Nitrogen and energy partitioning in two genetic groups of pigs fed low-protein diets at 130 kg body weight

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Abstract

The aim was to evaluate the effect of low-protein (LP) or low-amino acid diets on digestibility, energy and nitrogen (N) utilisation in 2 genetic groups (GG) of pigs (129±11 kg BW). Duroc Large White (A) pigs were chosen to represent a traditional GG for ham production, and Danbred Duroc (D) pigs to represent a GG with fast growing rate and high carcass yield. Dietary treatments: a conventional diet (CONV) containing 13.2% CP, and two LP diets, one with LP (10.4%) and low essential AA (LP1), the second with LP (9.7%) and high essential AA (LP2). Compared to CONV, LP2 had the same essential AA content per unit feed, while LP1 the same essential AA content per unit CP. Feed was restricted (DMI=6.8% BW75). Four consecutive digestibility/balances periods were conducted with 24 barrows, 12 A and 12 D. Metabolic cages and respiration chambers were used. No significant difference between diets was registered for digestibility. Nitrogen excreted: 41.3, 33.4 and 29.0 g/d (P=0.009), for CONV, LP1 and LP2 diets, respectively. Nitrogen retention was similar between the diets. Heat production (HP) was the lowest for LP diets. There was a tendency (P=0.079) for a lower energy digestibility in D group. The D pigs also had a higher HP and hence a lower retained energy in comparison with the A pigs. In conclusion: it is possible to reduce N excretion using very LP diets and LP low AA diets; Danbred GG have a higher heat production and a lower energy retention than A pigs.

Introduction

To be labelled as protected designation of origin (PDO) product, the Italian dry cured ham must be produced complying the Consortia guidelines establishing that at slaughter pigs must be at least 9 months old, 160 kg body weight (BW)±10%, with optimal carcass and ham fat coverings (European Commission, 1996). A restricted energy regime must be applied to achieve this goal (Mordenti et al., 2003), and a further protein restriction might be required for pigs with a high potential for a fast lean growth rate (Bosi and Russo, 2004). Recently, pigs of traditional genetic groups (GG) were partially replaced by commercial pigs with a better feed efficiency, but with carcass and hams too lean for PDO ham production (Lo Fiego et al., 2005). A reduction in the protein supply might be useful for these kinds of pigs. The use of low-protein diets was proposed to reduce the nitrogen (N) excretion from pig farms (Schiavon et al., 2009; Galassi et al., 2010; Gallo et al., 2014); however, different GG pigs would perform differently when exposed to the same diet (Bosi and Russo, 2004; Pelosi et al., 2010). The effects of low-protein diets or low-amino acid diets on nutrient digestion, metabolism (Hoffmann et al., 1999; Jentsch et al., 1993; Noblet et al., 1994; Schiennmann et al., 1989) and excretion (Scipioni and Martelli, 2001; Prandini et al. 2013; Zanfi et al., 2014) on pigs above 120 kg BW have been studied. From 120 to 160 kg BW the use of low-protein diets might exert relatively small effects on body protein and energy partitioning, as the pigs are approaching their physiologic maturity. However, as the feed consumed in this BW range is about 50% that required for the whole production cycle, the effects of energy and protein restriction in heavy pigs of different GG need to be investigated. In this experiment was investigated the effects of diets with conventional or reduced crude protein (CP) and essential amino acids (AA) contents on the energy and N partitioning of 130 kg BW pigs belonging to a traditional or a commercial crossbred type GG were investigated.

Materials and methods

All animals were cared for in accordance with the guidelines on animal welfare in animal research of the Italian Legislative decree no. 116/1992 (Italian Regulation, 1992).
national breeders association: ANAS) by breeding Italian Duroc boars with Italian Large White sows and Danbred Duroc GG (D). The first genetic line was chosen because it received a genetic pressure based on carcass and ham quality traits (Fontanesi et al., 2012; Cecchinato et al., 2008), the second line was chosen because of the high selective pressure to increase daily gain, lean meat percentage and feed efficiency. The animals of the 2 GG were acquired from 2 commercial herds taking care that all the piglets were born within the same week.

Twenty four pigs of 101±8.4 kg BW, 12 per each of the 2 GG, were allotted in 6 pens (4 animals per pen) and fed the 3 experimental diets (2 pens per diet: 1 of A pigs and 1 of D pigs). After 3 weeks 6 pigs (1 pig A and 1 pig D per each of the 3 diets) were housed individually in metabolic cages for the first of 4 digestibility/balances periods. Each digestibility period lasted 14 days: 7 days of cage adaptation, and 7 days of measurements to determine the digestibility of the diets and the N and energy balances. The experimental design was a factorial design, with 3 diets 2 GG 4 periods. Globally, the experiment involved 12 pigs per GG and 8 pigs per diet, and lasted 56 days. The average BW of the pigs in the trial period was 129±11 kg.

Digestibility, nitrogen and energy balances

During each of the 4 measurement periods the animals in the cages were placed individually in an open-circuit respiration chamber described by Crovetto (1984) to measure respiratory exchange over three consecutive 24 h cycles.

Heat production (HP) for each animal was calculated from Brouwer’s equation (1965):

\[ HP \text{ (kJ/d)} = (16.175\ O_2 + (5.021\ CO_2) - (2.167\ CH_4)) \times (5.987\ N) \]

where: O₂, CO₂ and CH₄ are the volumes (l/d) of the gases at standard temperature (0°C) and pressure (760 mm Hg) conditions, consumed or produced during respiration and N is the urinary nitrogen (g/d). Corrections for personnel entrance were applied.

During the digestibility and metabolic trial pigs were fed at 08:00 and at 17:00 h. During each measurement period urine was collected individually in a vessel containing 150 mL of a 20% (vol/vol) H₂SO₄ solution to maintain the pH below 2.5 and avoid ammonia loss. Urine was weighed daily, sampled (10% of total weight), pooled per pig and frozen (-20°C) for subsequent chemical analysis. Individual faeces were weighed daily, sampled (10% of total weight), pooled per pig and frozen (-20°C) for subsequent chemical analysis. Individual faecal weights, pooled per pig and frozen (-20°C) for subsequent chemical analysis.

Calculated SID amino acid content°

| Ingredient° | Diet | CONV | LP1 | LP2 |
|-------------|------|------|-----|-----|
| Corn grain  |      | 381.3| 382.4| 541.3|
| Barley grain|      | 200.0| 200.0| 340.0|
| Wheat grain |      | 106.7| 200.9| 0.0  |
| Soybean meal|      | 91.7 | 0.0  | 0.0  |
| Wheat bran  |      | 80.0 | 80.0 | 40.0 |
| Wheat middling |    | 60.0 | 60.0 | 0.0  |
| Can mouse  |      | 40.0 | 40.0 | 40.0 |
| Beef tallow |      | 14.0 | 11.0 | 8.0  |
| Calcium carbonate | | 13.5 | 13.7 | 11.5 |
| Dicalcium phosphate |   | 2.0 | 2.2 | 6.0 |
| Sodium bicarbonate |    | 2.5 | 2.5 | 2.5 |
| Sodium chloride |    | 3.0 | 3.0 | 3.0 |
| Vitamin and mineral premix° | | 2.0 | 2.0 | 2.0 |
| Choline HCl |      | 0.4 | 0.4 | 0.4 |
| L-Lysine |      | 0.0 | 1.4 | 2.8 |
| L-Threonine |    | 0.0 | 0.5 | 1.5 |
| L-Tryptophan |    | 0.0 | 0.1 | 0.5 |
| DL-Methionine |   | 0.0 | 0.0 | 0.5 |

CONV, conventional diet; LP1, low protein and low essential amino acids content diet; LP2, low protein and conventional essential amino acids content diet. °Actual daily loads of feed ingredients recorded by the weighing platforms of the feed firm. Providing the following per kilogram of diet: vitamin A, 7200 IU; vitamin D₃, 1600 IU; vitamin E, 32 mg; vitamin K₃, 1.68 mg; vitamin B₁, 1.2 mg; vitamin B₂, 3.2 mg; vitamin B₆, 2.4 mg; vitamin B₁₂, 0.016 mg; d-pantothenic acid, 16.2 mg; zinc, 105 mg; copper, 16 mg; iodine, 1.5 mg; iron, 182 mg; manganese, 75 mg; selenium, 0.36 mg.

Table 1. Ingredient composition (g/kg as fed) of the experimental diets.

Table 2. Nutrient content (g/kg as fed, unless otherwise indicated) of the experimental diets.

| Diet | CONV | LP1 | LP2 |
|------|------|-----|-----|
| Analysed nutrient composition DM | 882 | 883 | 885 |
| CP (N×6.25) | 132 | 104 | 97 |
| Starch | 443 | 486 | 519 |
| NDF | 121 | 129 | 120 |
| ADF | 38 | 39 | 36 |
| EE | 40 | 38 | 35 |
| Ash | 44 | 40 | 40 |
| ME, MJ/kg | 13.85 | 13.79 | 13.82 |
| Calculated nutrient composition° | | | |
| ME, MJ/kg | 13.17 | 13.19 | 13.21 |
| NE, MJ/kg | 10.02 | 10.16 | 10.26 |
| CP (N×6.25) | 133 | 101 | 96 |
| Fermentable fibre | 101 | 84 | 83 |
| Calculated total amino acid content° | | | |
| Lysine | 5.5 | 4.3 | 5.1 |
| Methionine | 2.2 | 1.9 | 2.2 |
| Threonine | 4.6 | 3.8 | 4.5 |
| Tryptophan | 1.5 | 1.2 | 1.4 |
| Calculated SID amino acid content° | | | |
| Lysine | 4.4 | 3.5 | 4.4 |
| Methionine | 1.9 | 1.6 | 1.9 |
| Threonine | 3.6 | 3.0 | 3.8 |
| Tryptophan | 1.1 | 0.8 | 1.1 |

CONV, conventional diet; LP1, low protein and low essential amino acids content diet; LP2, low protein and conventional essential amino acids content diet; DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; EE, ether extract; ME, metabolisable energy (determined by respiratory chambers); NE, net energy; SID, standardised ileal digestible amino acid.

°According to the National Research Council (2012).
ces were daily weighed and sampled (20% of total weight), pooled per pig and frozen (-20°C) for subsequent chemical analysis.

**Feed and excreta analysis**

All the diets were daily sampled to determine the DM content after drying at 55°C in a forced ventilation oven until constant weight. Furthermore, diet, faeces and urine daily samples were pooled for each period for further analysis. Before feeding, all remaining feed was removed from the trough, weighed and analysed for DM content. Analytical DM was determined by heating at 105°C for 3 h (AOAC, 1995, method 945.15), ash by incineration at 550°C for 2 h (AOAC, 1995, method 942.05), ether extract by solvent extraction (AOAC, 1995, method 920.29), N (wet faecal samples and urine) by the Kjeldahl method (AOAC, 1995, method 984.13), starch content was determined using Megazyme kit K-TSTA (Megazyme International Ireland Ltd., Wicklow, Ireland) for total starch assay procedure according to the method 996.11 (AOAC, 1998), NDF and ADF by Ankom® Fibre Analyzer (Ankom Technology Corporation, Fairport, NY) following the procedure of Mertens (2002) for NDF and Van Soest et al. (1991) for ADF. The GE of feeds, faeces and urine was measured using an adiabatic bomb calorimeter (IKA 4000; Ika, Staufen, Germany).

**Statistical analysis**

Data were analysed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA) according to the following linear model:

\[
y_{ijk} = \mu + \text{diet}_{i} + \text{GG}_{j} + \text{period}_{k} + e_{ijk}
\]

where \(y_{ijk}\) is the observed trait; \(\mu\) is the overall intercept of the model, diet\(_{i}\) is the fixed effect of the \(i\)th feeding treatment (\(i = 1, \ldots, 3\)), \(\text{GG}_{j}\) is the fixed effect of the genetic group (\(j = 1, 2\)), period\(_{k}\) is the fixed effect of the measurement period (\(k = 1, \ldots, 4\)), and \(e_{ijk}\) is the random residual. Residuals was assumed to be independently and normally distributed with a mean of zero and variance \(\sigma^2_e\).

For all data, the model initially included diet×period, GG×period, and diet×GG interactions as main effects; later the effects of the interactions were excluded from the model since they were not significant. For all statistical analyses, significance was declared at \(P \leq 0.05\) and trends at \(P \leq 0.10\).

**Results and discussion**

### Diets and excreta

Table 2 reports the analyses of the experimental diets in comparison with the expected data. The CP contents of the three diets were similar to those expected. On the contrary, the ME content of the three diets was higher than foreseen: on average 13.82 vs 13.19 MJ/kg. This has probably to be ascribed to the fact that the ME determined in the present experiment was obtained from heavy animals fed restricted, whilst the ME predicted by the National Research Council 2012 from the chemical analysis of the feedstuffs (Le Goff and Noblet, 2001; Noblet and Perez, 1993) is based on data obtained in literature and usually referred to light pigs fed ad libitum. As pointed out by Noblet and Shi (1994) the digestive ability of a heavy pig is higher than that of a lighter pig. Moreover, in the present experiment the urinary energy is low due to the low protein concentration of the diets, and this increases the ME concentration of the diets.

The LP diets did not include the soybean meal in order to decrease the CP concentration (and hence hopefully N excretion) in a physiological stage where the requirement of protein is low in comparison with previous stages (Bosi and Russo, 2004; National Research Council, 2012). Considering the feed intake of the animals fed the CONV, LP1 and LP2 diets (on average 2623, 2605 and 2580 g DM daily) and the calculated SID amino acid content of the diets (Table 2), the SID-lysine ingested with the 3 diets was 13.1, 10.3 and 12.8 g/d for the CONV, LP1 and LP2 diets, respectively. For the other essential AA supplied as crystalline AA in the LP diets (methionine, threonine and tryptophan) the ratios with lysine were similar to those expected. On the contrary, the SID-isoleucine, ingested with the CONV diet was equal to 392 g/d. Particularly, for the SID-isoleucine, intake of 11.6, 7.7 and 7.0 g/d can be predicted (National Research Council, 2012) respectively with CONV, LP1 and LP2 diets, compared to 8.4 g/d recommended by the National Research Council (2012) for pigs of 100-135 kg BW with a protein accretion of 115 g/d.

The amounts of faeces and urine produced by the animals on experiment do not show significant differences between diets, whilst a difference is registered between the 2 GG for the individual urinary yield: 3002 and 4854 g/d for A and D, respectively (\(P = 0.018\)). The higher intake which determined the higher urinary yield might be attributed to the high voracity of the D group, as suggested by Schiavon and Emmans (2000). For environmental reasons it is preferable to have smaller volumes of slurry and therefore a genotype seems better than D under this point of view.

### Apparent faecal digestibility

No significant difference between the experimental diets was registered for digestibility, (Table 3).

Looking at the differences between the 2 GG, the D group had a lower digestibility of EE (\(P = 0.010\)) and a trend for a lower digestibility of DM (\(P = 0.074\)) and energy (\(P = 0.079\)) in comparison with the A group. A possible explanation of this phenomenon might be a higher transit rate of the feed in the gastro-intestinal tract in the D pigs. However, to our knowledge there are no papers in literature comparing the feeding behavior of these 2 GG. Indeed, the Danbred genotype has been selected to grow faster under an ad libitum feed regime: the high feed transit rate can reduce digestibility, but this negative effect can be counterbalanced by a higher feed intake to attain similar or even better growth performance. In the present experiment pigs were fed restricted (the daily DM intake for the 2 GG was similar, on average 2605 g), but the D pigs could have had a faster gastro-intestinal transit rate anyway, for the genetic selection applied. Moreover, the presumably higher water intake (given the higher urinary yield) might have increased the GI transit time.
Nitrogen balance

The results of the N balance are reported in Table 4. Due to the lower CP concentration, the pigs fed the LP1 and LP2 diets had lower N intakes in comparison with the pigs fed the CONV diet (-22% and -28%, respectively; P<0.001). Faecal N was similar for the 3 diets in terms of both absolute values and as percentage of the N intake.

On the contrary, the amounts of urinary N with the LP diets were much lower (P=0.006) than the amount excreted with the diet CONV. Considering the N urinary excretion as a percentage of the intake N, the reduction in comparison with the CONV diet is not significant.

Globally, the N excreted by pigs fed the CONV, LP1 and LP2 diets, was respectively 41.3, 33.4 and 29.0 g/d, with significant differences between CONV and the LP diets (P=0.009).

Nitrogen retention was similar among all the diets when expressed both in absolute values and in percentage of the N intake.

Considering the N balance of the 2 GG, the only significant difference is the N intake, slightly in favor of the A group. This is due to the fact that one pig of the D group had some orts during the digestibility period. It has to be underlined that, with a restricted feed regime even a little difference in feed intake can be statistically significant, although not so important under a practical point of view.

Energy balance

The results of the energy balance are reported in Table 5. Consistently with the low N content of the LP diets seen above, the urinary energy loss was smaller (P=0.009) with the LP diets as compared to CONV diet. However, the ME content of the 3 diets is not different. On the contrary, for HP different losses were registered between the experimental diets. Particularly, the HP associated to the LP2 diet was lower (P=0.041) than that related to the LP1 diet, probably due to the less metabolic work required by diet LP2 to excrete N as urea.

No difference was registered between the diets in terms of retained energy, whilst the respiratory quotient (RQ) was higher (P=0.008) for the LP diets (1.24) in comparison with the CONV diet (1.18). A high RQ means a high fat deposition and this is consistent with a numerically lower N retention (g/d) of the LP diets in comparison with the CONV diet. This in turn is in agreement with Bunger et al. (2014), which found a slightly greater fat deposition in muscle of pigs fed with low-protein diet compared with control diet.

Looking at the energy balance of the 2 GG, the trend (P=0.079) for a lower energy digestibility in the D group led to a ME for the D group significantly lower (P=0.007) in absolute values and numerically lower

### Table 3. Apparent digestibility (%) of the experimental diets at 2 genetic lines of 129 (±9) kg body weight pigs.

| Diet | Genetic line   | DM | OM | CP | EE | ADF | Energy |
|------|----------------|----|----|----|----|-----|---------|
| CONV | ANAS | DANBRED | SEM | P | SEM | P |
| 87.2 | 86.9 | 88.3 | 0.75 | 0.314 | 88.0 | 86.8 | 0.44 | 0.074 |
| 88.8 | 88.5 | 90.0 | 0.67 | 0.218 | 89.6 | 88.7 | 0.39 | 0.114 |
| 84.9 | 81.8 | 82.9 | 1.24 | 0.159 | 83.4 | 83.0 | 0.72 | 0.760 |
| 72.9 | 73.4 | 71.8 | 2.16 | 0.817 | 75.3 | 70.1 | 1.24 | 0.010 |
| 56.1 | 54.9 | 57.7 | 2.58 | 0.427 | 57.3 | 55.2 | 1.49 | 0.340 |
| 41.8 | 36.2 | 36.7 | 3.80 | 0.868 | 39.9 | 36.5 | 2.19 | 0.507 |
| 87.1 | 86.8 | 88.8 | 0.72 | 0.309 | 88.0 | 86.8 | 0.43 | 0.079 |

CONV, conventional diet; LP1, low protein and low essential amino acids content diet; LP2, low protein and conventional essential amino acids content diet; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre. Values refer to 24 animals with 8 replications per diet and 12 replications per genetic lines. No Diet×Genetic line interaction was recorded.

### Table 4. Effects of dietary protein and essential amino acid content on nitrogen balance in 2 genetic lines of 129 (±9) kg body weight pigs.

| Diet | Genetic line   | NI, g/d | Faecal N g/d | % NI | Urinary N g/d | % NI | Excreted N g/d | % NI | Retained N g/d | % NI |
|------|----------------|---------|--------------|------|--------------|------|----------------|------|----------------|------|
| CONV | ANAS | DANBRED | SEM | P | SEM | P | SEM | P | SEM | P |
| 62.8 | 4.9 | 45.9 | 0.31 | <0.001 | 52.7 | 51.8 | 0.20 | 0.003 |
| 9.30 | 8.89 | 7.69 | 0.607 | 0.269 | 8.57 | 8.68 | 0.350 | 0.823 |
| 32.6 | 24.3 | 21.0 | 1.93 | 0.006 | 25.4 | 26.6 | 1.53 | 0.517 |
| 52.0 | 49.2 | 46.0 | 3.58 | 0.623 | 47.8 | 50.4 | 2.41 | 0.400 |
| 41.3 | 33.5 | 29.0 | 2.19 | 0.009 | 34.0 | 35.2 | 2.00 | 0.568 |
| 33.8 | 32.3 | 36.3 | 4.20 | 0.738 | 35.6 | 32.7 | 3.25 | 0.453 |

CONV, conventional diet; LP1, low protein and low essential amino acids content diet; LP2, low protein and conventional essential amino acids content diet; NI, nitrogen intake. *Within a row, means without a common superscript differ (P<0.05). Values refer to 24 animals with 8 replications per diet and 12 replications per genetic lines. No Diet×Genetic line interaction was recorded.
Table 5. Effects of dietary protein and essential amino acid content on energy balance in 2 genetic lines of 129 (±9) kg body weight pigs.

| Diet          | Genetic line | Metabolised E | CH4 E | Urinary E | Faecal E |
|---------------|--------------|---------------|-------|-----------|----------|
|               |              | EI (%)        | MJ/d  | EI (%)     | MJ/d     |
| CONV          |              | 84.6          | 41.1  | 41.1       | 20.7     |
| EI, MJ/d      |              | 84.1          | 40.7  | 40.3       | 21.4     |
| LP1           |              | 47.1          | 40.3  | 52.0       | 19.9     |
| LP2           |              | 0.26          | 0.52  | 0.82       | 0.40     |
| SEM           |              | 0.026         | 0.18  | 0.99       | 0.09     |
| P             |              | 0.009         | 0.06  | 0.055      | 0.04     |
| ANAS          |              | 48.3          | 41.4  | 85.6       | 41.2     |
| DANBRED       |              | 47.5          | 40.0  | 84.3       | 45.1     |
| SEM           |              | 0.15          | 0.30  | 0.47       | 0.57     |
| P             |              | 0.001         | 0.007 | <0.001     |          |

Conclusions

The overall experimental data obtained indicate that the LP diets are effective in decreasing N excretion significantly with no detrimental influence on nitrogen retention. Between the two low-protein diets, the LP2 had a lower energy loss in comparison with the LP1. Looking at the genetic groups, no difference was registered for the N balance, whilst A pigs had better energy utilization as compared to D pigs. The A pigs fed LP diets seem the most promising solution in view of a good dietary energy and nitrogen utilisation in the last fattening period of the heavy pigs.

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