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# Effect of low-protein diets in heavy pigs on dry-cured ham quality characteristics

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ABSTRACT

The present work aimed at evaluating if heavy pig low-protein diets balanced for amino acid (AA) profile affect the quality characteristics of dry cured hams. To the aim, 40 hams obtained by Italian Duroc × Italian Large White pigs fed three different dietary crude protein and indispensable AA levels were compared with those obtained by a conventional Parma ham Protected Designation of Origin diet (C). No physico-chemical (aw, pH), chemical (gross composition, NaCl, lipid peroxidation, non-protein nitrogen, total volatile bases) or sensory characteristics of hams were systematically affected by the administered diet, animal sex or their interaction with the exception of total and subcutaneous fat (the latter measured by an image analysis procedure). Considering gilts, low-protein diets resulted in samples with higher fat content and subcutaneous fat thickness with respect to hams obtained with C diet. In conclusion, low protein diets in the finishing phase of pig breeding could reduce the environmental impact due to nitrogen excretion without significantly affecting ham quality.

## 1. Introduction

Intensive animal farming can exert a severe potential impact on air, water and soil, arising from the consumption of resources associated with production processes as well as from various excretions and emissions of nutrients in the environment (Djekic, 2015). The contribution of the pig production system to the environmental impact has been the subject of many evaluations. Feed components, mainly nitrogen compounds, are the main contributors to environmental load of pork production. Nitrogen (N) excretion of pig breeding, as determined in different European countries, contributes to about 15% of total N excretion from livestock (Hou et al., 2016).

Heavy pig production is a traditional and significant activity in many countries worldwide (e.g. Spain, Italy, France, Germany, Poland and Greece), because it provides thighs for dry-cured ham, a traditional processed meat product (Bava, Zucali, Sandrucci, & Tamburini, 2017) that requires meat with an excellent suitability for salting and seasoning (Bosi & Russo, 2004). In heavy pig production, N excretion has been found to be surprisingly similar to that of light pigs, in the order of 13.8 to 13.5 kg N excreted/place/year (Xiccato, Schiavon, Gallo, Bailoni, & Bittante, 2005), even if at the highest body weights (BW, from 120 kg up to slaughtering) the efficiency of N retention is the lowest (Bava et al., 2017). The reason of this apparent inconsistence is due to the fact that in the heavy pig production system the dietary crude protein (CP) levels are much lower compared to those used in the light pig systems (Xiccato et al., 2005), and they are furtherly reduced with increasing BW (Schiavon et al., 2015).

Thus, a more efficient use of nitrogen, through decreasing the dietary protein level and optimizing the amino acid (AA) profile on the basis of the physiological phase, will reduce the environmental impact of heavy pig production (Bava et al., 2017). Supplying synthetic AA might reduce the protein content of feeds while maintaining an AA balance in pig diets. In the meantime, the incorporation of AA in feeds reduces the quantity of protein-rich ingredients, and to some extent modifies the environmental impacts associated with pig production. Moreover, the use of low protein diets would decrease the impacts on eutrophication, terrestrial eco-toxicity and cumulative energy demand of heavy pig production (Garcia-Launay, van der Werf, Nguyen, LeTutour, & Dourmad, 2014).

Feeding strategy may be important for animal growth rate and muscle proteolytic activity (Van den Hemel-Grooten et al., 1997). Although a certain level of proteolytic activity is required in order to develop the characteristic sensory traits of a dry-cured ham, problems associated with extensive proteolysis can be encountered (Toldrá & Flores, 1998). Contradictory information about the influence of CP on the calpain system has been found. Some Authors (van den Hemel-Grooten et al., 1997), reported that a protein-free diet did not influence the calpain system in skeletal muscle, whereas others (Tang et al., 2010) found that a moderate reduction of CP and energy in the diet caused an increase in Calpain 1 expression suggesting a higher

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myofibrillar protein degradation and pork tenderization. However, the influence of dietary regimen on muscle proteolytic enzymes has been scarcely studied.

Although the benefits of using AA and a low-protein diet as a measure to reduce the environmental burden of pig production are becoming evident, there is still a major potential to reduce environmental impacts at low cost as for current pig feeding practices (Garcia-Launay et al., 2014). Moreover, it has to be considered that some Protected Designation of Origin (PDO) hams impose feed restrictions. For instance, PDO Parma ham requires heavy pigs slaughtered not before 9 months of age, with restricted feeding and long finishing phases (MIPAAF, 2014; Mordenti et al., 2003). This is a crucial point, since PDO Parma ham, with an annual production of about 88 thousands of tons, is the most important PDO label among cured-meat products registered by the European Union, accounting for 44% of the total production of PDO and Protected Geographical Indication (PGI) cured-meat products (Ismea-Qualivita, 2015).

This research aims at evaluating if a reduction in the dietary CP content of the diets would influence the quality of hams in terms of composition and sensory traits. To the aim, different feeding strategies based on low-protein diets with addition of synthetic AA (eventually alternate on week basis) have been compared with a conventional PDO Parma ham diet.

## 2. Materials and methods

### 2.1. Samples

The research was carried out on 40 dry-cured hams chosen among 80 hams obtained by processing selected left thighs of 80 pigs equally divided into barrows and gilts. Animal care and use practices during the study were conformed to Dir. 86/609/CEE, as adopted by D.Lgs. 116/1992.

Italian Duroc × Italian Large White pigs were grown in the same farm and fed the following diets from 40 kg to approximately 175 kg live weight:

- C (control), standard Parma ham PDO diet containing 16 g/100 g CP during growth and 13 g/100 g CP during the fattening phase;
- 2AA, diet with a CP reduction of about 2 g/100 g with respect to C, while maintaining the level of indispensable AA by using two synthetic AA (lysine and tryptophan);
- 6AA, diet with the maximum possible reduction of protein content (ca. 11 g/100 g CP during growth and 9 g/100 g during fattening), ensuring the level of indispensable AA through the administration of six synthetic AA (lysine, methionine, threonine, tryptophan, valine, isoleucine);
- ALT, 6AA diet weekly alternated with the same diet without synthetic AA (methionine, threonine, tryptophan, valine, isoleucine).

The specific diet compositions are reported in Table 1. Diets 6AA and ALT provided a protein content lower than feed protein content allowed by the Parma ham PDO specifications (MIPAAF, 2014). For each diet, pigs were divided in 2 pens of gilts (5 animals each) and 2 pens of barrows (5 animals each).

Pigs were slaughtered according to industry-accepted procedures. After 24 h chilling period (0–2 °C), hams were trimmed to the typical Parma ham round-shape and cured according to the Parma ham PDO production regulations (MIPAAF, 2014), until 17 months curing. Discarding hams with defects, 40 cured hams (10 for each diet, belonging to the four different pens and representing equally gilts and barrows) were selected for the analyses on the basis of the mean weight (about 14.5 kg).

### 2.2. Sample preparation

Hams were mechanically deboned and manually sectioned at about 8 cm above the femoral head, in order to derive a portion of about 6 cm thick and 1.5–2 kg weight. After cutting, samples were immediately placed under vacuum and stored at 4 °C until the analyses. Each sample was then skinned and a first slice (10 mm) was cut out by a slicer and discarded. Afterwards, two slices of 5 mm for the image analysis, three slices of 1 mm for electronic nose evaluation and six slices of 1 mm for sensory analysis were obtained. The remainder of each sample was diced and passed three times in a meat grinder (TRD23050M, Fimar, Italy). An aliquot of the ground ham was immediately used to determine water activity, moisture, pH and ash. The residue was vacuum packed, stored at − 18 °C until the performance of chemical analyses, and then homogenized using a heavy duty blender (Waring Laboratory, USA) for 10 s at the lowest speed (15,800 rpm).

### 2.3. Physico-chemical and chemical analyses

pH was determined by a pH-meter (Seven Easy, Mettler Toledo AG, Switzerland) equipped with an electrode for solid samples. Water activity ($a_w$) was measured at room temperature by a dew-point hygrometer (Acqualab 3TE, Decagon devices Inc., USA) previously calibrated with a NaCl standard solution ($a_w$ = 0.760). Moisture (g/100 g) was determined gravimetrically by drying about 2 g of sample in an oven at 105 °C until constant weight (AOAC 950.46). Ash (g/100 g) evaluation was performed according to AOAC 920.153 procedure on the same sample previously weighed for moisture determination. NaCl content (g/100 g) was determined by Carpentier-Volhard titration (Haouet, Altissimi, Framboas, & Galarini, 2006). Fat content (g/100 g) was determined by a Soxhlet extraction with petroleum ether as described in AOAC 991.36 method. Kjeldahl method (AOAC 981.10) was performed to determine total nitrogen (g/100 g), then the results were expressed as protein by using 6.25 as conversion factor. Lipid peroxidation was evaluated by TBARs (2-thiobarbituric reactive substances) assay, according to Koutina, Jongberg, and Skibsted (2012). Results were expressed in milligrams of malondialdehyde equivalent (MDA) per kilogram of dry matter. The amount of non-protein nitrogen (NPN; g/100 g) was determined as an index of proteolysis, using the method of Careri et al. (1993). The determination of total volatile bases (TVB-N; mg/100 g) was performed according to the official method of the Commission of European Communities (Reg. EC 2074/2005), which consists of an acid-base titration after extraction of volatile bases with perchloric acid.

For each ham, all the analyses were carried out in duplicate on the homogenized samples and results are reported as mean and standard error of the mean (SEM).

### 2.4. Image analysis

The images of 5 mm thickness slices were acquired in duplicate using a flatbed scanner (HP Scanjet 8300, Hewlett Packard Enterprise, USA) directly interfaced with a computer by SylverFast software v.6.1 (LaserSoft Imaging Inc., Germany). Images were acquired at a resolution of 600 dpi, with a colour depth of 24-bit. During acquisition, the samples were covered with a black box to prevent light leakage. The images were saved in jpg format.

Image analysis was performed by a semi-automatic procedure through a self-build Matlab routine (Matlab R2014a, v. 8.3, The MathWorks Inc., USA). The background removal and the threshold for lean and fat area distinction were outlined by the intensity histogram values of the red channel. The measurements taken on the slice images were: the total fatty area expressed as percentage of the whole slice area, and the subcutaneous fat layer width (cm). The latter was measured vertically at the head of the femur, as reported in the Parma ham PDO specifications. For each ham, data were obtained from the analysis of two slices. Results are reported as mean and standard error of the mean (SEM).