Effects of a soybean-free diet supplied to Italian heavy pigs on fattening performance, and meat and dry-cured ham quality

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Abstract

The aim of this study was to investigate the effects of a diet containing non-conventional (i.e. alternative to soybean meal) vegetable protein sources on fattening performance, and meat and dry-cured ham quality of heavy pigs. Fifty-six (Landrace x Large White) castrated males with an initial average body weight of 50 kg were allocated to two experimental groups: a control group in which pigs received a traditional soybean meal-based diet, and a treatment group in which soybean meal was replaced by vegetable protein sources (i.e. sunflower meal, potato protein, corn gluten feed, faba beans and dehydrated alfalfa meal), mainly locally grown and not genetically modified. Pigs were slaughtered at approximately 160 kg body weight. Dietary treatment had no significant effect on fattening performance, or meat, fat or dry-cured ham properties. Results suggest that it is possible to feed heavy pigs a soybean-free diet without impairing fattening performance or the quality of meat and Italian PDO (Protected Designation of Origin) hams.

Introduction

European livestock production largely depends on imported (approx. 35-40 million tons per year) soy and soy raw materials (FEFAC, 2007; GMO Compass, 2011).

Soy meal is used widely in some types of animal feed. In the case of swine, soybean is an excellent protein source when fed to growing-finishing pigs (100 kg final weight) as the sole source of supplemental protein in the diet (Shelton et al., 2001).

To limit soybean imports from non-EU countries, which weigh negatively upon the trade balance, for some years economic incentives have been provided across Europe to encourage the production and use of alternative protein sources such as legume seeds and oil crops (Wiseman and Cole, 1998). Moreover, most of the soy and soy raw materials come from countries such as the USA, Brazil and Argentina where the cultivation of genetically modified (GMO) crops is permitted. Figures for 2010 indicate that approximately 93% of the US soybean and 98% of Argentinean soybean is GMO (GMO Compass, 2011).

It is well known that consumer attitudes toward GMO food products are largely negative in many of the developed countries in the European Union, as well as in Japan (Aruga, 2011), and several producers have taken a cautious stance towards these organisms and banned their use in some animal production chains. The organic production chain, implemented according to European Commission Regulation n. 834 (European Commission, 2007) is a very good example of this. Italy has a large soybean deficit, although this is less than the rest of Europe, and it has been estimated that approximately 400,000 tons of GMO soybean seed and approximately 2.3 million tons of flour obtained from GMO seed are imported each year (Mordenti and De Castro, 2003). Well before the spread of GMO crops, the use of alternative feedstuffs as replacements for soybean had been investigated around the world as part of efforts to reduce the costs of feed in swine production (Thacker and Kirkwood, 1999). However, it should be remembered that the supply of these alternative raw materials on the market is not always optimal, as their availability is still tied to the seasonal nature of the crops themselves.

Non-conventional protein sources can be separated into two main categories: feedstuffs that derive directly from crop production and raw materials obtained from crop fractionation (by-products and co-products) (Zijlstra and Beltranena, 2007). The first group includes all crops suitable for swine feeding that are usually cultivated in the Mediterranean area: pea bean (Pisum sativum), lupin (Lupinus spp.), common bean (Phaseolus vulgaris), sunflower (Helianthus annuus), faba bean (Vicia faba), grass pea (Lathyrus sativus) and dehydrated alfalfa meal (Medicago sativa). Crop fractionation is the separation of grain stock into two or more fractions: corn and wheat gluten meal, concentrated potato protein, wheat and corn distiller’s dried grain are examples of the second group of feedstuffs. Both groups are generally combined for use to reduce energetic and amino acid supplementation, to limit the concentration of anti-nutritional factors in diets and, in the specific case of the Italian heavy pig, to comply with the dietary inclusion limits established by the Consortium for Parma Ham (1992).

The use of non-conventional protein sources has been investigated in pig feeding by many researchers in all European Union (EU) countries but unfortunately the majority of these studies have mainly focused on nitrogen digestibility and nitrogen balance (Jansman et al., 1993; Flis et al., 1999; Seabra et al., 2001; Mariscal-Landin et al., 2002; Trombetta and Matti, 2005; Trombetta et al., 2006; Martelli et al., 2009; Zeiher et al., 2010) rather than on assessing carcass and meat (raw and cured) qualitative traits. Furthermore, available information mainly refers to the outcomes of applying extensive rearing systems and/or the organic method (Sundrum et al., 2000; Hoffman et al., 2003; Olsson et al., 2003; Milet et al., 2004; Trombetta et al., 2009), in which the use of alternative protein source interacts with rearing conditions influencing the final outcomes.

The aim of this study was to provide some new insights into the possible effects of a soybean-free diet containing a pool of alternative protein sources on the development of pigs used to produce Italian PDO hams.

Key words: Pig, Soybean-free diet, Fattening performance, Meat quality, Cured ham.

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locally grown protein sources on the quality of the carcass and fresh and cured meat of Italian heavy pigs, and its applicability in the dry-currying PDO (Protected Designation of Origin) ham production chain.

Materials and methods

The University of Bologna Ethics Committee on the care and use of laboratory animals reviewed and approved the experimental protocol. The experiment was conducted in the growing-finishning facilities of the Faculty of Veterinary Medicine of the University of Bologna, northern Italy, in accordance with current Italian legislation implementing European Council Directive n. 120 on the protection of pigs (European Commission, 2008).

Animals, housing and feeding

Fifty-six crossbred (Landrace x Large White) castrated males, taken from 6 litters, with an initial average body weight (BW) of 50 kg (average age 110 days) were used. The piglets were individually identified at birth; during the pre-experimental period (from birth to 50 kg live weight) they were all reared under the same conditions and fed with commercial diets. Subsequently, pigs were allocated (on the basis of litter and BW) to two experimental groups, each containing four collective pens with a completely slatted floor. Each pen (i.e. replicate) contained 7 pigs and was equipped with a bite drinker and a collective stainless steel feeder (0.3 m wide, 3.5 m long). The environment was enriched by providing steel hanging chains.

Pens were placed in temperature-controlled rooms (22°C) equipped with a forced-air ventilation system. Water was available ad libitum. Feed was offered in meal form twice a day (at 08:30 and 16:30) and pigs were fed at 9% of their metabolic body weight (BW^{0.75}); starting from 120 kg of BW feed intake was restricted to 2.9 kg dry matter (DM) per pig per day. The two groups were fed as follows (Table 1): the control group received a traditional soybean meal-based diet (20% in the first phase and 14% in the second); the treatment group (soybean-free) received a diet containing raw ingredients alternative to soybean, i.e. sunflower meal (7%), corn gluten feed (5%, supplied only in the first phase), faba beans (5%), dehydrated alfalfa meal (2%, supplied only in the first phase) and potato protein (2% of the first phase and 3% in the second). Each single ingredient was included at a low percentage (i.e. not exceeding 8% dry matter) according to the rules established by the Consortium for Parma Ham (1992).

The different feed formulations (50-80 kg and 80-160 kg) were all able to meet dietary requirements both in the growing and fattening periods according to the French Institut National de la Recherche Agronomique recommendations (INRA, 1989). All the diets were completely vegetable-based and were formulated so as to supply the same amounts of digestible energy (DE) and protein. To meet the pigs’ requirements, both formulations provided different amounts of amino acids.

Parameters measured during the growing phase

To calculate the average daily gain (ADG), the animals were individually weighed at the beginning of the trial, after 42 days (live weight: approx. 80 kg), after 102 days (live weight: approx. 120 kg) and at the end of the trial (174 days). The feed intake (FI) of every pen was recorded daily in order to calculate the feed conversion rate (FCR). The trial lasted up to the 174th day, by which time the pigs had reached a BW of approximately 160 kg and were ready for slaughter according to the rules established by the Consortium for Parma Ham (1992) for PDO Parma Ham production.

| Table 1. Composition of the diets. |
|-----------------------------------|
| Ingredients, % | Growing, 50-80 kg BW | Fattening, 80-160 kg BW |
|----------------|----------------------|-------------------------|
|                | Control | Soybean-free | Control | Soybean-free |
| Corn meal      | 50.00   | 49.50       | 53.00   | 55.00        |
| Soybean meal   | 20.00   | -           | 14.00   | -            |
| Barley meal    | 12.00   | 14.70       | 24.05   | 22.04        |
| Wheat bran     | 14.00   | 10.00       | 6.00    | 5.00         |
| Sunflower meal | -       | 7.00        | -       | 7.00         |
| Corn gluten feed | -     | 5.00        | -       | -            |
| Faba beans     | -       | 5.00        | -       | 5.00         |
| Dehydrated alfalfa meal | - | 2.00 | - | 3.00 |
| Potato protein | -       | 2.00        | -       | 3.00         |
| Soybean oil    | 0.50    | 1.10        | -       | -            |
| Calcium carbonate | 1.65 | 1.55       | 1.20    | 1.20         |
| Dicalcium phosphate | 0.85 | 0.90    | 0.75    | 0.70         |
| Premix (trace elements and vitamins) | 0.50 | 0.50 | 0.50 | 0.50 |
| Sodium chloride | 0.42    | 0.40        | 0.40    | 0.40         |
| L- Lysine      | 0.08    | 0.35        | 0.10    | 0.16         |
| Crude protein  | 15.72   | 15.69       | 13.84   | 13.75        |
| Ether extract  | 3.18    | 3.88        | 2.85    | 2.92         |
| Etherv fibre   | 4.67    | 4.88        | 3.71    | 4.15         |
| Calcium, %     | 5.93    | 5.43        | 4.76    | 4.51         |
| Sodium, %      | 0.18    | 0.18        | 0.17    | 0.17         |
| Ash, %         | 0.92    | 0.91        | 0.72    | 0.72         |
| Phosphorus, %  | 0.65    | 0.62        | 0.51    | 0.51         |
| DE, kcal/kg    | 3183    | 3182        | 3164    | 3148         |
| NE, kcal/g     | 2297    | 2291        | 2314    | 2315         |
| Lysine, %      | 0.84    | 0.84        | 0.70    | 0.71         |
| Methionine+cystine, % | 0.55 | 0.61 | 0.50 | 0.52 |
| Threonine, %   | 0.62    | 0.57        | 0.58    | 0.52         |
| Fatty acid composition |
| C 14:0, % TFA | 1.39    | 1.26        | 1.72    | 1.83         |
| C 16:0, % TFA | 14.57   | 14.02       | 15.24   | 14.62        |
| C 16:1, % TFA | 0.08    | 0.12        | 0.09    | 0.10         |
| C 18:0, % TFA | 2.43    | 2.53        | 2.16    | 2.05         |
| C 18:1, % TFA | 20.92   | 22.72       | 20.98   | 23.29        |
| C 18:2, % TFA | 55.74   | 54.35       | 55.80   | 54.92        |
| C 18:3n-3, % TFA | 4.07 | 4.15   | 3.05    | 2.17         |
| Others, % TFA | 0.77    | 0.79        | 0.96    | 1.01         |

BW, body weight; DE, digestible energy; NE, net energy; TFA, total fatty acid.
Slaughtering parameters, meat and fat quality

All pigs were slaughtered on the same day following 12-h fasting. After slaughter, carcass weight and lean meat content were measured using a Fat-o-Meater according to the European Commission’s Directive n. 468 (European Commission, 2001) and the dressing percentage calculated. At 45 min post mortem, the pH value of both the Semimembranosus and Longissimus dorsi muscles was measured in duplicate by means of an Orion portable pH meter (model 250A, Orion Research, Boston, MA, USA). Thereafter, each carcass was dissected into the main commercial cuts (thigh, loin and neck, shoulder, belly, jowl, and lard). At 24 h post mortem, a second reading was taken of the pH value of the Semimembranosus muscle. The color of the thighs (Semimembranosus muscle) was determined in the fresh cut according to the L*a*b* system (McLaren, 1980) using a Minolta Chroma Meter CR-200 (Minolta Camera, Osaka, Japan). Hue and chroma were subsequently calculated as follows: hue=arctan (b*/a*); chroma=\sqrt{(a*^2+b*^2)}.

A 20-mm thick slice (150 g) of Longissimus dorsi muscle at the last rib was taken from all pigs along with samples of subcutaneous fat from the area overlying the Biceps femoris muscle. Subcutaneous fat samples were randomly taken from 28 pigs (14 per group), vacuum packed, frozen and stored at -20°C. Drip loss in the Longissimus dorsi muscle was determined using Honikel’s gravimetric method (Honikel, 1998) according to which a meat sample of approximately 100 g (±0.5 g) was placed inside a plastic box on a supporting mesh (to ensure that the sample was not in contact with the box) and sealed. After 24 h storage at 4°C, the samples were taken out of the box, dabbed lightly on filter paper and weighed again. Drip loss was expressed as a percentage of the initial weight.

The proximate chemical composition of the Longissimus dorsi muscle was determined in freeze-dried milled samples according to the standard methods of the Association of Official Analytical Chemists (AOAC, 2000).

Total lipids were extracted from each sample of subcutaneous fat by means of the chloroform/methanol (2:1, v/v) method described by Folch et al. (1957) and measured gravimetrically. The fatty acid compositions of subcutaneous fat (outer and middle/inner layers) were determined by gas chromatography (HRGC 8560 Series Mega 2 gas chromatograph; Fisons Instruments, Milan, Italy). Fatty acids were esterified using 5% methanolic hydrogen chloride. The fatty acid methyl esters were separated by gas chromatography using a Supelco SP-2330 capillary column (length 30 m, internal diameter 0.25 mm, film thickness 0.2 μm; Supelco, Bellefonte, PA, USA). Injector and detector temperatures were kept at 220°C and 280°C, respectively.

The column was programmed as follows: 140°C for 1 min after which the temperature was then raised to 220°C (3°C/min) and held constant for 15 min. Fatty acids were identified by comparing the retention times of the peaks with those of known standards. Results are expressed as percentages of total fatty acids. The iodine number was determined according to the AOAC method (2000).

Processing weight losses, fatty acid composition and panel test of cured hams

Twenty-eight thighs per group, derived from the right side, were cured according to Parma Ham production methods. Thighs were monitored over an 18-month curing period, and weight losses were recorded after trimming, after salting and at the end of seasoning. At the end of seasoning, a subsample of 28 cured hams (14 per experimental group) was randomly selected for dissection. With the same techniques (Minolta colorimeter) as described for the raw thighs, the color of semimembranosus muscle and subcutaneous fat was measured. Samples of Biceps femoris muscle were collected to determine moisture, protein, fat and ash content according to AOAC methods (AOAC, 2000). Sodium chloride content and proteolysis indexes (non-protein N/Total N) were determined in the same samples of muscle of the cured hams, according to Baldini et al. (1992). The fatty acid content of the subcutaneous fat was analyzed by gas chromatography, according to the method described above for the subcutaneous fat of the raw thighs. The selected cured hams were evaluated in one single session by a panel of 5 trained experts, who rated hams on the basis of a checklist for subjective visual and tactile assessment of ham characteristics: the lean portion was assessed for firmness, color homogeneity, brightness and marbling, and the fat portion for firmness and fat thickness. The evaluation was expressed on a scale ranging from 1 (absence) to 10 (maximum). Overall evaluation was assessed as the general impression of the panelist when evaluating a ham, and was likewise expressed on a scale ranging from 1 to 10, 10 being attributed to good quality ham (optimal characteristics) and 1 to very poor quality ham.

Statistical analysis

The experimental data obtained were submitted to one-way analysis of variance (GLM procedure) with diet as the assumed main effect (SAS, 1999). The pen (7 pigs) was taken as experimental unit for BW, ADG and FCR values; individual data were taken to be the experimental unit for slaughtering parameters, meat, fat and thighs (weight losses and chemical analyses) qualitative traits. Panel test results from seasoned hams were analyzed by Kruskall-Wallis one-way analysis of variance (SAS, 1999). Agreement among panelists was assessed with the Kendall’s W coefficient. P<0.05 was considered significant.

Results and discussion

Diets, growing and slaughtering parameters

In the present trial, to comply with the production requirements for PDO ham, it was necessary to use a mix of alternative protein sources (sunflower, fava beans, alfalfa, corn gluten and potato protein concentrate) in order to meet nutritional needs.

From the point of view of sustainability, it is worth noting that under present Italian market conditions both the use of quite expensive ingredients, such as potato protein and the dietary supplementation with lysine, led to a 15% increase in the cost of the experimental feed. Nevertheless, on a European level, one might suppose that such an increase could be, at least partially, offset by willingness on the part of the consumer to pay more for GM-free foods (Chern et al., 2002). The palatability of the diets was similar among the experimental groups and no feed refusals were observed.

Trial conditions did not appear to influence the pigs’ health (no therapeutic treatments were needed) and no deaths were recorded during the trial period (data not shown). The mixture of alternative ingredients (sunflower meal, corn gluten feed, fava beans, dehydrated alfalfa meal and potato protein), each included at a low percentage in the diets fed to heavy pigs, did not affect (P>0.05) any of the production parameters (BW, ADG, FCR) or carcass traits (dressing out, lean meat yield, lean and fat cuts) studied (Tables 2 and 3). With respect to fattening performance, they were within the standard values for Italian heavy pigs (Mordenti et al., 1994; Sardi et al., 2006; Martelli et al., 2005; Trombetta et al., 2009). The lack of any specific literature dealing with the effects on pigs’ fattening performance of a
mixture of non-conventional protein sources similar to that used in the present experiment, led us to address separately the possible role of each single ingredient on growth and carcass parameters. However, the existence of positive or negative associative effects between ingredients cannot be ruled out. In agreement with the findings of Thacker and Haq (2008), who concluded that the inclusion of alfalfa meal up to a maximum of 7.5% in diets for pigs (112 kg of live weight) has no effect on rearing performances or on some carcass characteristics (gross yield, lean meat content, backfat thickness), the results from the present trial indicate that the inclusion of 2% dehydrated alfalfa meal in the diet does not negatively influence either daily feed intake or growing and slaughtering parameters. With respect to faba bean, studies by Partanen et al. (2003), as well as both the reviews by Thacker (1990) and Crepon et al. (2010), concluded that moderate levels of faba beans (i.e. not exceeding 20%) can be used in pig diets without any negative impact on growth parameters (ADG and FCR) or lean meat content. Also in this case, our results agree with the data available in the literature. As far as sunflower meal is concerned, Cortamira et al. (2000) found no differences in growing performance (ADG and FCR) or carcass quality (dressing percent, fat and lean tissue percentage) when twice decorticated sunflower meal was used in total replacement of soybean. Furthermore, in a preliminary trial on heavy pigs, Trombetta and Mattii (2005) did not observe any differences in growth parameters when using sunflower meal in total replacement of soybean. Nevertheless, the results of Thacker and Haq (2008), who replaced soya bean with a mixture of faba beans (11% of the diet), indicate a reduction in the lean meat yield measured using a Fat-o-Meater.

### Table 2. Fattening performance.

|                        | Control group, n=28 | Soybean-free group, n=28 | RMSE |
|------------------------|---------------------|--------------------------|------|
| Starting BW, kg        | 55.1                | 56.3                     | 5.6  |
| BW after 42 d, kg      | 83.1                | 83.9                     | 8.5  |
| BW after 102 d, kg     | 121.5               | 121.6                    | 7.1  |
| Final BW at 174 d, kg  | 158.7               | 160.7                    | 12.5 |
| ADG 0-42 d, g/d        | 666                 | 657                      | 85.3 |
| ADG 43-102 d, g/d      | 639                 | 628                      | 89.7 |
| ADG 102-174 d, g/d     | 522                 | 544                      | 142.7|
| ADG 0-174 d, g/d       | 595                 | 600                      | 70.2 |
| FCR 0-42 d, kg/kg      | 3.30                | 3.32                     | 0.43 |
| FCR 0-42 d, kg/kg      | 3.63                | 3.70                     | 0.25 |
| FCR 102-174, kg/kg     | 5.37                | 5.27                     | 0.68 |
| FCR 0-174 d, kg/kg     | 4.22                | 4.11                     | 0.46 |

RMSE, Root Mean Square Error; BW, body weight; ADG, average daily body weight gain; FCR, feed conversion rate. Data analysis evidenced no statistically significant difference (P>0.05) between the experimental groups.

### Table 3. Carcass traits.

|                        | Control group, n=28 | Soybean-free group, n=28 | RMSE |
|------------------------|---------------------|--------------------------|------|
| Carcass weight, kg     | 131.8               | 133.8                    | 10.9 |
| Dressing out, %        | 82.86               | 83.20                    | 1.8  |
| Lean meat yield (F-o-M), % | 49.31              | 48.61                    | 2.8  |
| Loin+neck, % CW        | 23.75               | 23.08                    | 0.8  |
| Thigh, % CW            | 23.28               | 23.45                    | 1.1  |
| Shoulder, % CW         | 13.65               | 13.66                    | 0.7  |
| Lean cuts, % CW        | 60.67               | 60.19                    | 1.8  |
| Fat cuts, % CW         | 31.85               | 32.39                    | 2.1  |
| Lean/fat cuts rate, % CW | 1.93               | 1.87                     | 0.3  |

RMSE, Root Mean Square Error; CW, carcass weight. Data analysis evidenced no statistically significant difference (P>0.05) between the experimental groups.

### Table 4. Meat quality.

|                        | Control group, n=28 | Soybean-free group, n=28 | RMSE |
|------------------------|---------------------|--------------------------|------|
| pH 45° LD muscle       | 6.45                | 6.29                     | 0.1  |
| pH 45° SM muscle       | 6.59                | 6.42                     | 0.1  |
| pH 24 h SM muscle      | 5.87                | 5.86                     | 0.1  |
| Colour SM muscle       | L*                  | 40.27                    | 3.8  |
| Hue                    | 0.18                | 0.19                     | 0.1  |
| Chroma                 | 14.48               | 14.74                    | 3.1  |
| Drip loss, %           | 1.96                | 1.92                     | 0.6  |
| Chemical composition of LD muscle |          |                          |      |
| Moisture, %            | 73.44               | 73.82                    | 0.9  |
| Protein, %             | 21.27               | 21.05                    | 0.6  |
| Fat, %                 | 4.44                | 4.21                     | 0.9  |
| Ash, %                 | 1.12                | 1.12                     | 0.1  |

RMSE, Root Mean Square Error; LD, Longissimus Dorsi; SM, Semimembranosus. Data analysis evidenced no statistically significant difference (P>0.05) between the experimental groups.
Meat, fat and dry-cured ham quality

Table 4 shows the main qualitative parameters of meat (color, pH values and water holding capacity) and the chemical composition (protein, fat and ash content) of the Longissimus dorsi muscle; no significant differences were found between the groups. The fatty acid composition of the subcutaneous fat of thighs at the beginning of the seasoning period is presented in Table 5. Likewise, none of these parameters showed any significant difference between the two groups.

The main findings of the present trial as regards the qualitative traits of meat (color, pH values and water holding capacity) generally agree with those reported by other authors for heavy pigs receiving conventional formulations (i.e. based on soya bean as the main protein source) (Scipioni and Martelli, 2001; Corino et al., 2002; Sardi et al., 2006; Della Casa et al., 2009). The fat content of the loin was the only parameter that showed some differences when compared to the results of other studies: it was shown to be lower than that observed by Della Casa et al. (2009) and moderately higher than the values reported by Corino et al. (2002) and Sardi et al. (2006). Concerning the fatty acid composition of the subcutaneous fat of thighs (raw ham), the absence of differences between groups reflects, as expected, the similarity of the fatty acid composition of the diets. Furthermore, the fatty acid content of the raw thigh fat (linoleic acid <15%, iodine number <70) appears to be perfectly suitable for dry curing (Consortium for Parma Ham, 1992).

The weight losses of thighs after the different phases (trimming and salting) and over an 18-month seasoning period (final resting) and the results of the chemical analyses of cured hams are shown in Table 6. Our data concerning weight losses are consistent with the standard reported by Mordenti et al. (1994) for Parma hams after a 12-month curing period (28-28%) and, with respect to the present experiment, it is worth noting that the seasoning period was even longer (18 months). For dry-cured hams, their quality depends, as is well known, on many different factors, such as animal breed, animal age, feeding, environmental conditions prior to slaughtering (antemortem factors), product handling at the slaughterhouse and ripening conditions, the raw material quality and the ripening conditions being the most important factors (Gonzalez and Ockerman, 2000; Virgili and Schivazappa, 2002; Bosi and Russo, 2004, Candek-Potokar and Skrlep, 2011). With respect to the qualitative parameters of cured hams (moisture, proteolysis and NaCl content), our results are in agreement with those reported by other authors for Parma Ham, 1992.

Table 5. Fatty acid composition (% of total fatty acids) of thigh subcutaneous fat.

| Fatty Acid       | Control group, n=14 | Soybean-free group, n=14 | RMSE |
|------------------|----------------------|--------------------------|------|
| C 14:0, %        | 1.35                 | 1.31                     | 0.2  |
| C 16:0, %        | 23.07                | 22.89                    | 0.8  |
| C 16:1, %        | 1.92                 | 1.88                     | 0.3  |
| C 18:0, %        | 12.15                | 12.04                    | 1.0  |
| C 18:1, %        | 39.94                | 39.90                    | 1.9  |
| C 18:2, %        | 14.94                | 14.87                    | 1.3  |
| C 18:3 n-3, %    | 0.84                 | 0.81                     | 0.1  |
| SFA, %           | 38.20                | 38.01                    | 1.9  |
| MUFA, %          | 44.57                | 44.74                    | 1.6  |
| PUFA, %          | 17.23                | 17.24                    | 1.5  |
| Iodine, no.      | 68.21                | 68.06                    | 0.3  |

Table 6. Ham weight processing losses and chemical composition (Biceps femoris muscle) of dry-cured hams.

| Parameter                  | Control group | Soybean-free group | RMSE |
|----------------------------|---------------|--------------------|------|
| Thighs, n                  | 28            | 28                 |      |
| Initial weight, kg         | 15.20         | 15.69              | 1.25 |
| Weight after trimming, kg  | 12.52         | 12.83              | 1.09 |
| Weight after salting, kg   | 11.66         | 11.97              | 1.02 |
| Final weight (end of curing), kg | 9.05      | 9.30               | 0.84 |
| Weight loss after trimming, % | 17.65   | 18.27              | 1.80 |
| Weight loss (end of curing), % | 27.71   | 27.48              | 1.99 |
| Samples, n                 | 14            | 14                 |      |
| Dry matter, %              | 40.35         | 39.95              | 1.31 |
| Crude protein, %           | 28.98         | 28.54              | 0.72 |
| Fat, %                     | 4.14          | 4.05               | 1.24 |
| Ash, %                     | 7.24          | 7.36               | 0.59 |
| Salt, %                    | 5.58          | 5.51               | 0.49 |
| Proteolysis index, %       | 27.40         | 27.60              | 2.57 |

Table 7. Visual and tactile assessment of dry-cured hams.

| Parameter               | Control group, n=14 | Soybean-free group, n=14 | RMSE |
|-------------------------|----------------------|--------------------------|------|
| Colour of fat           |                       |                          |      |
| L                       | 74.84                 | 75.55                    | 1.97 |
| Hue                     | -1.21                 | -1.32                    | 0.56 |
| Chroma                  | 8.55                  | 8.87                     | 0.61 |
| Colour of muscle        |                       |                          |      |
| L                       | 39.27                 | 39.98                    | 1.96 |
| Hue                     | 0.70                  | 0.72                     | 0.87 |
| Chroma                  | 14.31                 | 14.22                    | 1.22 |
| Evaluation of the lean portion |            |                          |      |
| Firmness                | 6.14                  | 5.63                     | 0.79 |
| Colour homogeneity      | 5.89                  | 6.64                     | 1.23 |
| Bitonality              | 2.96                  | 3.39                     | 1.43 |
| Marbling                | 3.71                  | 4.54                     | 1.85 |
| Evaluation of the fat portion |            |                          |      |
| Ham fatness             | 5.79                  | 5.84                     | 0.77 |
| Overall evaluation      | 6.36                  | 6.93                     | 1.21 |

RMSE, Root Mean Square Error. Data analysis evidenced no statistically significant difference (P>0.05) between the experimental groups.
Table 8. Fatty acid composition of total lipids of cured ham subcutaneous fat.

| Group | Control group, n=14 | Soybean-free group, n=14 | RMSE |
|-------|---------------------|--------------------------|------|
| C 14:0, % | 1.48 | 1.60 | 0.22 |
| C 16:0, % | 23.06 | 23.10 | 1.37 |
| C 16:1, % | 2.60 | 2.70 | 0.45 |
| C 18:0, % | 9.89 | 9.31 | 1.01 |
| C 18:1, % | 45.07 | 44.59 | 1.16 |
| C 18:2, % | 13.51 | 14.37 | 1.66 |
| C 18:3 n-3, % | 0.67<sup>a</sup> | 0.77<sup>b</sup> | 0.01 |
| C 20:4, % | 0.59 | 0.67 | 0.10 |
| SFA, % | 35.59 | 35.03 | 1.82 |
| MUFA, % | 49.11 | 48.74 | 1.15 |
| PUFA, % | 15.30 | 16.24 | 1.92 |

RMSE, Root Mean Square Error; SFA, saturated fatty acids; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids.
<sup>a</sup>Values in the same row with different superscripts are different (P<0.05).

Conclusions

Our findings suggest that a diet containing non-conventional vegetable protein sources (i.e. sunflower meal, corn gluten feed, faba beans, dehydrated alfalfa meal and potato protein), as compared to the conventional soybean meal, may be used for heavy pig production serving as a raw material for dry-curing of hams. We also need to gain more knowledge about the possible use of alternative protein sources that could be locally grown for developing GMO-free feed formulations.

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