



## AperTO - Archivio Istituzionale Open Access dell'Università di Torino

# Effects of diets with increasing levels of Spirulina Platensis on carcass characteristics, meat quality and fatty acid composition of growing rabbits.

This is the author's manuscript
Original Citation:
Availability:
This version is available http://hdl.handle.net/2318/101267 since
Published version:
DOI:10.1016/j.livsci.2011.03.031
Terms of use:
Open Access
Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use

of all other works requires consent of the right holder (author or publisher) if not exempted from copyright

(Article begins on next page)

protection by the applicable law.



# UNIVERSITÀ DEGLI STUDI DI TORINO

This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in *Livestock Science, Volume 140, Issues 1–3, September 2011, Pages 218–224* http://dx.doi.org/10.1016/j.livsci.2011.03.031

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

(1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.

(2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.

(3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license (<u>http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en</u>), <u>http://dx.doi.org/10.1016/j.livsci.2011.03.031</u>

# Effects of diets with increasing levels of *Spirulina platensis* on the carcass characteristics, meat quality and fatty acid composition of growing rabbits

P.G. Peiretti<sup>a,\*</sup> G. Meineri<sup>b</sup>

<sup>a</sup> Institute of Sciences of Food Production, National Research Council, Grugliasco, Turin, Italy
 <sup>b</sup> Department of Animal Production, Epidemiology and Ecology, University of Turin, Grugliasco, Turin, Italy.

\*Corresponding author. Tel.: +39.11.6709230; fax +39.11.6709297; e-mail address: piergiorgio.peiretti@ispa.cnr.it

#### The proofs should be sent to:

Dr. Pier Giorgio PEIRETTI, Istituto di Scienze delle Produzioni Alimentari, National Research Council, Via L. da Vinci 44, 10095 Grugliasco (Torino), Italy

#### Abstract

Spirulina platensis (SP) is a useful raw material for human and animal nutrition due to its high protein content. It is also a source of minerals, carotenoids, chlorophyl, pigments and essential polyunsaturated fatty acids, such as  $\gamma$ -linolenic acid (GLA). The effects of four levels 0, 50, 100, or 150 g/kg of SP, included in the diet to replace soybean and alfalfa, on some carcass characteristics and the meat quality of growing rabbit have been investigated. The modifications of the diet composition did not significantly influence the carcass yield or the proportions of the various carcass parts and organs. The chemical composition of the meat was unaffected, with the exception of the lipid content, which was lower in the control group than in the groups fed the SP diets. The fatty acid (FA) profile of the perirenal fat and *longissimus dorsi* muscle was determined to evaluate the effect of SP supplementation on the GLA content of these tissues. The content of this FA

increased in the perirenal fat and meat with increasing SP supplementation. As a direct result of the diet composition, the FA profiles and the atherogenic and thrombogenic indexes of the rabbit tissues showed significant differences. These indexes were lower in the meat of the rabbits fed the SP diets than those found in the control group. The results of this study suggest that SP could potentially be used in rabbit nutrition with consequent benefits on the nutritional quality of rabbit meat for consumers.

Keywords: Rabbit; Spirulina platensis; Meat quality; Fatty acid

#### 1. Introduction

Current research is directed toward developing feeding strategies to increase the nutritional value of rabbit meat as a "functional food" by including vitamins, antioxidants and essential fatty acids in rabbit diets and assessing their effects on quality of both raw and stored/processed meat (Cavani et al., 2009; Petracci et al., 2009). *Spirulina platensis* (SP) is a blue-green alga that can supply several phytochemicals which have potential health benefits. SP has been used as an animal feed, due to its protein and lipid content, or in nutritional integrator production, due to its potential antiviral, antioxidant, hepatopreserver, antiallergenic and immunomodulator activities (Khan et al., 2005). It is contains 60.0-70.0 % crude protein and is a rich source of vitamins (especially vitamin  $B_{12}$ ), minerals, chlorophyll, carotenoids, carbohydrates, sterols, the pigments phycocyanin and allophycocyanin, which are mainly responsible for the antioxidant activities (Belay et al., 1996; Henrikson, 1997; Miranda et al., 1998) and some fatty acids, such as  $\gamma$ -linolenic acid (GLA, C18:3 n-6). This fatty acid (FA) has been shown to have important effects on several aspects of human health and nutrition (Fan and Chapkin, 1998).

SP has been evaluated widely as fresh meal for mollusc bivalve and fish larvae (Harel et al., 2002; Lu et al., 2002) or as raw meal for juveniles and adults (Nandesha et al., 2001; Takeuki et al., 2002; Palmegiano et al., 2005; Palmegiano et al., 2008).

The dietary use of SP has also been proposed by many authors as a protein feed supplement for swine (Yap et al., 1982; Grinstead et al., 2000).

The effects of various raw materials (alfalfa, linseed and other oilseeds), as suitable unsaturated FA sources, have been the subject of many experiments concerning the quality of rabbit meat (Bianchi et al., 2006, 2009; Peiretti et al., 2007; Peiretti and Meineri, 2008a). Rabbit meat has a high nutritional value and is highly valued because of its dietary properties, since it is a lean meat with a low-fat content and less saturated FA and cholesterol than other meats (Hernandez, 2008). Its quality is rather stable. Unlike other species, no specific alterations of meat texture or physical properties have been observed (Cavani et al., 2003). Moreover, rabbit meat consumption could become a good way of providing bioactive compounds to human consumers, since the rabbit meat FA profile may be favorably modified by the inclusion of raw materials rich in unsaturated FAs in the diet (Hernandez, 2008; Dal Bosco et al., 2004; Kouba et al., 2008).

Rabbits, like other monogastric animals, are able to directly incorporate dietary FAs into adipose and intramuscular tissue lipids, making it possible to modify the FA profile of rabbits through strategic use of unsaturated dietary fat sources (Dalle Zotte, 2002).

The aim of the present research was to evaluate the effects of SP based diets on the carcass characteristics and meat quality of rabbits. The FA profile of the perirenal fat and *longissimus dorsi* muscle has been studied to evaluate the effects of SP supplementation on their GLA content and on indexes related to human health.

#### 2. Materials And Methods

#### 2.1. Animals, housing and diets

The study was carried out at the CISRA (Centro Interdipartimentale Servizio Ricovero Animali) experimental rabbitry at the University of Turin. Forty weaned crossbred rabbits aged nine weeks, with a mean weight of  $2034\pm174$  g, were randomly assigned to four groups of 10 (five male and five female rabbits each) with equal initial weight variability. The animals were housed individually, at a temperature of  $22\pm2$ °C and 16 h daily lighting, in wire cages at a height of 90 cm from the concrete floor. An automatic feeder and nipple-drinker were provided for each rabbit. Four isoproteic and isoenergetic diets were formulated with increasing levels of SP (0, 50, 100 and 150 g/kg), which was obtained from Ornitalia Product Service s.a.s. (Colleredo di Monte Albano, Italy). All the diets were pelleted and stored in darkness to avoid auto-oxidation of the lipid sources. The experimental period for each diet lasted 31 days in each trial with 1 week for adaptation to the diets and cages. Food and water were available *ad libitum* to the animals. The performance and apparent digestibility have been previously reported in another paper (Peiretti and Meineri, 2008b).

#### 2.2. Measured traits

At the end of the experimental period, the rabbits from each group were weighed and slaughtered without fasting. The carcasses were prepared by removing the skin, feet, paws, genital organs, urinary bladder and digestive tract, as recommended by Blasco et al. (1993). The carcasses were weighed and the weights of the skin and limbs, head, liver, kidneys, heart and lungs were recorded and expressed as g/kg of slaughter weight (SW). The forelegs, hind legs, breast and ribs, loins and abdominal wall were weighed. Their weights were expressed as g/kg of commercial carcass weight (CCW).

*Longissimus dorsi* muscle and perirenal fat samples were collected from the carcass 24 h post mortem. The right *longissimus dorsi* muscle was dissected, trimmed of all visible extramuscular fat. All the tissue samples were immediately frozen at  $-20^{\circ}$ C until analyzed.

#### 2.3. Analytical determinations

The meat and diet samples were analyzed in duplicate to determine dry matter, ash, crude protein (AOAC 955.04) and ether extract (AOAC 963.15), according to the methods of the Association of Official Analytical Chemists (1990). The diet samples were also analyzed to determine neutral detergent fibre (NDF) without sodium sulfite or  $\alpha$ -amylase, and acid detergent fibre (ADF), as described by Van Soest et al. (1991), expressed exclusive of residual ash, and gross energy (GE) by means of an adiabatic bomb calorimeter (IKA C7000, Staufen, Germany).

Lipid extraction was performed on the SP, the diets and the fat samples according to Hara and Radin (1978), while the transesterification of the FAs was carried out according to Christie (1982), with the modifications described by Chouinard et al. (1999).

The FAs were analyzed as their methyl esters. The analysis was carried out by gas chromatography, as reported by Peiretti et al. (2007). The saturation (SI), atherogenic (AI) and thrombogenic (TI) indexes of the fat were calculated according to Ulbricht and Southgate (1991) as follows:

 $SI = (C14:0 + C16:0 + C18:0) / [\Sigma MUFA + \Sigma PUFA]$ 

AI =  $(C12:0 + 4 \times C14:0 + C16:0) / [\Sigma MUFA + \Sigma(n-6) + \Sigma(n-3)]$ 

 $TI = (C14:0 + C16:0 + C18:0) / [0.5 \text{ x } \Sigma \text{MUFA} + 0.5 \text{ x } \Sigma(\text{n-6}) + 3 \text{ x } \Sigma(\text{n-3}) + \Sigma(\text{n-3}) / \Sigma(\text{n-6})]$ 

where MUFA and PUFA are monounsaturated fatty acids and polyunsaturated fatty acids, respectively.

These indexes can indicate whether a food is suitable for the prevention of human cardiovascular disease. The atherogenic or hyperlipidaemic saturated FAs (C12:0, C14:0 and C16:0) and the thrombogenic FAs (C14:0, C16:0 and C18:0) must be low, while high LA and ALA contents of the meat are thought to be anti-atherogenic and anti-thrombogenic (Ulbricht and Southgate, 1991).

2.4. Statistical analyses

The statistical analyses were performed using the SPSS (1999) software package (version 11.5.1 for Windows, SPSS Inc., USA). An analysis of variance was used to evaluate the effects of concentrations of SP on the carcass characteristics, meat composition and FA profile of the meat and fat of the rabbits. The differences were tested using Duncan's Multiple Range Test.

#### **3. Results and Discussion**

#### 3.1. Composition of the raw spirulina and diets

The ingredients, chemical composition and nutritive value of the SP and of the four diets are shown in Table 1 (the data have previously been reported by Peiretti and Meineri (2008b)). The four diets were formulated to have similar protein and energy contents.

The raw SP material had a crude protein content similar to that found in other studies (Ross and Dominy, 1990; Grinstead et al., 2000), while the fat content was similar to that found by Ross and Dominy (1990) and was lower than that found by Grinstead et al. (2000). The higher fat content of this SP strain could be explained by the fact that it is grown under different conditions.

The FA pattern of the experimental diets are reported in Table 2. The GLA and stearidonic acid (SDA, C18:4 n-3) contents increased and  $\alpha$ -linolenic acid (ALA, C18:3 n-3) decreased with an increasing SP inclusion level. This is due to the increasing GLA and SDA contribution of SP and to the decrease in dehydrated alfalfa meal inclusion with increasing levels of SP, respectively.

#### 3.2. Carcass traits

The initial and final weight of the rabbits, as reported by Peiretti and Meineri (2008b), ranged from 2024 to 2047 g and from 2983 to 3184 g, respectively. Modification of the diet composition and inclusion of SP in the diets did not significantly influence the carcass yield or the proportions of the various carcass parts and organs of the rabbits (Table 3). This is in agreement with other studies on vegetable sources of added fat, which do not seem to affect the main carcass traits (Peiretti et al., 2007; Peiretti and Meineri, 2008a; Kouba et al., 2008). The carcass yield values obtained in the present study are similar to those reported by Peiretti and Meineri (2008a) and by Gondret et al. (1998).

## 3.3. Chemical composition of the longissimus dorsi muscle

The dry matter, protein and ash of the *longissimus dorsi* muscle were not affected by the diets (table 4). The total lipid content was higher (P<0.05) in the *longissimus dorsi* muscle of rabbits fed the SP-supplemented diets than those fed the control diet, probably because the ether extract content was lower in this diet than that of the SP diets.

3.4. Fatty acid profile and indexes related to human health of the perirenal fat and *longissimus dorsi* muscle

The FA profile of the perirenal fat and the *longissimus dorsi* muscle differed (Tables 5 and 6).

The modification of the diet composition and the increasing SP inclusion level are related to a decrease (P<0.05) in the perirenal fat for C14:0, C15:0, C16:0, C18:0, C18:1n-9, and C18:1 n-7, while C10:0, LA, GLA, C20:2 n-6 or C21:0 increased (P<0.05) with an increase in the SP content of the diet (Table 5). No significant differences were detected among the treatments for the other FAs. The low ALA or PUFA n-3 contents in the fat of the rabbits fed a diet with 150 g/kg of SP are probably a consequence of the substitution of the dehydrated alfalfa meal with pelletted ground hay in the diet ingredients, which was necessary to obtain four isoproteic and isoenergetic diets (Table 1).

As a direct results of the changing diet composition, the saturated FA (SFA) and monounsaturated FA (MUFA) contents were significantly higher in the perirenal fat of the rabbits fed the control diet than in those of the rabbits fed the SP diets. However, the PUFA content was higher in the fat of the rabbits fed diets supplemented with SP than in rabbits fed the control diet without SP.

Meineri et al. (2009) evaluated the changes in the FA profile of the tissues of rabbits fed diets with a low or a high fat content and with or without SP supplementation (10 g/kg of the diet) and found that the FA profile of the perirenal fat of rabbits fed the diets supplemented with SP had a higher (P<0.05) content of GLA than the rabbits fed the diets without SP.

As shown in Table 6, there was also a dietary effect on the FA profile of the *longissimus dorsi*. The muscle fat had a significantly higher proportion of C15:0, C16:0, C20:4 n-6, and unidentified FAs and a lower proportion of LA in the control group in comparison to the groups fed SP. A low content of ALA (P<0.05) was found in the muscle fat of the rabbits fed the 150 g/kg SP diet because alfalfa was totally omitted from the diet which led to the decreased ALA content. The GLA content was nil in the *longissimus dorsi* of the rabbits fed the control diet and increased (P<0.05) with increasing SP supplementation, due to the high GLA content of the microalgae.

Meineri et al. (2009) showed significant differences for the ALA content in the *longissimus dorsi* FAs of rabbits fed two low-fat isoproteic diets supplemented or not supplemented with SP.

In the present study, the n-6/n-3 ratio ranged from 9.1 and 9.4 in the perirenal fat and meat of the rabbits fed the control diet, and to 21.4 and 20.8 in the meat and perirenal fat of the rabbits fed 150 g/kg of SP. Our values are higher than the results published by Polak et al. (2006), who reported an n-6/n-3 ratio of 8.1 for rabbit meat, while Dalle Zotte (2002) and Ramirez et al. (2005) found values of 11.6 and 11.5 for the hind leg, respectively. The n-6/n-3 ratio is high in our rabbit

meat, due to the high LA content in the diets; moreover, the increasing GLA content in the SP diets contributed to the increase in the n-6/n-3 ratio in the meat of rabbits fed these diets. SP is less efficient than alfalfa or linseed meal in decreasing the n-6/n-3 ratio in the meat, due to its PUFA n-6 content. The inclusion of PUFA n-6 sources, such as grains that are normally high in LA, in rabbit diets, increases the total PUFA n-6 content. This is almost concomitant with a lowerPUFA n-3 supply resulting in decreased PUFA n-3 in the muscle. This results in an increased n-6/n-3 ratio.

Different vegetable oil sources have been used in rabbit diets to increase the level of lipid unsaturation and to lower the indexes of the rabbit tissues related to human health (Dalle Zotte, 2002). Dal Bosco et al. (2004) studied the dietary use of linseed oil as a way of raising the PUFA n-3 content of the meat of rabbits and found that the n-6/n-3 ratio of the *longissimus dorsi* muscle of rabbits fed a linseed diet was less than half that of rabbits fed a control diet. Peiretti et al. (2007) found that the n-6/n-3 ratio significantly decreased from 3.9 and 3.1 in the perirenal fat and meat of rabbits fed a control diet, to 1.2 and 1.1 in the meat and perirenal fat of rabbits fed 150 g/kg of false flax seed.

Peiretti and Meineri (2008a) found that the n-6/n-3 ratio significantly decreased from 4.5 and 4.2 in the meat and perirenal fat of rabbits fed a control diet, to 1.0 and 0.9 in the meat and perirenal fat of rabbits fed 150 g/kg of chia seed.

The atherogenic index showed significant variations and was lower in the meat and perirenal fat of the rabbits fed the SP diets than that found in the control group, with consequent benefits on the nutritional quality of rabbit meat for consumers. This index is similar to those found by Peiretti et al. (2007) and Peiretti and Meineri (2008a) in two experiments conducted to study the effect of various levels of false flax seed or chia seed in fattening rabbit diets.

The thrombogenic index of the meat of the rabbits fed the SP diets was significantly lower than that found in the control group and similar to that found by Dal Bosco et al. (2001) in the *longissimus dorsi* muscle of rabbits supplemented with vitamin E. Rabbits fed diets rich in ALA have shown a good capability of elongating and desaturating ALA and these diets enriched the PUFA n-3 content of the meat, reducing its thrombogenic index (Dal Bosco et al., 2004; Peiretti et al., 2007; Peiretti and Meineri, 2008a). Castellini et al. (1999) found that alfalfa diets increased the level of ALA and decreased the atherogenic and thrombogenic indexes in the *longissimus dorsi* muscle of rabbits with values similar to those found in the muscle of rabbits fed herring meal.

Therefore, the meat of rabbits fed SP supplemented diets presents lower atherogenic and thrombogenic indexes than the meat of rabbits fed a control diet. These advantageous indexes of SP supplemented diets come from the lower concentration of the atherogenic saturated FAs as well as from the higher concentration of the PUFA n-6. The optimal values of these indexes have been found in the Eskimo fish based diet which is rich in unsaturated PUFA n-3 and presents an atherogenic and thrombogenic index of 0.39 and 0.28, respectively. These indexes are very low, compared to the indexes found in the Danish diet, which is rich in saturated fat, where the atherogenic and thrombogenic index are 1.29 and 1.51, respectively (Ulbricht and Southgate, 1991).

The FA profile of rabbit meat may be favourably modified through the inclusion of raw materials rich in PUFA. Administration of an enriched diet during the last two weeks of fattening is sufficient to increase the PUFA content of meat to the higher values, thus reducing costs in comparison to a longer treatment (Cavani et al., 2009).

Recently, simplified production techniques and the development of alternative nutrient sources have been adopted to obtain large quantities of SP at competitive prices in comparison to other raw materials, and this could increase the use of this microalgae in animal nutrition (Viera Costa et al., 2004; Raoof et al., 2006). In order to reduce costs even more, the SP dietary supply could be limited to the fattening period to increase the value of rabbit meat with improved indexes related to human health.

#### 4. Conclusions

The modifications of the diet have not significantly influenced the carcass yield or the proportions of the various carcass parts and organs. The meat quality was unaffected by diet type, with the exception of the lipid content, which was lower in the control group than in the groups fed SP-containing diets. The fatty acid profile was influenced by diet composition and microalgae supplementation and the results showed that modifications of the diet were effective in reducing the atherogenic and thrombogenic indexes, with consequent benefits on the nutritional quality of rabbit meat for consumers, mainly concerning the well known relationship between meat fat composition and human cardiovascular diseases.

## References

- AOAC, 1990. Official Method of Analysis, 15th Edn. AOAC, Washington, DC: Association of Official Analytical Chemists.
- Belay, A., Kato, T., Ota, Y., 1996. Spirulina (*Arthrospira*): Potential application as an animal feed supplement. J. Appl. Phycol. 8, 303-311.
- Bianchi, M., Petracci, M., Cavani, C., 2006. Effects of dietary inclusion of dehydrated lucerne and whole linseed on rabbit meat quality. World Rabbit Sci. 14, 247-258.
- Bianchi, M., Petracci, M., Cavani, C., 2009. The influence of linseed on rabbit meat quality. World Rabbit Sci. 17, 97-107.
- Blasco, A., Ouhayoun, J., Masoero, G., 1993. Harmonization of criteria and terminology in rabbit meat research. World Rabbit Res. 1, 3-10.
- Castellini, C., Dal Bosco, A., Bernardini, M., 1999. Effetto dell'integrazione alimentare di acidi grassi polinsaturi della serie n-3 sulla composizione lipidica e sulla stabilità ossidativa della carne di coniglio. Zoot. Nutr. Anim. 15, 63–70.
- Cavani, C., Betti, M., Bianchi, M., Petracci, M., 2003. Effects of the dietary inclusion of vegetable fat and dehydrated alfalfa meal on the technological properties of rabbit meat. Vet. Res.

Commun. 27 (Suppl. 1), 643-646.

- Cavani, C., Petracci, M., Trocino, A., Xiccato, G., 2009. Advances in research on poultry and rabbit meat quality. Ital. J. Anim. Sci. 8 (Suppl. 2), 741-750.
- Chouinard, P.Y., Corneau, L., Sæbø, A., Bauman, D.E., 1999. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. J. Dairy Sci. 82, 2737-2745.
- Christie, W.W., 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. J. Lipid Res. 23, 1072-1075.
- Dal Bosco, A., Castellini, C., Bernardini, M., 2001. Nutritional quality of rabbit meat as affected by cooking procedure and dietary vitamin E. J. Food Sci. 66, 1047-1051.
- Dal Bosco, A., Castellini, C., Bianchi, L., Mugnai, C., 2004. Effect of dietary α-linolenic acid and vitamin E on the fatty acid composition, storage stability and sensory traits of rabbit meat. Meat Sci. 66, 407-413.
- Dalle Zotte, A., 2002. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. Livest. Prod. Sci. 75, 11-32.
- Fan, Y., Chapkin, R.S., 1998. Importance of dietary γ-linolenic acid in human health and nutrition?J. Nutr. 128, 1411-1414.
- Fernández-Carmona, J., Cervera, C., Blas, E., 1996. Prediction of the energy value of rabbit feeds varying widely in fibre content. Anim. Feed Sci. Technol. 64, 61-75.
- Polak, T., Gašperlin, L., Rajar, A., Žlender, B., 2006. Influence of genotype lines, age at slaughter and sexes on the composition of rabbit meat. Food Technol. Biotechnol. 44, 65-73.
- Gondret, F., Mourot, J., Lebas, F., Bonneau, M., 1998. Effects of dietary fatty acids on lipogenesis and lipid traits in muscle, adipose tissue and liver of growing rabbits. Anim. Sci. 66, 483-489.
- Grinstead, G.S., Tokach, M.D., Dritz, S.S., Goodband, R.D., Nelssen J.L., 2000. Effects of *Spirulina platensis* on growth performance of weanling pigs. Anim. Feed Sci. Technol. 83, 237-247.
- Hara, A., Radin, N.S., 1978. Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem.

90, 420-426.

- Harel, M., Koven, W., Lein, I., Bar, Y., Beherens, P., Stubbleffeld, J., Zohar, Y., Place, A.R., 2002. Advanced DHA, EPA and ArA enrichment materials for marine aquaculture using single cell heterotrophs. Aquaculture 213, 347-362.
- Hernández, P., 2008. Enhancement of nutritional quality and safety in rabbit meat. Proc. 9th World Rabbit Congress, Verona, Italy, pp. 1287-1299.
- Henrikson, R., 1997. Earth food Spirulina: how this remarkable blue-green algae can transform your health and our planet. Renore Enterprises, Kenwood, CA.
- Khan, Z., Bhadouria, P., Bisen, P.S., 2005. Nutritional and therapeutic potential of Spirulina. Curr Pharm Biotechnol 6, 373-379.
- Kouba, M., Benatmane, F., Blochet, J.E., Mourot, J., 2008. Effect of a linseed diet on lipid oxidation, fatty acid composition of muscle, perirenal fat, and raw and cooked rabbit meat. Meat Sci. 80, 829-834.
- Lu, J., Yoshizaki, G., Sakai, K., Takeuchi, T., 2002. Acceptability of raw *Spirulina platensis* by larval tilapia *Oreochromis niloticus*. Fish. Sci. 68, 51-58.
- Meineri, G., Ingravalle, F., Radice, E., Aragno, M., Peiretti, P.G., 2009. Effects of high fat diets and *Spirulina platensis* supplementation in New Zealand White Rabbits. J. Anim. Vet. Adv. 8 (12), 2735-2744.
- Miranda, M.S., Cintra, R.G., Barros, S.B., Mancini Filho, J., 1998. Antioxidant activity of the microalga *Spirulina maxima*. Braz. J. Med. Biol. Res. 31, 1075-1079.
- Nandeesha, M.C., Gangadhara, B., Manissery, J.K., Venkataraman, L.V., 2001. Growth performance of two Indian major carps, catla (*Catla catla*) and rohu (*Labeo rohita*) fed diets containing different levels of *Spirulina platensis*. Bioresource Technol. 80, 117-120.
- Palmegiano, G.B., Agradi, E., Forneris, G., Gai, F., Gasco, L., Rigamonti, E., Sicuro, B., Zoccarato,
  I., 2005. Spirulina as a nutrient source in diets for growing sturgeon (*Acipenser baeri*).
  Aquacult. Res. 16, 188-195.

- Palmegiano, G.B., Gai, F., Daprà, F., Gasco, L., Sabia, C., Pazzaglia, M., Peiretti, P.G., 2008. Effects of Spirulina and plant oils on the growth and lipids traits of white sturgeon (*Acipenser transmontanus*) fingerlings. Aquacult. Res. 39, 587-595.
- Peiretti, P.G., Mussa, P.P., Prola, L., Meineri, G., 2007. Use of different levels of false flax (*Camelina sativa* L.) seed in diets for fattening rabbits. Livest. Sci. 107, 192-198.
- Peiretti, P.G., Meineri, G., 2008a. Effects on growth performance, carcass characteristics, and the fat and meat fatty acid profile of rabbits fed diets with chia (*Salvia hispanica* L.) seed supplements. Meat Sci. 80, 1116-1121.
- Peiretti, P.G., Meineri, G., 2008b. Effects of diets with increasing levels of *Spirulina platensis* on the performance and apparent digestibility in growing rabbits. Livest. Sci. 118, 173-177.
- Petracci, M., Bianchi M., Cavani, C., 2009. Development of rabbit meat products fortified with n-3 polyunsaturated fatty acids. Nutrients 1, 111-118.
- Ramírez, J.A., Díaz, I., Pla, M., Gil, M., Blasco, A., Oliver, M.A., 2005. Fatty acid composition of leg meat and perirenal fat of rabbits selected by growth rate. Food Chem. 90, 251–256.
- Raoof, B., Kaushik, B.D., Prasanna, R., 2006. Formulation of a low-cost medium for mass production of *Spirulina*. Biomass Bioenerg. 30, 537-542.
- Ross, E., Dominy, W., 1990. The nutritional value of dehydrated, blue-green algae (*Spirulina platensis*) for poultry. Poult. Sci. 69, 794-800.
- SPSS, 1999. Statistical Package for the Social Sciences. Mc Graw-Hill, New York, USA.
- Takeuchi, T., Lu, J., Yoshizaki, G., Satoh, S., 2002. Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw Spirulina. Fish. Sci. 68, 34-40.
- Ulbricht, T.L., Southgate, D.A.T., 1991. Coronary heart disease: seven dietary factors. The Lancet 338 (8773), 985-992.
- Van Soest. P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3591.

Vieira Costa, J.A., Colla, L.M., Duarte Filho, P.F., 2004. Improving Spirulina platensis biomass

yield using a fed-batch process. Bioresource Technol. 92, 237-241.

Yap, T.N., Wu, J.F., Pond, W.G., Krook, L., 1982. Feasibility of feeding *Spirulina maxima*, or *Chlorella sp.* to pigs weaned to a dry diet at 4 to 8 days of age. Nutr. Rep. Int. 25, 543-552.

Ingredients and composition of the experimental diets (from Peiretti and Meineri, 2008)

	Spirulina powder			
_	0	50	100	150
Ingredients (g/kg of diet)				
Spirulina powder <sup>1</sup>	0	50	100	150
Soybean seed meal	150	70	10	-
Corn	270	300	300	300
Dehydrated alfalfa meal	380	390	310	-
Pelleted ground hay	120	110	200	460
Corn oil	40	40	40	40
Lignosulphite	20	20	20	30
Vitamin-mineral premix <sup>2</sup>	20	20	20	20
Chemical composition (%)				
Dry matter (% as fed)	89.9	90.3	90.8	88.2
Organic matter	92.1	91.7	91.8	92.0
Crude protein	18.8	19.1	19.6	19.1
Crude fibre	14.5	15.8	15.9	15.7
Ether extract	4.8	7.0	6.9	7.5
Ash	7.9	8.3	8.2	8.0
Neutral detergent fibre	30.1	29.8	34.2	32.5
Acid detergent fibre	17.0	19.3	19.4	18.6
Gross energy (MJ/kg DM)	18.1	18.9	18.9	19.8
Nutritive value				
Digestible Energy, MJ/kg DM <sup>3</sup>	12.5	11.8	11.9	11.6
Digestible Protein, g/kg DM	120.5	127.7	134.7	130.0
DP/DE, g/MJ	9.6	10.8	11.3	11.2

<sup>-1</sup> Composition: moisture 7.3 %, crude protein 61.8 %, fat 1.7 %, ash 98 %, crude fibre 4 %, nitrogen free extract 263 %, gross energy 20.1 MJ/kg DM, and γ-linolenic acid 241 g/kg of total FA

<sup>2</sup> per kg of the complete diet: Vit. A 200 IU; α-tocopheryl acetate 16 mg; Niacin 72 mg; Vit. B<sub>6</sub> 16 mg; Choline 0.48 mg; DL-methionine 600 mg; Ca 500 mg; P 920 mg; K 500 mg; Na 1 g; Mg 60 mg; Mn 1.7 mg; Cu 0.6 mg

<sup>3</sup> The digestible energy content of the diets was calculated according to the regression proposed by Fernández-Carmona et al. (1996)

Fatty acid pattern (g/kg of total FA) of the experimental diets

	Spirulina powder (g/kg of diet)			
	0	50	100	150
C14	2	1	1	1
C16	113	113	117	115
C16:1	2	2	3	3
C18	21	19	19	17
C18:1 n-9	250	254	250	247
C18:1 n-7	7	6	6	6
C18:2 n-6 (LA)	521	536	524	513
C18:3 n-6 (GLA)	0	4	9	15
C18:3 n-3 (ALA)	43	34	34	22
C18:4 n-3	7	7	11	13
C20	4	4	4	4
C20:1 n-9	3	2	2	2
Unidentified	27	18	22	42

Carcass yield and proportions (means±S.E.) of various carcass parts and organs of rabbits (n=10 for

	Spirulina powder (g/kg of diet)			
	0	50	100	150
Slaughter weight (SW), g	2983±138	3149±65	3184±73	3019±78
Hot carcass weight (HCW), g	$1731 \pm 81$	$1862 \pm 48$	$1885 \pm 48$	$1749 \pm 48$
Commercial carcass weight (CCW), g	$1721 \pm 80$	1853±48	$1875 \pm 48$	1737±48
Carcass yield, %	57.7±0.3	$58.8 \pm 0.6$	$58.9 \pm 0.4$	$57.5 \pm 0.5$
Head, g/kg SW	$54.0{\pm}1.7$	56.7±1.6	57.6±0.9	$54.5 \pm 1.1$
Liver, g/kg SW	$26.0{\pm}1.2$	26.9±0.6	$27.4 \pm 0.8$	27.4±1.6
Kidney, g/kg SW	5.6±0.3	6.0±0.3	$6.0\pm0.2$	5.4±0.3
HLTTO <sup>1</sup> g/kg SW	8.1±0.4	9.1±0.3	$10.0\pm0.4$	8.7±0.3
Skin and limbs g/kg SW	$150 \pm 10$	170±6	176±5	162±9
Hind leg, g/kg CCW	269±13	290±7	290±8	272±7
Foreleg, g/kg CCW	131±6	139±4	138±3	124±3
Breast and ribs, g/kg CCW	$170 \pm 11$	185±7	200±5	$184 \pm 8$
Loin and abdominal wall, g/kg CCW	227±12	249±8	243±12	221±10

<sup>1</sup>HLTTO: Heart, lung, thymus, trachea and oesophagus

Chemical composition (g/kg on a dry matter basis; means±S.E.) of the *longissimus dorsi* muscle of rabbits (n=10 for each group) fed four levels of *Spirulina platensis* after 1 d of storage at 4°C

		Spirulina powder (g/kg of diet)				
	0	50	100	150		
Water	738.8±6.6	734.6±3.6	745.8±2.1	738.2±5.0		
Protein	243.0±5.6	243.0±3.2	234.1±2.4	$240.6 \pm 4.5$		
Ash	12.5±0.2	12.5±0.2	12.0±0.1	12.5±0.3		
Lipid	$5.7 \pm 0.6^{a}$	$9.9{\pm}0.8^{\mathrm{b}}$	$8.1{\pm}0.6^{ m b}$	$8.7{\pm}0.6^{\mathrm{b}}$		

<sup>a,b</sup> Means in the same row with unlike superscripts differ (P<0.05)

Lipid content (g/kg fresh matter; means±S.E.), fatty acid pattern (g/kg of total FA; means±S.E.) and indexes related to human health in the perirenal fat of rabbits (n=10 for each group) fed increasing levels of *Spirulina platensis* 

	Spirulina powder (g/kg of diet)			
	0	50	100	150
Lipid	707.3±13.0	699.7±14.0	724.6±19.5	722.6±12.8
C10:0	$0.3{\pm}0.1^{a}$	$1.0{\pm}0.3^{b}$	$0.6{\pm}0.1^{ab}$	$0.6{\pm}0.2^{ab}$
C12:0	0.7±0.1	1.3±0.3	$1.0\pm0.2$	$1.2 \pm 0.3$
C14:0	$21.5{\pm}0.9^{a}$	$16.1 \pm 0.5^{b}$	$16.4{\pm}0.5^{b}$	$16.9 \pm 0.7^{b}$
C14:1	1.5±0.3	$1.2 \pm 0.2$	$0.9\pm0.2$	$0.9\pm0.2$
C15:0	$5.1{\pm}0.2^{a}$	$4.1 \pm 0.1^{c}$	$4.6{\pm}0.2^{\rm b}$	$4.1\pm0.1^{c}$
C16:0	$240.6 \pm 8.4^{a}$	$205.2 \pm 3.2^{b}$	$214.3 \pm 3.6^{b}$	$216.5 \pm 2.9^{b}$
C16:1	$26.0 \pm 2.5$	21.5±1.7	19.3±1.7	$20.4{\pm}2.0$
C17:0	$6.0 \pm 0.3^{ab}$	$5.5 \pm 0.1^{a}$	$6.7 \pm 0.3^{\circ}$	$6.3 \pm 0.2^{bc}$
C18:0	$51.2{\pm}2.0^{a}$	$45.3 \pm 0.8^{bc}$	$49.4 \pm 2.1^{ab}$	$44.1 \pm 1.1^{c}$
C18:1 n-9	$254.6 \pm 2.8^{a}$	$244.2\pm2.1^{b}$	$240.1 \pm 3.1^{b}$	$241.5 \pm 2.0^{b}$
C18:1 n-7	$9.5 \pm 0.3^{a}$	$8.6 \pm 0.3^{b}$	$8.5{\pm}0.3^{\mathrm{b}}$	$7.7 \pm 0.2^{\circ}$
C18:2 n-6 (LA)	$319.2 \pm 11.0^{a}$	$384.3 \pm 6.2^{b}$	$372.0 \pm 4.9^{b}$	$387.9 \pm 5.8^{b}$
C18:3 n-6 (GLA)	$1.9{\pm}0.1^{a}$	$5.4{\pm}0.8^{\rm b}$	$8.0{\pm}0.9^{c}$	$11.7 \pm 0.5^{d}$
C18:3 n-3 (ALA)	$35.6 \pm 0.9^{a}$	$34.3 \pm 2.5^{a}$	$35.7 \pm 0.7^{a}$	$18.8 \pm 0.3^{b}$
C20:0	1.1±0.2	1.3±0.1	$1.2\pm0.2$	$1.4\pm0.1$
C20:1 n-9	2.2±0.2	$2.0\pm0.1$	$2.0\pm0.1$	$1.8\pm0.1$
C20:2 n-6	$1.7{\pm}0.1^{a}$	$2.2 \pm 0.1^{bc}$	$2.3 \pm 0.1^{\circ}$	$1.9{\pm}0.1^{ab}$
C21:0	$1.2{\pm}0.2^{a}$	$1.6 \pm 0.1^{ab}$	$1.6{\pm}0.1^{ab}$	$2.0{\pm}0.1^{b}$
Unidentified	$20.2{\pm}2.6^{a}$	$14.8 \pm 0.6^{b}$	$15.5 \pm 1.3^{b}$	$14.1 \pm 0.4^{b}$
$SFA^1$	327.6±9.1 <sup>a</sup>	$281.5 \pm 3.7^{b}$	$295.9 \pm 4.8^{b}$	293.3±3.3 <sup>b</sup>
MUFA <sup>2</sup>	$293.8 \pm 5.4^{a}$	$277.5 \pm 3.5^{b}$	$270.8 \pm 4.8^{b}$	$272.3 \pm 4.0^{b}$
PUFA <sup>3</sup>	$358.4{\pm}11.8^{a}$	$426.1 \pm 6.8^{b}$	$417.9 \pm 5.1^{b}$	$420.3 \pm 6.1^{b}$
PUFA n-3 <sup>4</sup>	$35.6 \pm 0.9^{a}$	$34.3 \pm 2.5^{b}$	$35.7{\pm}0.7^{b}$	$18.8 \pm 0.3^{b}$
PUFA n-6 <sup>5</sup>	$322.8 \pm 11.1^{a}$	$391.9 \pm 6.4^{b}$	$382.2\pm5.0^{b}$	$401.5 \pm 6.1^{b}$
$n-6/n-3^{6}$	$9.08 \pm 0.21^{a}$	$12.80 \pm 2.13^{b}$	$10.74 \pm 0.25^{ab}$	$21.42 \pm 0.40^{\circ}$
Atherogenic Index	$0.50{\pm}0.03^{a}$	$0.39{\pm}0.01^{b}$	$0.41{\pm}0.01^{b}$	$0.41 \pm 0.01^{b}$
Thrombogenic Index	$0.76{\pm}0.04^{a}$	$0.61 \pm 0.02^{c}$	$0.65 \pm 0.01^{bc}$	$0.71 {\pm} 0.01^{ab}$

<sup>a,b,c</sup> Means in the same row with unlike superscripts differ (P < 0.05)

<sup>1</sup> SFA: Saturated Fatty Acid

<sup>2</sup> MUFA: Monounsaturated Fatty Acid

<sup>3</sup> PUFA: Polyunsaturated Fatty Acid

<sup>4</sup> PUFA n-3: Polyunsaturated Fatty Acid series n-3

<sup>5</sup> PUFA n-6: Polyunsaturated Fatty Acid series n-6

<sup>6</sup> n-6/n-3: PUFA n-6/ PUFA n-3 ratio

Fatty acid pattern (g/kg of total FA; means±S.E.) and indexes related to human health in the *longissimus dorsi* of rabbits (n=10 for each group) fed increasing levels of *Spirulina platensis* 

		0 1 1		
	Spirulina powder (g/kg of diet)			
	0	50	100	150
C14:0	23.4±1.5	$19.8 \pm 0.5$	21.6±0.7	$21.7 \pm 1.2$
C15:0	$5.0\pm0.1^{a}$	$4.3 \pm 0.1^{\circ}$	$4.7 \pm 0.1^{b}$	$4.5 \pm 0.1^{bc}$
C16:0	$284.1\pm6.4^{a}$	$245.2\pm2.7^{c}$	$258.5 \pm 4.2^{b}$	$260.8 \pm 2.9^{b}$
C16:1	31.3±3.3	$28.4{\pm}1.9$	$30.5 \pm 3.6$	$27.4 \pm 2.9$
C17:0	$6.5 \pm 0.3^{ab}$	$5.9 \pm 0.1^{a}$	$7.0{\pm}0.3^{\rm b}$	$7.1 \pm 0.3^{b}$
C18:0	$66.6 \pm 4.5$	55.1±1.7	62.7±3.5	$57.2 \pm 3.5$
C18:1 n-9	$236.8 \pm 5.7$	234.9±3.1	$237.8 \pm 2.5$	$238.9 \pm 2.2$
C18:1 n-7	$9.2{\pm}0.3^{a}$	$8.4{\pm}0.2^{b}$	$8.6{\pm}0.1^{\mathrm{ab}}$	$9.2 \pm 0.3^{a}$
C18:2 n-6 (LA)	$239.7 \pm 5.3^{a}$	$308.9 \pm 8.0^{b}$	$297.9 \pm 4.8^{b}$	$307.3 \pm 4.8^{b}$
C18:3 n-6 (GLA)	$0.0^{\mathrm{a}}$	$4.4{\pm}0.3^{b}$	$6.6 \pm 0.2^{\circ}$	$8.9{\pm}0.4^{d}$
C18:3 n-3 (ALA)	$27.1 \pm 2.6^{a}$	$28.7 \pm 1.3^{a}$	$26.8 \pm 1.3^{a}$	$15.3 \pm 0.5^{b}$
C20:4 n-6	$22.8 \pm 2.5^{a}$	$17.8 \pm 1.8^{b}$	$15.6 \pm 0.7^{b}$	$17.2 \pm 1.1^{b}$
C22:4 n-6	6.7±1.1	$7.3 \pm 0.8$	5.7±0.7	$5.9 \pm 0.9$
Unidentified	$40.8 \pm 4.3^{a}$	$30.8 \pm 3.9^{a}$	$15.9 \pm 1.7^{b}$	$18.6 \pm 3.4^{b}$
$SFA^1$	$408.4{\pm}7.0^{a}$	$348.2 \pm 5.0^{\circ}$	$370.1 \pm 6.9^{b}$	$368.5 \pm 3.4^{b}$
$MUFA^2$	277.3±7.8	271.7±4.0	276.9±5.6	$275.5 \pm 5.1$
PUFA <sup>3</sup>	$266.8 \pm 6.4^{a}$	$342.1 \pm 7.8^{b}$	$331.3 \pm 5.7^{b}$	$331.5 \pm 5.4^{b}$
PUFA n-3 <sup>4</sup>	$27.1 \pm 2.6^{a}$	$28.7 \pm 1.3^{a}$	$26.8 \pm 1.3^{a}$	$15.3 \pm 0.5^{b}$
PUFA n-6 <sup>5</sup>	$239.7 \pm 5.3^{a}$	$313.3 \pm 7.9^{b}$	$304.5 \pm 5.0^{b}$	$316.2 \pm 5.1^{b}$
$n-6/n-3^{6}$	$9.42{\pm}0.69^{a}$	$11.1 \pm 0.49^{b}$	$11.52 \pm 0.42^{b}$	$20.77 \pm 0.60^{\circ}$
Atherogenic Index	$0.70{\pm}0.02^{a}$	$0.53{\pm}0.01^{b}$	$0.57 \pm 0.01^{b}$	$0.57 \pm 0.01^{b}$
Thrombogenic Index	$1.11 \pm 0.04^{a}$	$0.85 \pm 0.02^{\circ}$	$0.92 \pm 0.03^{bc}$	$0.99 {\pm} 0.01^{b}$

<sup>a,b,c</sup> Means in the same row with unlike superscripts differ (P < 0.05)

<sup>1</sup> SFA: Saturated Fatty Acid

<sup>2</sup> MUFA: Monounsaturated Fatty Acid

<sup>3</sup> PUFA: Polyunsaturated Fatty Acid

<sup>4</sup> PUFA n-3: Polyunsaturated Fatty Acid series n-3

<sup>5</sup> PUFA n-6: Polyunsaturated Fatty Acid series n-6

<sup>6</sup> n-6/n-3: PUFA n-6/ PUFA n-3 ratio