve production efficiency, animal health and farm sustainability while reducing production costs (1, 8, 9).

Blood samples are easy to collect in pig farms and blood biochemistry is a fast analysis method that could give us some parameters directly related to energy, protein and lipid metabolism (10–12). Dietary changes affect nutrient metabolism and this should be reflected in blood serum metabolites such as total protein (11–13), albumin (12–14), serum urea nitrogen (**SUN**) (15–17) and creatinine (17) for the protein metabolism, and glucose (11–13), triglycerides (14, 18) and cholesterol (12, 14, 19) for the energy and

lipid metabolism. Therefore, blood biochemistry may be a useful tool to detect dietary imbalances in pig farms and correct them through dietary modifications.

Faecal samples are also easy to collect in pig farms. Volatile fatty acids (**VFA**) and other fermentation components present in faeces can be easily measured in faecal samples (20). The nutrient composition of a diet is a key factor in the microbiome profile of the gastrointestinal tract, which will affect the faecal VFA profile (20–22). Carbohydrates are catabolized to short-chain fatty acids such as acetic, propionic or butyric acid, while protein results in a higher concentration of branched-chain fatty acids (**BCFA**) which are produced from the deamination of branched-chain amino acids such as leucine, isoleucine and valine (22). Therefore, high proportions of BCFA measured in faeces would indicate an excess of protein reaching distal parts of the intestine.

Describing the effects of different levels of energy and protein in the blood serum metabolite and faecal VFA profiles could help nutritionists to better understand the effect of dietary changes and improve feed efficiency in finishing pigs. Thus, the use of fast analysis methods from easy collection samples in pig farms could be useful to detect and correct dietary imbalances in a specific farm.

We hypothesise that blood metabolite and faecal VFA profiles will be affected due to dietary changes on energy and protein. Thus, the objective of the present study was to evaluate the effect of high and low net energy and crude protein diets on productive performance, blood serum metabolite and faecal VFA profiles on finishing pigs in a high sanitary status farm.

Methods

Animals, diets and experimental design

The study received ethical approval from the Teagasc Animal Ethics Committee (TAEC 244/2019) and it was conducted at the Teagasc Pig Research Facility in Fermoy, Co. Cork, Ireland. A total of 220 Danish Duroc × (Large White × Landrace) grower-finisher pigs born within one week were moved at 11 weeks of age and housed in mixed sex pens with fully slatted concrete floor (2.4 × 4.2 m) containing a single wet-dry feeder (330mm [Width] × 370mm [Depth] × 1000mm [Height]; MA37, Verba, Netherlands) and one supplementary nipple drinker. Water and pelleted feed were provided ad libitum. Temperature was controlled by a mechanical ventilation system with fan speed and air inlet area regulated by a climate controller. Pens were enriched with a larch wood post. Pigs were fed a single soybean meal-maize-wheat based grower-finisher diet (9.67 MJ of net energy (**NE**), 161.8 g crude protein (**CP**), and 9.2 g of standardized ileal digestive (**SID**) lysine (**Lys**) per kg of feed) from 11 to 18 weeks of age.

Pigs were weighed per pen as a group (n = 20; 11 pigs/pen; 87.0 ± 4.10 kg BW) at 18 weeks of age. Pigs were assigned per pen based on BW to five different dietary treatments and pigs were followed for 10 days. Diets were formulated to obtain a control diet [**C**; 10.03 MJ/NE, 160 g of CP, and 9.5 g of SID Lys per kg of feed] which met or exceed the minimum nutrient requirements (23), and 4 unbalanced diets which were: low crude protein (**LCP**; 10.03 MJ/NE, 132 g of CP, and 7.5 g of SID Lys per kg of feed), high crude

protein (**HCP**; 10.03 MJ/NE, 188 g of CP, and 11.5 g of SID Lys per kg of feed), low net energy (**LNE**; 9.61 MJ/NE, 160 g of CP, and 9.5 g of SID Lys per kg of feed) and high net energy (**HNE**; 10.45 MJ/NE; 160 g of CP, and 9.5 g of SID Lys per kg of feed). Diet composition is shown in Table 1. Blood and faecal samples were collected from 2 randomly selected pigs per pen at the end of the trial to obtain the blood serum and faecal VFA profiles. Pigs went back to the common management of the Teagasc Pig Research Facility after the 10-day trial period.

Table 1
Ingredient, calculated and analysed nutrient composition on an as-fed basis of the five dietary treatments.[1]

	Diets [2]				
	C	LCP	HCP	LNE	HNE
Ingredients, g/kg					
Wheat	350.0	350.0	350.0	330.0	306.2
Barley	282.5	345.0	0.0	310.5	200.0
Maize	150.0	150.0	286.6	100.0	275.5
Soybean meal 47.5	172.4	95.7	254.1	175.1	172.4
Soybean hulls	14.2	29.7	63.9	58.3	0.0
Vegetable Oil	5.0	5.0	17.6	0.0	21.5
Calcium carbonate	12.3	12.7	12.2	10.7	11.7
Dicalcium phosphate anhydrous	0.50	0.50	1.00	3.00	0.50
Sodium chloride	4.50	4.40	3.20	4.40	3.70
L-Lysine HCl	4.30	3.80	5.30	4.15	4.40
L-Threonine	1.60	1.20	2.20	1.15	1.60
DL-Methionine	1.30	0.70	2.20	1.30	1.20
L-Tryptophan	0.20	0.10	0.20	0.20	0.10
L-Valine	0.00	0.00	0.30	0.00	0.00
Vitamin and trace mineral mixture [3]	1.20	1.20	1.20	1.20	1.20
Calculated / Analysed Composition [4], % as fed or as specified					
Dry Matter, analysed	88.00	87.70	88.30	87.90	87.90

[1] Diets were fed in finishing pigs during 10 days at 18 weeks of age.

[2] C = Control; LCP = Low Crude Protein; HCP = High Crude Protein; LNE = Low Net Energy; HNE = High Net Energy

[3] Provided per each kg of feed: 60 mg Copper sulphate, 80 mg Ferrous sulphate monohydrate, 50 mg Manganese oxide, 100 mg Zinc oxide, 0.5 mg Potassium iodate, 0.4 mg Sodium selenite, 2 MIU Vitamin A, 0.5 MIU Vitamin D_3, 40 MIU Vitamin E, 4 mg Vitamin K, 0.015 mg Vitamin B_{12}, 2 mg Riboflavin, 12 mg Nicotinic acid, 10 mg Pantothenic acid, 2 mg Vitamin B_1, 3 mg Vitamin B_6.

[4] NE = Net Energy; SID = Standardized Ileal Digestible; NDF = Neutral Detergent Fiber.

	Diets [2]				
Ash, analysed	3.90	3.60	4.00	4.10	3.90
NE, MJ/kg	10.03	10.03	10.03	9.61	10.45
SID Lys:NE, g/MJ	0.95	0.75	1.15	0.99	0.91
Crude Protein, analysed	13.40	11.60	16.20	14.50	14.30
Total Lys, analysed	1.05	0.88	1.31	1.08	1.02
Total Thr / Lys ratio, analysed	0.58	0.58	0.56	0.57	0.62
Total Met-Cys / Lys ratio, analysed	0.64	0.64	0.63	0.65	0.68
Total Trp / Lys ratio, analysed	0.14	0.15	0.15	0.14	0.14
Total Val / Lys ratio, analysed	0.60	0.65	0.65	0.67	0.69
Total Leu / Lys ratio, analysed	1.09	1.14	1.08	1.14	1.14
Total Ile / Lys ratio, analysed	0.54	0.55	0.57	0.58	0.60
Total His / Lys ratio, analysed	0.34	0.38	0.37	0.36	0.38
SID Lys	0.95	0.75	1.15	0.95	0.95
SID Thr / Lys ratio	0.65	0.65	0.65	0.65	0.65
SID Met-Cys / Lys ratio	0.59	0.59	0.59	0.59	0.59
SID Trp / Lys ratio	0.19	0.19	0.19	0.19	0.19
SID Val / Lys ratio	0.66	0.67	0.65	0.66	0.66
SID Leu / Lys ratio	1.16	1.20	1.11	1.16	1.15
SID Ile / Lys ratio	0.56	0.55	0.57	0.57	0.57
SID His / Lys ratio	0.36	0.36	0.34	0.35	0.36
Fat, analysed	2.79	2.74	3.78	2.21	4.19

[1] Diets were fed in finishing pigs during 10 days at 18 weeks of age.

[2] C = Control; LCP = Low Crude Protein; HCP = High Crude Protein; LNE = Low Net Energy; HNE = High Net Energy

[3] Provided per each kg of feed: 60 mg Copper sulphate, 80 mg Ferrous sulphate monohydrate, 50 mg Manganese oxide, 100 mg Zinc oxide, 0.5 mg Potassium iodate, 0.4 mg Sodium selenite, 2 MIU Vitamin A, 0.5 MIU Vitamin D_3, 40 MIU Vitamin E, 4 mg Vitamin K, 0.015 mg Vitamin B_{12}, 2 mg Riboflavin, 12 mg Nicotinic acid, 10 mg Pantothenic acid, 2 mg Vitamin B_1, 3 mg Vitamin B_6.

[4] NE = Net Energy; SID = Standardized Ileal Digestible; NDF = Neutral Detergent Fiber.

	Diets [2]				
Crude Fiber, analysed	2.90	3.40	4.20	4.20	2.40
NDF	12.96	14.15	13.54	15.02	12.00
Calcium	0.75	0.75	0.80	0.77	0.72
Digestible Phosphorus	0.22	0.22	0.22	0.25	0.22

[1] Diets were fed in finishing pigs during 10 days at 18 weeks of age.

[2] C = Control; LCP = Low Crude Protein; HCP = High Crude Protein; LNE = Low Net Energy; HNE = High Net Energy

[3] Provided per each kg of feed: 60 mg Copper sulphate, 80 mg Ferrous sulphate monohydrate, 50 mg Manganese oxide, 100 mg Zinc oxide, 0.5 mg Potassium iodate, 0.4 mg Sodium selenite, 2 MIU Vitamin A, 0.5 MIU Vitamin D_3, 40 MIU Vitamin E, 4 mg Vitamin K, 0.015 mg Vitamin B_{12}, 2 mg Riboflavin, 12 mg Nicotinic acid, 10 mg Pantothenic acid, 2 mg Vitamin B_1, 3 mg Vitamin B_6.

[4] NE = Net Energy; SID = Standardized Ileal Digestible; NDF = Neutral Detergent Fiber.

Body Weight, Feed Intake and Feed Efficiency Traits

Pigs were weighed per pen at the beginning and at the end of the trial period. Feed intake was recorded per pen on a daily basis. Average daily gain (**ADG**), average daily feed intake (**ADFI**) and feed conversion ratio (**FCR**) were calculated for the overall trial period. Feed conversion ratio was calculated as $\frac{\mathrm{kgoffeedconsumed}}{\mathrm{BWgain}}$.

Feed Analysis

Feed samples of each diet were collected per duplicate from the feeders and analysed for dry matter, ash, crude protein, crude fiber, fat, and total amino acid profile at the Sciantec Analytical Services (Stockbridge Technology Centre, Cawood, Yorkshire, UK). Dry matter was measured by oven drying for 4 h at 103°C (24). Ash was measured via combustion in a muffle furnace at 550°C (Thiex, Novotny, & Crawford, 2012). Crude protein was determined as N × 6.25 based on the DUMAS method (26) using LECO FP-628 analyser (Leco Instruments Ltd., Stockport, UK). Crude fiber was determined by a Fibertec semi-automatic system (Tecator, Hoganas, Sweden) using the gravimetry method (Thiex 2009). Fat was measured using Randall/Soxtec/Submersion method (27). Total amino acid profile was determined based on ion exchange high performance liquid chromatography technique (28) using the Biochrom Amino Acid Analyser Sodium System (Biochrom Ltd., Cambridge, UK).

Blood sample collection and blood serum analysis

Blood samples were collected via venepuncture of the external jugular vein (approximately 10 ml/pig) from 2 pigs/pen selected randomly at the end of the trial, at 20 weeks of age approximately. Blood samples were collected early in the morning in a non-fasting state as per commercial practice. Blood

samples were kept immediately on ice at 4°C after collection until serum was separated by centrifugation for 15 min at 2000 rcf. The same day, blood serum samples were analysed using the ABX Pentra 400 Clinical Chemistry benchtop analyser (HORIBA Medical, Irvine, California, USA) at the Teagasc Chemistry Lab in Fermoy, Co. Cork, Ireland. Selected blood serum metabolites were albumin, glucose, triglycerides, cholesterol, SUN, creatinine and total protein, which were determined by colorimetry using their respective ABX Pentra 400 re-agents (HORIBA ABX SAS, Montpellier, France). Albumin assay was conducted using the bromocresol green dye-binding procedure (29). Glucose assay was performed using the glucose hexokinase method (30). The concentration of triglycerides was determined using an enzymatic method involving lipoprotein lipase, glycerol kinase, glycerol-3-phosphate oxidase, and peroxidase (31). Cholesterol concentration was also determined based on an enzymatic photometric test involving cholesterol esterase, cholesterol oxidase, and peroxidase (32). The assay of SUN was conducted using an enzymatic UV test using urease and glutamate dehydrogenase (33). Total protein concentration assay was determined based on the Biuret reaction (34). Finally, creatinine assay was conducted using an enzymatic method involving creatinine amidohydrolase, sarcosine oxidase and peroxidase (35).

Faecal sample collection and volatile fatty acid analysis

Faecal samples were collected using BioFreeze™ vials (Alimetrics Diagnostics Ltd, Espoo, Finland) from 2 pigs/pen selected randomly at the end of the trial, at 20 weeks of age approximately. BioFreeze™ vials enable to collect the fresh samples and stop all biological activity at ambient temperature until the analysis. Faecal VFA analysis was conducted via gas chromatography using pivalic acid as an internal standard (36) at Alimetrics Diagnostics. The VFA profile included acetic, propionic, butyric, valeric, BCFA and total VFA.

Data management and statistical analysis

All analyses were carried out using SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Plots were created using R v4.0.2 (37). Pen was considered as the experimental unit for all performance, blood serum and faecal VFA data analyses. Alpha level for determination of significance was 0.05 and trends were identified as alpha of 0.10. Data were tested for normality using the Shapiro-Wilk test and by examining the normal probability plot. Initial BW data were analysed using general linear models including treatment as fixed effect. Models for BW, ADG, ADFI and FCR variables were analysed using general linear models including treatment diet as fixed effect and initial BW as a co-variable. For blood serum, models for albumin, glucose, triglycerides, cholesterol, SUN, creatinine, and total protein were analysed using general linear models including treatment diet as fixed effect. For faecal VFA, models for acetic, propionic, butyric, valeric, BCFA and VFA were analysed using general linear models including treatment diet as fixed effect. Multiple means comparisons were done using Tukey-Kramer's correction in all cases. Results for fixed effects are reported as least square means ± standard error mean.

Results

Body Weight, Feed Intake and Feed Efficiency Traits

There were no differences on BW, ADG, ADFI and FCR between dietary treatments ($P > 0.05$; Table 2); although HCP had numerically higher ADG and lower FCR than LCP pigs, and HNE pigs had numerically lower ADFI and FCR than LNE pigs by the end of the trial.

Table 2

Productive performance from 220 finishing pigs grouped by dietary treatment (n = 4).[1]

	Dietary Treatment [2]						
	Control	LCP	HCP	LNE	HNE	SEM	*p*-Value
BW, kg							
128 d	87.1	88.1	87.8	85.4	86.9	2.32	0.926
138 d	100.2	99.9	101.0	100.2	100.2	0.70	0.828
ADG, g							
128–138 d	1315.0	1283.7	1399.1	1312.1	1317.7	71.49	0.825
ADFI, g							
128–138 d	3024.2	3078.4	3110.0	3284.8	2977.7	128.86	0.531
FCR							
128–138 d	2.33	2.39	2.23	2.48	2.27	0.08	0.228

[1] Initial and final body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) (Least square means ± Standard error mean [SEM]). Pigs were followed from 128 to 138 days of age.

[2] Dietary treatments: Control (10.03 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed), LCP (Low Crude Protein; 10.03 MJ/kg of NE; 132.0 g of CP; 7.5 g of SID Lys per kg of feed), HCP (High Crude Protein; 10.03 MJ/kg of NE; 188.0 g of CP; 11.5 g of SID Lys per kg of feed), LNE (Low Net Energy; 9.61 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed), and HNE (High Net Energy; 10.45 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed).

[a–b] Within rows, significant differences between groups ($P < 0.05$).

Blood serum metabolites

Albumin and glucose did not differ between dietary treatments ($P > 0.05$; Fig. 1), although HNE pigs tended to have higher glucose (5.68 ± 0.273 mmol/L) than LNE pigs (4.61 ± 0.273 mmol/L; $P = 0.063$). Moreover, HNE pigs had higher triglycerides (0.42 ± 0.03 mmol/L) and creatinine (133.81 ± 3.966 µmol/L) than LNE pigs (0.28 ± 0.03 mmol/L; 117.20 ± 3.966 µmol/L; $P < 0.05$; Fig. 1); however, HNE pigs had lower total protein (60.86 ± 1.509 g/L) than C pigs (67.36 ± 1.509 g/L; $P = 0.033$; Fig. 1). Furthermore, LCP pigs had higher cholesterol (2.44 ± 0.084 mmol/L) than LNE pigs (2.03 ± 0.084 mmol/L; $P = 0.015$; Fig. 1); while HCP pigs had higher SUN (13.63 ± 0.951 mg/dL) than the other dietary treatments (7.47 ± 0.951 mg/dL; $P < 0.001$; Fig. 1).

Volatile fatty acids profile

Total VFA (mmol/kg) did not differ between dietary treatments ($P > 0.05$; Fig. 2). Nevertheless, C pigs had lower % of acetic (57.4 ± 1.01) of total VFA than LCP and HCP pigs (61.6 ± 1.01; 62.0 ± 1.01, respectively; $P < 0.05$; Fig. 2); but C pigs had higher % of valeric (2.8 ± 0.15) of total VFA than LNE pigs (2.1 ± 0.15; $P = 0.010$; Fig. 2). Finally, LNE pigs had lower % of BCFA (4.4 ± 0.38) of total VFA than C and HNE pigs (6.4 ± 0.38; 6.1 ± 0.38, respectively; $P < 0.01$; Fig. 2).

Discussion

Body Weight, Feed Intake and Feed Efficiency Traits

The productive performance of the pigs was not affected by the dietary treatments in the trial as expected. Previous literature reported that pigs improve their feed efficiency (38, 39) and reduce their feed intake (39, 40) with increasing levels of net energy in diets. An insufficient crude protein level should also limit the growth of the pigs (23). However, the 10-day trial period is perhaps too short to find differences in performance despite pigs fed the HNE diet showed numerically reduced feed intake and better feed efficiency than pigs fed the LNE diet, and pigs fed the HCP diet showed numerically better average daily gain and feed efficiency than pigs fed the LCP diet. Nevertheless, the 10-day trial period was enough time to observe differences in the blood metabolite profile between dietary treatments considering that blood metabolites may be quite constant/homogenous among pigs under the same feed, management and housing conditions (16, 17, 41). This shows the potential of blood metabolites to detect dietary imbalances even when no changes in productive performance have occurred.

Blood serum metabolites

The existing literature related on blood serum metabolites in pigs is scarce and reference laboratory values may be useful to detect diseases in pigs based on values derivate from healthy pigs complying different factors such as age, breed, sex, diet, geographical habitat, and methods of sample collection and laboratory measurement (42). Nevertheless, baseline blood metabolite values and how these are affected when pigs are fed different levels of protein and energy at a farm level at a specific age to evaluate the nutritional value of a diet are not available.

Differences in levels of protein and energy in diets had an effect on the blood metabolite profile in finishing pigs at 20 weeks of age. Serum total protein (45–70 g/L) is the sum concentration of all individual serum proteins (42). Prior studies suggested that serum total protein could be used as an indicator of the adequacy of dietary protein content in pigs (43). Several reports have shown that grower pigs fed insufficient crude protein diets up to 60% of the lysine requirements cause a decrease of serum total protein concentration in blood, which may persist during the subsequent finisher phases (13, 44). Moreover, Zeng et al. (11) observed a decrease of serum total protein concentration in blood when grower pigs were fed 65% of the lysine requirements without modifying the levels of crude protein between diets. However, no differences were observed between HCP and LCP diets in finishing pigs in the present study.

This result agrees with that of Regmi et al. (12) who did not observe differences in serum total protein concentration in finishing pigs fed insufficient (0.32%), adequate (0.60%) or excess (0.87%) SID lysine diets, and they suggested the pig age difference as the cause to explain the inconsistency in this aspect. Therefore, finishing pigs may be able to show an homeostatic control of serum total protein concentration in blood besides the dietary protein content (12). Moreover, finishing pigs have already reached the maximum protein deposition (45–47) and their metabolism may not be focused on protein turn-over contrary to early stages of the grower-finisher period. Then, serum total protein may be a good indicator of protein synthesis during the growing phase, but not in the finishing phase when pigs are fed insufficient levels of crude protein up to approximately 85% of the requirements such as the present study.

Serum albumin (19–40 g/L) is the most abundant circulating protein found in serum, which accounts for the 60% of the total plasma proteins (42). Albumin plays a major role as a modulator of the plasma colloid osmotic pressure, participating in the transport of hormones, enzymes, fatty acids, metal ions and medicinal products (48). Albumin is considered being a sensitive indicator of protein synthesis capacity of the liver (49) and dietary protein nutrition in pigs (43). However, albumin concentration did not differ in finishing pigs between dietary treatments in the present study, while previous research observed differences between low and high crude protein and amino acids diets when fed in growing pigs (13, 14, 44). Pigs fed insufficient crude protein diets up to 60–80% of the requirements have a reduced albumin concentration in blood (13, 14, 44) leading to a hypoalbuminemia in cases of severe protein restrictions with levels far below 60% of the pigs' requirements (50–52); while hyperalbuminemia was reported with high crude protein diets (53). Moreover, Regmi et al. (12) observed reduced plasma albumin concentration when finishing pigs were fed a 0.32% SID lysine diet during 4 weeks, but no differences were observed between pigs fed 0.60 and 0.87% SID lysine diets, which are more similar to the SID lysine levels of the dietary treatments of the present study. Thus, serum albumin concentration could be a good indicator of protein synthesis during the growing phase, but not in the finishing phase where differences between levels of crude protein dietary treatments are not observed if pigs are not subjected to a severe low protein diet for a prolonged time.

Serum urea nitrogen is the principal end product of protein catabolism. Amino acid catabolism results in ammonia that is transformed into urea, which it will be transported via blood circulation to the kidney for filtration and posterior excretion via urine (54). Serum urea nitrogen has been used previously as a predictor of efficiency of dietary crude protein utilization (15, 17, 55) and dietary amino acid requirements (11, 12, 16). Serum urea nitrogen was increased in pigs fed the HCP diet in the present study. This finding is in agreement with previous literature that observed increased SUN concentration due to excessive consumption of protein, which was then inefficiently used (14, 15, 17). The same happens when pigs are fed lysine insufficient diets up to 60% of the pigs' requirements due to an increase of extra free amino acids which will be catabolized through deamination, after the first limiting amino acid is used up (11, 12, 16). The latter was not observed in pigs fed the low crude protein dietary treatment probably because the crude protein and amino acid levels were not so restrictive for finishing pigs, and because amino acids were formulated based on the ideal protein concept (56). Coma et al. (16) stated that the feeding time

required to obtain a constant SUN concentration after changing the diet is 3 days. Overall, SUN may be a good indicator of protein efficiency in pigs. Nevertheless, it is worth mentioning that SUN may be altered due to an increase of energy intake (57) or by the use of low crude protein diets supplemented with synthetic amino acids as they could decrease the cation:anion ratio obtaining lower SUN concentrations (16).

Serum creatinine was lower when pigs were fed the LNE diet versus the HNE diet. This result is contrary to that of Hong et al. (17) who found that finishing pigs fed a low energy diet (13.65 MJ/ME) had higher levels of creatinine than pigs fed a high energy diet (14.07 MJ/ME) because of more lean tissue and less fat depositions after 13 weeks of changing the diet. Creatinine (90–240 μmol/L) is produced as the result of normal muscle metabolism (42). Thus, serum creatinine has a positive correlation with total and striated muscle (58, 59). Nevertheless, the discrepancy between the present study and that of Hong et al. (17) could be attributed to the duration of the feeding regime. The present study fed the diets for 10 days to finishing pigs. The difference in added fat and dietary protein/energy ratio between the low and high energy diet could have had a role in changing the protein/lipid metabolism, increasing the serum creatinine concentration due to an increase of carcass fatness and a reduction of carcass leanness (60, 61). This change in the protein/lipid metabolism might be exacerbated based on the protein deposition curve over the grower-finisher period, which declines in finishing pigs (45–47).

Glucose (4.7–8.3 mmol/L) is the principal source of energy for animal cells (42). Pigs fed the LNE diet tended to have lower serum glucose concentration than pigs fed the HNE diet in the present study. Previous literature did not find differences in serum glucose concentration when diets had the same energy levels (11–13) which is consistent with the results obtained in the present study. Nevertheless, Mule et al. (14) found decreased serum glucose concentration at the end of the finisher phase in pigs fed a HCP diet. The same authors attributed this difference due to the decreased amount of carbohydrates in the HCP diet, as glucose is the most important product of carbohydrate metabolism. However, this finding was not observed in the present study, maybe due to a short term feeding regime of the dietary treatments in comparison to that of Mule et al. (14).

Triglycerides are involved in lipid metabolism and the major source of lipid comes from the diet. Pigs fed the HNE diet showed increased serum triglycerides concentration than pigs fed the LNE diet. Lipids are a concentrated energy source and supplemental fats and oils may be added to swine diets to increase energy density of the diet (18). Fats and oils are highly digestible energy sources for pigs and apparent total tract digestibility of lipids is increased with age (18). Therefore, increased serum triglycerides concentration in pigs fed the HNE diet might be explained because of the increased added oil in the diet compared to no added oil in the low net energy diet, affecting the lipid metabolism. Mule et al. (14) reported lower serum triglycerides concentration in finishing pigs fed a HCP diet which seems to be consistent with other research that found a significant correlation between dietary protein restriction and body fat deposition (62). This outcome was not observed in the present study, maybe because of the short term feeding regime of the dietary treatments and/or that HCP diet had a higher inclusion of oil than low crude protein diet.

Cholesterol (1.4–3.1 mmol/L) it is also involved in lipid metabolism and it is derived from dietary sources and synthesized in vivo from acetyl-CoA in the liver as the main site (42). Pigs fed the LCP diet had numerically high serum cholesterol concentration. Previous literature reported a hypercholesterolemic effect when pigs are subjected to dietary protein restriction (19) and amino acid deficient diets (12, 14). It is not clear yet the exact mechanism for the increased serum cholesterol in LCP diets, although earlier studies indicated the insulin/glucagon ratio as an early metabolic index of the effect of dietary proteins and serum amino acids on serum cholesterol levels (63), or that serum albumin acts as a shuttle to enhance cholesterol efflux from cells (64). However, no differences were observed in serum cholesterol concentration between HCP and LCP dietary treatments in the present study. This discrepancy could be attributed to the high added fat in the HCP diet or because of the amino acid levels were not as restricted as previous studies (12, 14). Nevertheless, pigs fed the LNE diet had lower serum cholesterol concentration than pigs fed the LCP diet, which could be related to the no added fat in the LNE diet.

Taken together, the blood serum metabolite profile might be useful towards understanding dietary imbalances and SUN seems to be the best indicator in terms of protein efficiency. Nevertheless, further work needs to be done to establish standard intervals of each serum metabolite to understand whether pigs are fed over or below nutrient requirements, taking into account the pig genetics (14, 55). Further research could also be conducted to determine the separate effect of crude protein and amino acids in the blood metabolite profile.

Volatile fatty acids profile

No differences in the total VFA profile were observed between the dietary treatments. Previous literature reported that a reduction of crude protein in the diet reduced the production of short- and branched-chain fatty acids in manure in growing and finishing pigs (20, 65, 66). Nevertheless, the VFA profile changes from the colon to the manure after a few days of storage (67), so faecal VFA profile might not be comparable to the manure VFA profile. Moreover, the non-difference in total VFA might be explained by the fact that finishing pigs have already a developed gastrointestinal tract with a high fermentation capacity that makes it difficult to observe differences between the dietary treatments of the present study. On this line, a recent study reported that the increased body weight and age of the pigs resulted in an improved digestibility of dietary fiber fractions, which will influence the VFA production (68). In addition, previous literature also showed that the concentration of VFA in faeces is positively correlated with the apparent total tract digestibility of insoluble dietary fiber and cellulose, which are the best factors for predicting faecal VFA concentration (69). Therefore, the difference observed in the BCFA between pigs fed the LNE and HNE diet could be related to the added fiber in the LNE diet. Soybean hulls have a great fermentation capacity (69), which could have produced a shift in the VFA profile reducing the BCFA production by the microbial population. On the same page, the differences observed from pigs fed the control dietary treatment could be attributable to a low fiber content which was an unexpected outcome. Ziemer et al. (70) reported that changes in the faecal VFA profile are influenced by the percentage of cellulose added to the diet, while the level of crude protein in the diet influences the manure VFA profile. Therefore, it could be useful to add the analysis of manure as an indicator of dietary imbalances.

Moreover, the non-analysed microbiome gastrointestinal tract could have given a clue of the VFA profile (20).

Thus far, the evidence presented in the present study suggests that blood metabolite and VFA profiles may be affected even before a visual change in growth performance is observed. Furthermore, the changes observed in the blood metabolite and VFA profile could give us a clue whether pigs are fed over or below the nutrient requirements and the high or low inclusion of some nutrients, such as fat or fiber in the diets. Ultimately, the early detection of blood metabolites and/or VFA changes in pigs could improve performance and health and prevent economic losses for pig producers because of inefficiency use of the diets or changes in carcass composition.

Conclusion

The present study enhances our understanding of changes in the blood serum metabolite and faecal VFA profiles due to nutrient dietary modifications in finishing pigs. Dietary changes affect the blood serum metabolite profile in a short-term feeding regime. Serum urea nitrogen seems to be the best indicator related to protein efficiency. Low crude protein and amino acid diets may cause a hypercholesterolemic effect that should be further researched. The addition of fat in the diets causes increased levels of triglycerides and creatinine in pigs fed a high net energy diet. Faecal VFA profile was affected due to dietary changes. Fiber and not crude protein may play a key role in the composition of faecal VFA in finishing pigs with a high gastrointestinal tract fermentation capacity. Further studies are needed to fully understand the implications of specific dietary nutrients on blood serum and faecal VFA profile and establish standard intervals of each serum metabolite and VFA to detect whether pigs are fed over or below nutrient requirements at farm level and improve feed efficiency and farm sustainability.

Abbreviations

ADFI: Average Daily Feed Intake; ADG: Average Daily Gain; BCFA: Branched-Chain Fatty Acids; SUN: Serum Urea Nitrogen; BW: Body Weight; C: Control; CP: Crude Protein; FCR: Feed Conversion Ratio; HCP: High Crude Protein; HNE: High Net Energy; LCP: Low Crude Protein; LNE: Low Net Energy; Lys: Lysine; NE: Net Energy; SID: Standardized Ileal Digestible; VFA: Volatile Fatty Acids.

Declarations

Authors' contributions

J.C.M and E.G.M conceived and designed the study. J.C.M collected the data. J.C.M and E.G.M analysed the data. J.C.M drafted the manuscript. E.G.M, D.S.O, R.M, J.G and N.L. reviewed and edited the manuscript. E.G.M supervised the study. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study received ethical approval from the Teagasc Animal Ethics Committee (TAEC 244/2019). The authors confirm that they have followed the European Union Directive 2010/63 on the protection of animals used for scientific purposes.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

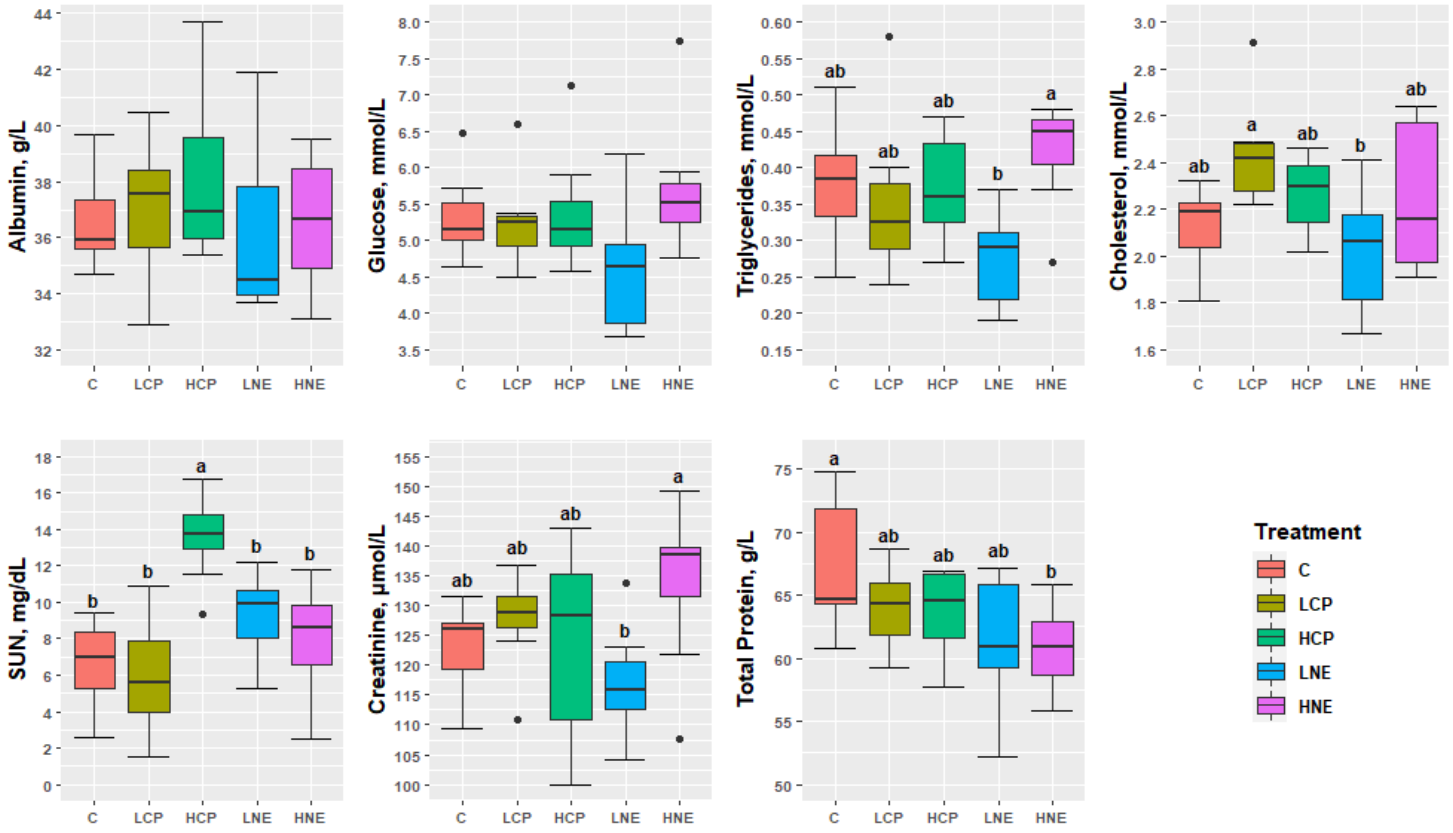

Figure 1

Blood serum metabolites profile from 40 finishing pigs grouped by dietary treatment (n = 4) at 20 weeks of age. Blood serum metabolites profile included albumin (g/L), glucose (mmol/L), triglycerides (mmol/L), cholesterol (mmol/L), serum urea nitrogen (SUN; mg/dL), creatinine (mmol/L), and total protein (g/L) (Means ± Standard error mean); Dietary treatments: Control (10.03 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed), LCP (Low Crude Protein; 10.03 MJ/kg of NE; 132.0 g of CP; 7.5 g of SID Lys per kg of feed), HCP (High Crude Protein; 10.03 MJ/kg of NE; 188.0 g of CP; 11.5 g of SID Lys per kg of feed), LNE (Low Net Energy; 9.61 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed), and HNE (High Net Energy; 10.45 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed); a, b Significant differences between treatments ($P < 0.05$).

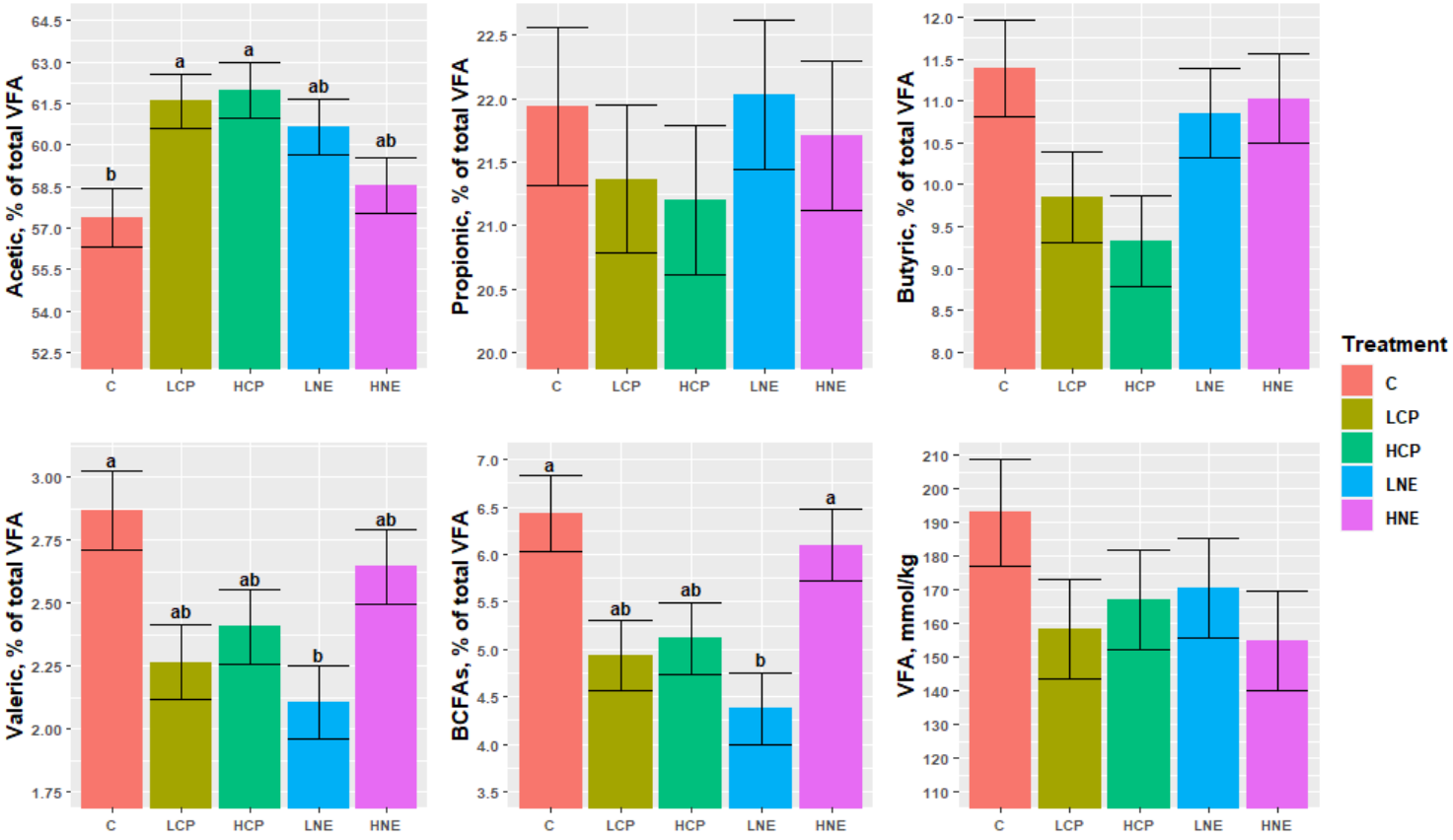

Figure 2

Volatile fatty acid (VFA) profile from 40 finishing pigs grouped by dietary treatment (n = 4) at 20 weeks of age. VFA profile (mmol/kg) included acetic, propionic, butyric, valeric, and branched-chain fatty acids (BCFAs) as % of total VFA (Means ± Standard error mean); Dietary treatments: Control (10.03 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed), LCP (Low Crude Protein; 10.03 MJ/kg of NE; 132.0 g of CP; 7.5 g of SID Lys per kg of feed), HCP (High Crude Protein; 10.03 MJ/kg of NE; 188.0 g of CP; 11.5 g of SID Lys per kg of feed), LNE (Low Net Energy; 9.61 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed), and HNE (High Net Energy; 10.45 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed); a, b Significant differences between treatments ($P < 0.05$).