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# Effect of a cauliflower (Brassica oleraceae var. Botrytis) leaf powder-enriched diet on performance, carcass and meat characteristics of growing rabbit



MEAT SCIENCE

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# ABSTRACT

Phenolic content, antioxidant activity

The aim of this study was to investigate the effect of a cauliflower leaf powder (CLP)-enriched diet on the performance, quality and antioxidative potential of rabbit meat. No significant differences were found for live performance parameters between rabbits fed with standard (SD) and CLP diet. Dietary supplementation influenced the meat traits of rabbits: CLP meat showed significantly lower drip loss after 48 h, cooking loss, and a significantly higher lightness (L\*) and redness (a\*) values, vitamin A and vitamin E content, and oxidative stability, compared to SD meat. Moreover, the CLP supplementation caused a significant decrease in SFA and increase in PUFA percentage of rabbit intramuscular fat. The statistical analysis also showed a significant effect of dietary fortification on phenolic content and antioxidant activity of rabbit meat which resulted higher in meat of CLP group. This study highlighted that dietary fortification with CLP is a valid strategy to produce rabbit meat with better technological and functional quality.

## 1. Introduction

Meat may be considered a functional food since it is a source of numerous bioactive components which have been demonstrated to have several biological activities (Ahhmed & Muguruma, 2010; Simonetti, Gambacorta, & Perna, 2016). In fact, meat is a source of bioactive peptides (Samaranayaka & Li-Chan, 2011) and vital nutrients such as B vitamins, P, Mg, Co, Zn, Fe, and Se; furthermore, it contribute to the intake of components which are often under-consumed by adults such as vitamin E and omega-3 fatty acids (DHHS, 2010). Rabbit meat shows high nutritive and dietetic properties because of its richness in proteins of high biological value due to high essential amino acid levels, high unsaturated fatty acids proportion and low cholesterol, and high amounts of micronutrients (Dalle Zotte & Szendrő, 2011; Hermida, Gozalez, Miranda, & Rodríguez-Otero, 2006). However, the main problem linked to high polyunsaturated fatty acids content in rabbit meat is the susceptibility of these unsaturated fatty acids to oxidative deterioration, resultantly decreasing meat quality. In fact, lipid oxidation of PUFA is among the main causes of meat quality degradation because it produces intermediate reaction products which will react further to form aldehydes and ketones indicative of rancid odor, off-flavor and surface discoloration of meat (Ahn, Nam, & Lee, 2009). In addition, intramuscular fat, antioxidant, heme pigment, iron contents and fatty acid composition influence the rate of lipid oxidation of meat. To

improve the functional value of rabbit meat and, at the same time, its shelf life, the animal dietary fortification with nutraceutical components is a valid and sustainable strategy. Some herbs and spices are important sources of natural bioactive components such as polyphenols which are phytochemicals produced by plants (secondary metabolites) to defend against environmental stress and pathogens (Havsteen, 2002) and they exert in human various therapeutic activities (Kennedy, 2014; Russo et al., 2018). Moreover, polyphenols for their antioxidant properties could be considered as an useful tool to improve the shelf life of meats vulnerable to oxidative changes. The Brassicaceae or Cruciferae family is comprised of 350 genera and about 3500 species (Sasaki & Takahashi, 2002). Brassicaceae vegetables are an inexpensive food source that provides nutrients and a complex mixture of health-promoting phytochemicals that reduce the risk of diseases such as polyphenols, minerals, vitamin E and C (Heimler, Vignolini, Dini, Vincieri, & Romani, 2005; Vallejo, Gil-Izquierdo, Perez-Vicente, & Garcia-Viguera, 2004), glucosinolates (Fowke et al., 2003), and amino acids (Ayaz et al., 2006). However, glucosinolates are very unpalatable and therefore, they could cause a reduction of their feed intake, which would lead to reduce growth; moreover, some glucosinolates and their degradation products originating from enzymatic cleavage by myrosinase, have been linked to toxic and/or anti-nutritional effects (Larocca et al., 2017). Tripathi and Mishra (2007) reported that a glucosinolates content of 7.9 mmol/kg, at most, in the rabbit dietary

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has no apparent negative effects on the growth and health of animals. The beneficial effects of Brassicacea vegetables on human health are generally associated with their antioxidant capacity and, undoubtedly, flavonoids and phenolic acids are the major antioxidants of this plant (Podsedek, 2005; Singh et al., 2006). The aim of this study was to investigate the effect of a cauliflower (*Brassica oleraceae* var. *Botrytis*) leaf powder (CLP)-enriched diet on the performance, quality and antioxidative potential of rabbit meat.

## 2. Materials and methods

#### 2.1. Samples

This study was evaluated and approved by the Ethical Institutional Committee on Animal Experimentation of the Institute for the Animal Production System in the Mediterranean Environment (CNR, Consiglio Nazionale delle Ricerche, Italy; www.ispaam.cnr.it) as per protocol number 6. One hundred male rabbits (New Zealand White), weaned at  $30 \pm 2$  days of age, with approximately equal mean body weight, were acclimatized for ten days in laboratory conditions and were randomly divided into two groups of 50 animals each and housed individually in cages (60  $\times$  25 cm length  $\,\times$  33 cm height; 22  $\,\pm\,$  2 °C of temperature,  $65 \pm 5\%$  relative humidity) under a 16 h light/8 h dark cycle. One group was fed with commercial pelleted diet for growing rabbit (control, SD group), and the second group was fed with diet supplemented with cauliflower leaves powder at 30% (CLP group; 300 g CLP/kg diet). For CLP preparation, after harvesting, the leaves were washed, dried in an oven at 50 °C for 7 h, and finely minced. Proximate analysis using Association of Official Analytical Chemists (AOAC, 2000) methods showed that CLP contained on a dry matter basis: 21.16% protein, 10.64% ash, 5.93% total lipid and 62.27% total carbohydrate. The total phenolic content in CLP was extracted and determined spectrophotometrically using Folin-Ciocalteu reagent as described by Larocca et al. (2017), and the content was 8.27 mg of gallic acid equivalents (GAE) per g of dry weight. The experimental diet was prepared by adding the CLP to the commercial diet before the pelleting process. The glucosinolates in CLP was extracted according to the Rosa (1997) procedure and determined by HPLC analysis as described by Spinks, Sones, and Fenwick (1984). This content was below 7.9 mmol/kg, as recommended by Tripathi and Mishra (2007) to avoid negative effects on animal growth and health. The ingredients and chemical composition (AOAC, 2000) of two diets are reported in Table 1. The animals were fed ad libitum and had free access to food and water until the end of the trial. Weekly throughout the 60 days of duration of the study, all rabbits and feed residues were weighed to determine the following live performance parameters: average body weight (BW), average daily gain (ADG), average feed intake (FI), and feed conversion ratio (FCR). On day 60 the rabbits were slaughtered and hot carcass weight was recorded according to Blasco, Ouhayoun, and Masoero (1992) recommendations. After 24 h of cold storage (4 °C) Longissimus lumborum (LL) muscle was removed from each carcass. Finally, samples were vacuum packed and stored at -20 °C until analysed.

# 2.2. Chemical composition and rheological parameters

The pH measurement was performed 24 h after slaughter using a pH-meter (model PHM 92, Radiometer, Copenhagen, Denmark) in distilled water extract with a 1:1 meat to water ratio (20 °C), after 1 h of extraction. Dry matter (DM), protein, intramuscular fat (IMF), and ash contents of LL muscle were determined according to AOAC (1995) methods. DM was determined by oven drying method at 105 °C until constant weight (method 950.46), protein by Kjeldahl method (method 990.03) using a 6.25 factor to convert the nitrogen content into total protein, IMF by Soxhlet extraction (method 920.39), and ash by using a muffle furnace for 12 h at 550 °C (method 920.153). Haem iron content was determined according to the method described by Hornsey (1956).

Table 1

I	ngredients	and	chemical	composition	of diet.
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	Standard diet	Diet supplemented with $\mbox{CLP}^1$
Ingredients, g/kg diet		
Soybean meal	230	160
Alfalfa hay	220	140
Wheat bran	210	130
Alfalfa meal dehydratated, 17% CP	100	86.8
Beet pulp	100	70
Barley	77	60
Wheat	20	10
Calcium carbonate	20	20
Soybean oil	10	10
Sodium Chloride	4	4
Dicalcium phosphate	1.8	2
Vitamin and mineral premix <sup>2</sup>	2.5	2.5
Methionine (99%)	2.3	2.3
Lysine (78.5%)	1.4	1.4
Choline (75.0%)	1	1
Cauliflower leaves powder	0	300
Chemical composition, g/kg <sup>3</sup>		
Dry matter (DM)	$909.0 \pm 1.0$	915.7 ± 1.4
Crude protein	$165.8 \pm 0.6$	$166.2 \pm 0.8$
Crude fiber	$171.4 \pm 1.1$	$171.6 \pm 1.0$
Ether extract	$26.8 \pm 0.3$	$29.2 \pm 0.5$
Crude ash	$61.8~\pm~0.3$	$62.6 \pm 0.1$
NDF	$366.9 \pm 1.1$	$365.3 \pm 1.1$
ADF	$207.3 \pm 1.7$	$205.1 \pm 1.2$

<sup>1</sup> CLP: cauliflower leaf powder.

 $^2$  Supplied per kg of feed: vitamin A 12000 I. U., vitamin D3 2200 I. U., vitamin E 10.0 mg, vitamin B2 4.0 mg, vitamin B6 1.5 mg, vitamin B1 1.0 mg, vitamin B1 2 0.001 mg, vitamin K 2.0 mg, vitamin B8 0.07 mg, vitamin PP 6.7 mg, folic acid 1.67 mg, D-pantotenic acid 6.67 mg, Choline chloride 400 mg, magnesium 133.4 mg, copper 1.67 mg, manganese 10.0 mg, iron 25.0 mg, zinc 22.3 mg, iodine 0.25 mg, selenium 0.033 mg.

<sup>3</sup> n = 3; mean  $\pm$  SD.

The water holding capacity (WHC) of LL muscle at 48 h post mortem, measured as Percent Drip Loss, was carried out as described by Simonetti, Perna, Giudice, Cappuccio, and Gambacorta (2018). The cooking loss was evaluated as described by Tartrakoon, Tartrakoon, and Kitsupee (2016) and the results were expressed as a percentage of the initial sample weight (Vergara, Gallego, García, & Landete-Castillejos, 2003). All samples were analysed in triplicate.

## 2.3. Lipid oxidative status

Lipid oxidation was evaluated with a spectrophotometer (Shimadzu UV–vis Spectrophotometer 1204, Kyoto, Japan) that measured the absorbance of thio-barbituric acid-reactive substances (TBARS; Ke, Ackman, Linke, & Nash, 1977). TBARS were determined using the method described by Botsoglou et al. (1994). The standard curve was prepared as described by Perna, Simonetti, Intaglietta, and Gambacorta (2015a), and the oxidation products were quantified as mg of malondialdehyde/kg muscle. All samples were analysed in triplicate.

## 2.4. HPLC analysis of vitamins E and A

The saponification and extraction of vitamins E and A was carried out as described by Perna, Simonetti, Intaglietta, and Gambacorta (2015b) and measured by liquid chromatography equipped with Varian ProStar Pump model 210, Rheodyne injector with a 20 mL loop, fluorescence detector Varian ProStar model 363 and using Galaxie™ Chromatography Software (Varian, Inc., Walnut Creek, CA, USA) as described by Perna et al. (2015b). Identification and quantification of the peaks were done by comparison with vitamin E and A standards and the results were expressed as mg/kg fresh matter .

#### 2.5. Instrumental colour measurement

Instrumental colour (CIE  $L^*$ ,  $a^*$ ,  $b^*$ ) was measured as described by Simonetti et al. (2018) using a MINOLTA Chromameter CR-300 (Minolta Camera Corp., Meter Division, Ramsey, NJ, USA) equipped with a D65 illuminant, the 10° Observer and zero and white calibration. The measuring head has an 8 mm-diameter measuring area. All samples were analysed in triplicate.

## 2.6. Fatty acids profile

The lipid fraction for the fatty acid evaluation was extracted from rabbit meat following the method reported by Folch, Lees, and Stanley (1957), and fatty acid methyl esters (FAMEs) were prepared according to the ISO (1978) method. Analysis was performed as described by Perna et al. (2015a), using a Varian 3400 gas chromatograph (Varian, Turin, Italy), equipped with a split-splitless injector, a TR-FAME capillary column (120 m × 0.25 mm i.d. × 0.25 µm film thickness; Thermo Fisher Scientific, Milan, Italy), a flame ionization detector (FID) and a Galaxie<sup>™</sup> Chromatography Software (Varian, Inc., Walnut Creek, CA/USA) for chromatogram acquisition and data reporting. The results were expressed as percentage of the total fatty acids analysed.

Indexes of atherogenicity (AI) and thrombogenicity (TI) were calculated as suggested by Ulbricht and Southgate (1991):

AI =  $[(4 \times C14:0) + C16:0]/[\Sigma MUFA + \Sigma PUFA-n6 + \Sigma PUFA-n3];$ TI =  $(C14:0 + C16:0 + C18:0)/(0.5 \quad MUFA + 0.5 \quad PUFA-n6 + 3$ PUFA-n3 + PUFA-n3/PUFA-n6).

#### 2.7. Total phenol content (TPC) and antioxidant activity of meat samples

For meat extract preparation, 5 g of each meat sample was homogenized with 10 mL of 0.05 M phosphate buffer (pH 7) using a Polytron (PT-MR 2100, Kinematica AG, Littau, Luzern, Switzerland) at 13,500 rpm for 15 s. The homogenates were placed in an ultrasound (US) water bath apparatus (Elma Transsonic 460/H, Singen, Germany) for 10 min at 25 °C and centrifuged at 5000  $\times$  g at 4 °C for 20 min. The supernatant was filtered through a 0.45 µm cellulose acetate membrane filter (Sigma-Aldrich, Milan, Italy) and was kept a -55 °C until analysis. TPC in meat samples was determined using Folin-Ciocalteu reagent, following the method of Qwele et al. (2013). Gallic acid (0-200 mg/L) was used as standard to derive the calibration curve and the results were expressed as mg of GAE/ 100 g of meat. The 2,2'-azinobis-3-thylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging and ferric-reducing antioxidant power (FRAP) assays were carried out according to Perna, Intaglietta, Simonetti, and Gambacorta (2014). Results were expressed as micrograms of Trolox equivalents (TE) per gram of sample. Each determination and measurement were made in triplicate.

#### 2.8. Statistical analysis

Data were analysed according to the following linear model (SAS Institute, 1996):

 $y_{ik} = \mu + \alpha_i + \varepsilon_{ik}$ 

where  $y_{ik}$  is the observation;  $\mu$  is the overall mean;  $\alpha_i$  is the fixed effect of the *i*th diet (i = 1, 2); and  $\varepsilon_{ik}$  is the random error. Before setting the values, expressed in percentage terms, they were subjected to arcsine transformation. Differences between means at the 95% (P < .05) confidence level were considered statistically significant.

#### 3. Results and discussion

#### 3.1. Productive performance

Growth performance parameters of rabbits fed with standard (SD) and cauliflower leaves powder (CLP) diet are shown in Table 2. No

#### Table 2

Body weight, average daily gain, feed intake, and feed conversion ratio of rabbits fed according to the diet (SD: standard diet, CLP: cauliflower leaves powder diet).

	Diet		SEM <sup>1</sup>	<i>P</i> -value <sup>2</sup>
	SD	CLP		
N° of rabbits	50	50		
Age, days	Body weight, kg			
40	0.89	0.93	0.012	0.604
70	1.94	1.86	0.014	0.280
100	3.25	3.27	0.016	0.574
	Average daily gain, g/d			
40–70	37.36	33.21	0.604	0.059
70-100	43.66	46.96	0.521	0.737
40-100	40.62	39.81	0.209	0.195
	Feed intake, g			
40–70	68.15	66.75	0.426	0.062
70-100	130.54	126.48	0.736	0.060
40-100	100.42	97.64	0.455	0.061
	Feed conversion ratio, g feed/g of live weight			
40–70	1.82	2.07	0.090	0.609
70-100	2.99	2.69	0.081	0.252
40–100	2.47	2.45	0.069	0.566

<sup>1</sup> SEM: standard error of means.

<sup>2</sup> *P*-value is significant at P < .05.

differences were observed between the two dietary groups (P > .05) for analysed growth performance parameters; although, average feed intake (FI) tended to be lower (P = .061) in the CLP group than control group. Dal Bosco et al. (2014)) found a lower (P < .001) FI in rabbits fed with natural bioactive compounds (fresh alfalfa). On the contrary, Palazzo et al. (2015) in rabbits fed with natural extract from *Lippia* citriodora and Chrenková et al. (2013) in rabbits fed with dry extract of Siberian ginseng (Eleutherococcus senticosus) detected a higher FI compared to control group; even if no improvement in the final body weight and feed conversion ratio in rabbits of supplemented group were observed. However, Zhao, Xu, Du, Li, and Zhang (2005) detected an improvement in appetite and productive performance in growing rabbits fed with Chinese herbs. The effect of dietary supplementation on carcass traits of rabbits is reported in Table 3. The results relating to hot carcass weights and slaughter yield are similar in two groups (P > .05). Our results are supported by Dalle Zotte and Cossu (2009) who found no significant effect of diet on above mentioned carcass traits in rabbits fed with tannin extract from red quebracho trees (Schinopsis spp.). Drip loss after 48 h highlighted a significant effect of dietary treatment with a lower value in the CLP group compared with the SD group (P < .05). Even Dal Bosco et al. (2014) showed a positive effect of thymus supplementation in the rabbit diet on drip loss of meat. This was probably due to the positive effect of antioxidants on maintenance of the muscle fibres integrity, which implement their capability to retain water. Cheah, Cheah, and Krausgrill (1995) reported that the antioxidants are able to stabilize membranes by the inhibition of the phospholipase A2 activity and by lowering Ca<sup>2+</sup> release, this leads to a reduction of the rate of post-mortem glycolysis with an increase pH and

## Table 3

Effect of diet (SD: standard diet, CLP: cauliflower leaves powder diet) on carcass traits of rabbits.

	Diet		$SEM^1$	<i>P</i> -value <sup>2</sup>
	SD	CLP		
N° of rabbits	50	50		
Hot carcass weight, kg	2.18	2.09	0.027	0.621
Slaughter yield, %	61.06	61.17	0.258	0.387
Drip loss after 48 h, %	2.55	2.28	0.016	0.049
Cooking loss, %	32.44	30.03	0.154	0.036

<sup>1</sup> SEM: standard error of means.

<sup>2</sup> *P*-value is significant at P < .05.

a positive effect on drip loss. The our results were similar to those reported by Mitsumoto, Arnold, Schaefer, and Cassens (1995), and Monahan, Buckley, Gray, and Morrissey (1990), in beef and pork meat, respectively. Asghar et al. (1989), in poultry meat, reported that the antioxidants improve the capacity of membranes to function as semi permeable barriers against exudative loss. Cooking process affects meat quality, because some nutrients may be lost in the cooking juice with effects on juiciness and tenderness of meat. In this study, CLP group showed a lower cooking loss of meat compared to SD group (P < .05). To support this, several studies (Cardinali et al., 2012; Kołodziej-Skalska et al., 2011: Symeon, Zintilas, Avoutanti, Bizelis, & Deligeorgis, 2009) highlighted the positive effect of dietary supplementation with sources rich in polyphenols on cooking loss of meat. On the contrary, Peiretti and Meineri (2008) detected an increased cooking loss on rabbit meat with a dietary supplementation of 10% of Chia seeds (Salvia hispanica L.).

#### 3.2. Chemical composition and colourimetric parameter

No statistically significant differences (P > .05) were found for proximate composition of meat between SD and CLP groups (Table 4). These findings are consistent with what is reported by many authors in meat of animals fed with several polyphenol-rich supplementation (Cardinali et al., 2015; Peiretti, Gai, Rotolo, Brugiapaglia, & Gasco, 2013; Peiretti, Gasco, Brugiapaglia, & Gai, 2011). As expected, vitamin E and A content was higher in CLP rabbits than in the SD ones (P < .001). On the other hand cauliflower leaves are a very good source of these vitamins (Li, Pang, & Piao, 2017; Vallejo et al., 2004). In particular, in meat of CLP group vitamin A and E content was 1.8 times and 1.5 times, respectively, higher than meat of SD group. This increase in vitamin E extends meat shelf life and improve the characteristics of rabbit meat as water holding capacity (Zhang, Xiao, Samaraweera, Lee, & Ahn, 2010). Furthermore, vitamin E plays a fundamental role as biological antioxidant (Dalle Zotte & Szendrő, 2011; Rooke, Robinson, & Arthur, 2004). It is known that meat oxidative stability is greatly influenced by the diet of animals (Aouadi et al., 2014). Oxidation in meat can be assessed by measuring the amount of thiobarbituric acidreactive substances (TBARS) which were significantly affected by dietary supplementation (P < .01). In this study, CLP meat showed lower TBARS value compared to SD meat (P < .001; Table 4). Even Botsoglou, Florou-Paneri, Christaki, Giannenas, and Spais (2004) found a lower TBARS value in meat of rabbits fed with oregano essential oil supplementation compared to control meat. Meat colour is a important visible parameter that reflects the meat quality. The meat colour of SD and CLP group, as expressed in terms of lightness (L\*), redness (a\*), and yellowness (b\*) is reported in Table 5. L\* and a\* values were higher in meat from CLP group than meat from SD group (P < .05), whereas no

#### Table 4

Chemical composition of *Longissiumus lumborum* muscle of rabbits fed according to the diet (SD: standard diet, CLP: cauliflower leaves powder diet).

	Diet		SEM <sup>1</sup>	<i>P</i> -value <sup>2</sup>
	SD	CLP		
N° of rabbits	50	50		
pH after 24 h	5.74	5.70	0.058	0.238
Dry matter, g/100 g meat	26.65	27.17	0.140	0.082
Fat, g/100 g meat	2.96	3.04	0.050	0.102
Protein, g/100 g meat	22.62	23.00	0.117	0.135
Ash, g/100 g meat	1.18	1.12	0.014	0.071
Haem iron, ppm	3.26	3.38	0.073	0.269
Vitamin A, mg/g meat	0.31	0.57	0.005	2.18E-05
Vitamin E, mg/g meat	2.67	3.86	0.036	1.62E-07
TBARS <sup>3</sup> , mg MDA/kg meat	0.11	0.09	0.001	2.13E-05

<sup>1</sup> SEM: standard error of means.

<sup>2</sup> *P*-value is significant at P < .05.

<sup>3</sup> TBARS: thio-barbituric acid-reactive substances.

#### Table 5

Colourimetric parameters of *Longissiumus lumborum* muscle of rabbits fed according to the diet (SD: standard diet, CLP: cauliflower leaves powder diet).

	Diet		$SEM^1$	<i>P</i> -value <sup>2</sup>
N° of rabbits L* a* b*	SD 50 54.37 1.95 2.98	CLP 50 58.21 2.09 3.04	0.279 0.102 0.176	0.002 0.019 0.327

<sup>1</sup> SEM: standard error of means.

<sup>2</sup> *P*-value is significant at P < .05.

significant difference was found for b\* value between the two groups. Even Mancini, Secci, Preziuso, Parisi, and Paci (2018) found a higher L\* value in L. lumborum muscle of rabbits fed with ginger supplementation. On the contrary, no significant differences for L\* value was detected by Cardinali et al. (2015) in rabbit meat fed with oregano and rosemary extract supplementation, by Peiretti et al. (2013) in rabbit meat fed with byproducts of tomato processing supplementation, and by Alagón et al. (2015) in rabbit fed with wheat dried distillers grains with soluble of barley, wheat and corn. Red colour of meat is mainly due to myoglobin oxygenation and the oxidative status of the haem iron (Faustman, Sun, Mancini, & Suman, 2010). Antioxidant compounds delay the oxidative process with consequent reduction in the production of meta-myoglobin (gray-brown). In line with our results, Alagón et al. (2015) showed a increase of a\* value in rabbits meat fed with a dietary supplementation of 20% of wheat dried distillers grains with soluble. However, contrary to what is reported in our study, a significant effect of dietary supplementation on b\* value was detected by Dalle Zotte and Cossu (2009) in rabbits fed with tannin extract from red quebracho trees (Schinopsis spp.) and by Peiretti et al. (2013) in rabbit meat fed with 6% of byproducts of tomato processing supplementation.

## 3.3. Fatty acid profile

The fatty acid profiles of the rabbits IMF fed on SD and CLP diet are reported in Table 6. Overall, LL muscle of rabbits fed with standard diet showed a SFA (51.11%), MUFA (29.82%), and PUFA (19.06%) content in line with what found by other authors in rabbit meat (Dal Bosco et al., 2012; Kouba, Benatmane, Blochet, & Mourot, 2008). The CLP supplementation caused a significant decrease (P < .001) in SFA percentage due to lower proportion of C14:0, C16:0, C18:0 and C20:0 acids, and a significant increase (P < .001) in PUFA percentage mainly due to higher proportion of linoleic (+16.82%), linolenic (+18.69%) arachidonic (ARA; > 100%), eicosapentaenoic (EPA; > 37.5%) docosapentaenoic (DPA; 75%), and docosahexaenoic (DHA; > 100%) acids. Consequently, n-6 and n-3 fatty acids content in the IMF of the experimental group is increased of 21.38 and 38.10% (P < .001), respectively, compared to SD group. These results were in agreement with what reported by other authors in meat of rabbit fed with Lippia citriodora extract (Palazzo et al., 2015) and Salvia hispanica L. seeds (Meineri, Cornale, Tassone, & Peiretti, 2010). In fact, rabbit, as a monogastric, is able to incorporate the long chain fatty acids, coming from the diet, in the adipose tissue and IMF (Dalle Zotte, 2002), thus their derived long chain PUFAs are the result of the exogenous lipids influence, such as linolenic acid which is particularly present in cauliflower leaves (Bhandari & Kwak, 2015). Even Jung et al. (2010), in breast of broilers fed with a mixture of polyphenols (gallic acid) and linoleic acid, found a reduction in SFA and MUFA and an increase in PUFA content; these authors hypothesized that the increased of PUFAs level could reduce the synthesis of MUFA by inhibiting the activity of  $\Delta$ 9-desaturase enzyme that converts SFA into MUFA. In this study, the nutritional quality of the lipid fraction of foods was evaluate by n-6/n-3 ratio, that should not exceed 4.0 (Department of Heath, 1994), atherogenic index (AI) and thrombogenic index (TI), which must be as

#### Table 6

Fatty acid profile (% of total fatty acids), fatty acid ratios and nutritional indices of intramuscular fat of rabbits fed according to the diet (SD: standard diet, CLP: cauliflower leaves powder diet).

SD CLP   N° of rabbits 50 50   C14:0 2.36 2.00 0.027 0.001   C16:0 36.04 31.31 0.300 0.002   C18:0 10.23 8.46 0.105 0.001   C20:0 0.32 0.27 0.004 0.003   C22:0 0.70 0.67 0.009 0.206   Others 1.46 0.51 0.011 1.05E-08   SFA <sup>3</sup> 51.11 43.54 0.494 0.001   C14:1 0.30 0.28 0.004 0.74   C16:1 4.60 4.51 0.036 0.267   C18:1 24.22 23.89 0.219 0.343   C20:1 0.17 0.18 0.003 0.185   Others 0.53 0.58 0.007 0.059   MUFA <sup>3</sup> 29.82 29.43 0.235 0.333   C18:2 n-6 16.23 18.96 0.144 1.01E-02		Diet		SEM <sup>1</sup>	<i>P</i> -value <sup>2</sup>
C14:02.362.000.0270.001C16:036.0431.310.3000.002C18:010.238.460.1050.001C20:00.320.270.0040.003C22:00.700.670.0090.206Others1.460.510.0111.05E-08SFA <sup>3</sup> 51.1143.540.4940.001C14:10.300.280.0040.074C16:14.604.510.0360.267C18:124.2223.890.2190.343C20:10.170.180.0030.185Others0.530.580.0070.059MUFA <sup>3</sup> 29.8229.430.2350.333C18:2 n-616.2318.960.1441.01E-02C18:3 n-31.982.350.0021.01E-05C20:3 n-60.170.250.0041.30E-03C20:4 n-60.811.670.0204.04E-05C20:5 n-30.160.220.0043.40E-02C21:5 n-30.350.420.0060.001C22:5 n-30.360.850.0095.13E-07Others0.280.240.0040.007PUFA <sup>3</sup> 20.5825.400.1633.61E-04n-33.104.280.0242.68E-06n-6/n-35.554.890.0260.001Al <sup>4</sup> 0.910.720.0090.001		SD	CLP		
C16:0 $36.04$ $31.31$ $0.300$ $0.002$ C18:0 $10.23$ $8.46$ $0.105$ $0.001$ C20:0 $0.32$ $0.27$ $0.004$ $0.003$ C22:0 $0.70$ $0.67$ $0.009$ $0.206$ Others $1.46$ $0.51$ $0.011$ $1.05E-08$ SFA <sup>3</sup> $51.11$ $43.54$ $0.494$ $0.001$ C14:1 $0.30$ $0.28$ $0.004$ $0.74$ C16:1 $4.60$ $4.51$ $0.036$ $0.267$ C18:1 $24.22$ $23.89$ $0.219$ $0.343$ C20:1 $0.17$ $0.18$ $0.003$ $0.185$ Others $0.53$ $0.58$ $0.007$ $0.059$ MUFA <sup>3</sup> $29.82$ $29.43$ $0.235$ $0.333$ C18:2 n-6 $16.23$ $18.96$ $0.144$ $1.01E-02$ C18:3 n-3 $1.98$ $2.35$ $0.002$ $1.01E-05$ C20:3 n-6 $0.17$ $0.25$ $0.004$ $1.30E-03$ C20:4 n-6 $0.81$ $1.67$ $0.020$ $4.04E-05$ C20:5 n-3 $0.16$ $0.22$ $0.006$ $0.001$ C22:5 n-3 $0.35$ $0.42$ $0.006$ $0.001$ C22:5 n-3 $0.36$ $0.85$ $0.009$ $5.13E-07$ Others $0.28$ $0.24$ $0.004$ $0.007$ PUFA <sup>3</sup> $20.58$ $25.40$ $0.163$ $3.61E-04$ n-3 $3.10$ $4.28$ $0.024$ $2.68E-06$ n-6 $17.21$ $20.89$ $0.152$ $9.36E-04$ n	N° of rabbits	50	50		
C18:010.238.460.1050.001C20:00.320.270.0040.003C22:00.700.670.0090.206Others1.460.510.0111.05E-08SFA <sup>3</sup> 51.1143.540.4940.001C14:10.300.280.0040.074C16:14.604.510.0360.267C18:124.2223.890.2190.343C20:10.170.180.0030.185Others0.530.580.0070.059MUFA <sup>3</sup> 29.8229.430.2350.333C18:2 n-616.2318.960.1441.01E-02C18:3 n-31.982.350.0280.002C20:3 n-60.170.250.0041.30E-03C20:4 n-60.811.670.0204.04E-05C20:5 n-30.160.220.0043.40E-02C21:5 n-30.350.420.0060.001C22:5 n-30.120.210.0021.28E-04C22:6 n-30.360.850.0095.13E-07Others0.280.240.0040.007PUFA <sup>3</sup> 20.5825.400.1633.61E-04n-33.104.280.0242.68E-06n-617.2120.890.1529.36E-04n-6/n-35.554.890.0260.001Al <sup>4</sup> 0.910.720.0090.001	C14:0	2.36	2.00	0.027	0.001
C20:0 $0.32$ $0.27$ $0.004$ $0.003$ C22:0 $0.70$ $0.67$ $0.009$ $0.206$ Others $1.46$ $0.51$ $0.011$ $1.05E-08$ SFA <sup>3</sup> $51.11$ $43.54$ $0.494$ $0.001$ C14:1 $0.30$ $0.28$ $0.004$ $0.074$ C16:1 $4.60$ $4.51$ $0.036$ $0.267$ C18:1 $24.22$ $23.89$ $0.219$ $0.343$ C20:1 $0.17$ $0.18$ $0.003$ $0.185$ Others $0.53$ $0.58$ $0.007$ $0.59$ MUFA <sup>3</sup> $29.82$ $29.43$ $0.235$ $0.333$ C18:2 n-6 $16.23$ $18.96$ $0.144$ $1.01E-02$ C18:3 n-3 $1.98$ $2.35$ $0.002$ $0.002$ C20:3 n-6 $0.17$ $0.25$ $0.004$ $1.30E-03$ C20:4 n-6 $0.81$ $1.67$ $0.020$ $4.04E-05$ C20:5 n-3 $0.12$ $0.21$ $0.002$ $1.28E-04$ C21:5 n-3 $0.35$ $0.42$ $0.006$ $0.001$ C22:5 n-3 $0.12$ $0.21$ $0.002$ $1.28E-04$ C22:6 n-3 $0.36$ $0.85$ $0.009$ $5.13E-07$ Others $0.28$ $0.24$ $0.004$ $0.007$ PUFA <sup>3</sup> $20.58$ $25.40$ $0.163$ $3.61E-04$ n-3 $3.10$ $4.28$ $0.026$ $0.001$ Alf <sup>4</sup> $0.91$ $0.72$ $0.009$ $0.001$	C16:0	36.04	31.31	0.300	0.002
C22:00.700.670.0090.206Others1.460.510.0111.05E-08SFA $^3$ 51.1143.540.4940.001C14:10.300.280.0040.074C16:14.604.510.0360.267C18:124.2223.890.2190.343C20:10.170.180.0070.059MUFA $^3$ 29.8229.430.2350.333C18:2 n-616.2318.960.1441.01E-02C18:3 n-31.982.350.0021.01E-05C20:3 n-60.170.250.0041.30E-03C20:4 n-60.811.670.0204.04E-05C20:5 n-30.120.210.0021.28E-04C22:5 n-30.120.210.0021.28E-04C22:6 n-30.360.850.0095.13E-07Others0.280.240.0040.007PUFA $^3$ 20.5825.400.1633.61E-04n-33.104.280.0242.68E-06n-617.2120.890.1529.36E-04n-6/n-35.554.890.0260.001A1 $^4$ 0.910.720.0090.001	C18:0	10.23	8.46	0.105	0.001
Others1.460.510.0111.05E-08 $SFA^3$ 51.1143.540.4940.001C14:10.300.280.0040.074C16:14.604.510.0360.267C18:124.2223.890.2190.343C20:10.170.180.0070.059MUFA <sup>3</sup> 29.8229.430.2350.333C18:2 n-616.2318.960.1441.01E-02C18:3 n-31.982.350.0021.01E-05C20:3 n-60.170.250.0041.30E-03C20:4 n-60.811.670.0204.04E-05C20:5 n-30.160.220.0043.40E-02C21:5 n-30.360.850.0095.13E-07Others0.280.240.0040.007PUFA <sup>3</sup> 20.5825.400.1633.61E-04n-33.104.280.0242.68E-06n-617.2120.890.1529.36E-04n-6/n-35.554.890.0260.001Alf <sup>4</sup> 0.910.720.0090.01	C20:0	0.32	0.27	0.004	0.003
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C22:0	0.70	0.67	0.009	0.206
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		1.46	0.51	0.011	1.05E-08
C16:14.604.510.0360.267C18:124.2223.890.2190.343C20:10.170.180.0030.185Others0.530.580.0070.059MUFA <sup>3</sup> 29.8229.430.2350.333C18:2 n-616.2318.960.1441.01E-02C18:3 n-31.982.350.0020.002C20:3 n-60.170.250.0041.30E-03C20:4 n-60.811.670.0204.04E-05C20:5 n-30.160.220.0043.40E-02C21:5 n-30.350.420.0060.001C22:5 n-30.360.850.0095.13E-07Others0.280.240.0040.007PUFA <sup>3</sup> 20.5825.400.1633.61E-04n-33.104.280.0242.68E-06n-617.2120.890.1529.36E-04n-6/n-35.554.890.0260.001Al <sup>4</sup> 0.910.720.0090.001	SFA <sup>3</sup>	51.11	43.54	0.494	0.001
C18:124.2223.890.2190.343C20:10.170.180.0030.185Others0.530.580.0070.059MUFA <sup>3</sup> 29.8229.430.2350.333C18:2 n-616.2318.960.1441.01E-02C18:3 n-31.982.350.0020.002C20:3 n-30.130.230.0021.01E-05C20:3 n-60.170.250.0041.30E-03C20:4 n-60.811.670.0204.04E-05C20:5 n-30.160.220.0043.40E-02C21:5 n-30.350.420.0060.001C22:5 n-30.120.210.0021.28E-04C22:6 n-30.360.850.0095.13E-07Others0.280.240.0040.007PUFA <sup>3</sup> 20.5825.400.1633.61E-04n-33.104.280.0242.68E-06n-617.2120.890.1529.36E-04n-6/n-35.554.890.0260.001Al <sup>4</sup> 0.910.720.0090.001	C14:1	0.30	0.28	0.004	0.074
$\begin{array}{ccccccc} C20:1 & 0.17 & 0.18 & 0.003 & 0.185 \\ Others & 0.53 & 0.58 & 0.007 & 0.059 \\ MUFA^3 & 29.82 & 29.43 & 0.235 & 0.333 \\ C18:2 n-6 & 16.23 & 18.96 & 0.144 & 1.01E-02 \\ C18:3 n-3 & 1.98 & 2.35 & 0.028 & 0.002 \\ C20:3 n-3 & 0.13 & 0.23 & 0.002 & 1.01E-05 \\ C20:3 n-6 & 0.17 & 0.25 & 0.004 & 1.30E-03 \\ C20:4 n-6 & 0.81 & 1.67 & 0.020 & 4.04E-05 \\ C20:5 n-3 & 0.16 & 0.22 & 0.004 & 3.40E-02 \\ C21:5 n-3 & 0.35 & 0.42 & 0.002 & 1.28E-04 \\ C22:5 n-3 & 0.12 & 0.21 & 0.002 & 1.28E-04 \\ C22:5 n-3 & 0.36 & 0.85 & 0.009 & 5.13E-07 \\ Others & 0.28 & 0.24 & 0.004 & 0.007 \\ PUFA^3 & 20.58 & 25.40 & 0.163 & 3.61E-04 \\ n-3 & 3.10 & 4.28 & 0.024 & 2.68E-06 \\ n-6 & 17.21 & 20.89 & 0.152 & 9.36E-04 \\ n-6/n-3 & 5.55 & 4.89 & 0.026 & 0.001 \\ Al^4 & 0.91 & 0.72 & 0.009 & 0.001 \\ \end{array}$	C16:1	4.60	4.51	0.036	0.267
Others $0.53$ $0.58$ $0.007$ $0.059$ MUFA <sup>3</sup> $29.82$ $29.43$ $0.235$ $0.333$ C18:2 n-6 $16.23$ $18.96$ $0.144$ $1.01E-02$ C18:3 n-3 $1.98$ $2.35$ $0.028$ $0.002$ C20:3 n-3 $0.13$ $0.23$ $0.002$ $1.01E-05$ C20:3 n-6 $0.17$ $0.25$ $0.004$ $1.30E-03$ C20:4 n-6 $0.81$ $1.67$ $0.020$ $4.04E-05$ C20:5 n-3 $0.16$ $0.22$ $0.004$ $3.40E-02$ C21:5 n-3 $0.35$ $0.42$ $0.006$ $0.001$ C22:5 n-3 $0.12$ $0.21$ $0.002$ $1.28E-04$ C22:6 n-3 $0.36$ $0.85$ $0.009$ $5.13E-07$ Others $0.28$ $0.24$ $0.004$ $0.007$ PUFA <sup>3</sup> $20.58$ $25.40$ $0.163$ $3.61E-04$ n-3 $3.10$ $4.28$ $0.024$ $2.68E-06$ n-6 $17.21$ $20.89$ $0.152$ $9.36E-04$ n-6/n-3 $5.55$ $4.89$ $0.026$ $0.001$ Al <sup>4</sup> $0.91$ $0.72$ $0.009$ $0.011$	C18:1	24.22	23.89	0.219	0.343
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	C20:1	0.17	0.18	0.003	0.185
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Others	0.53	0.58	0.007	0.059
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	MUFA <sup>3</sup>	29.82	29.43	0.235	0.333
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C18:2 n-6	16.23	18.96	0.144	1.01E-02
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C18:3 n-3	1.98	2.35	0.028	0.002
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C20:3 n-3	0.13	0.23	0.002	1.01E-05
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C20:3 n-6	0.17	0.25	0.004	1.30E-03
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C20:4 n-6	0.81	1.67	0.020	4.04E-05
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C20:5 n-3	0.16	0.22	0.004	3.40E-02
$\begin{array}{cccccccc} C22:6 n-3 & 0.36 & 0.85 & 0.009 & 5.13E-07 \\ Others & 0.28 & 0.24 & 0.004 & 0.007 \\ PUFA^3 & 20.58 & 25.40 & 0.163 & 3.61E-04 \\ n-3 & 3.10 & 4.28 & 0.024 & 2.68E-06 \\ n-6 & 17.21 & 20.89 & 0.152 & 9.36E-04 \\ n-6/n-3 & 5.55 & 4.89 & 0.026 & 0.001 \\ Al^4 & 0.91 & 0.72 & 0.009 & 0.001 \\ \end{array}$	C21:5 n-3	0.35	0.42	0.006	0.001
Others $0.28$ $0.24$ $0.004$ $0.007$ PUFA <sup>3</sup> $20.58$ $25.40$ $0.163$ $3.61E-04$ n-3 $3.10$ $4.28$ $0.024$ $2.68E-06$ n-6 $17.21$ $20.89$ $0.152$ $9.36E-04$ n-6/n-3 $5.55$ $4.89$ $0.026$ $0.001$ Al <sup>4</sup> $0.91$ $0.72$ $0.009$ $0.001$	C22:5 n-3	0.12	0.21	0.002	1.28E-04
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C22:6 n-3	0.36	0.85	0.009	5.13E-07
n-3 3.10 4.28 0.024 2.68E-06   n-6 17.21 20.89 0.152 9.36E-04   n-6/n-3 5.55 4.89 0.026 0.001   Al <sup>4</sup> 0.91 0.72 0.009 0.001	Others	0.28	0.24	0.004	0.007
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	PUFA <sup>3</sup>	20.58	25.40	0.163	3.61E-04
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	n-3	3.10	4.28	0.024	2.68E-06
AI <sup>4</sup> 0.91 0.72 0.009 0.001	n-6	17.21	20.89	0.152	9.36E-04
	n-6/n-3	5.55	4.89	0.026	0.001
TI <sup>4</sup> 1.47 1.09 0.021 1.10E-03	AI <sup>4</sup>	0.91	0.72	0.009	0.001
	TI <sup>4</sup>	1.47	1.09	0.021	1.10E-03

<sup>1</sup> SEM: standard error of means.

<sup>2</sup> *P*-value is significant at P < .05.

<sup>3</sup> SFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids.

<sup>4</sup> AI: atherogenicity index; TI: thrombogenicity index.

low as possible and still < 1 (Ulbricht & Southgate, 1991). In particular, AI and TI indicate the different effects that the fatty acids might have on human health: AI assesses the risk of atherosclerosis, while TI evaluate the potential aggregation of blood platelets (Ulbricht & Southgate, 1991). Overall, rabbit meat showed a n-6/n-3 ratio above the recommended value because of the high content of C18:2 n-6 which is precursor of C20:4 n-6, fatty acid that brings health benefits of the consumer when present in low amounts (Parra, Bandarra, Kiely, Thorsdottir, & Martinez, 2007). CLP supplementation has markedly influenced the studied indices (P < .001) which were all lower in CLP group than SD group (P < .001). In particular, AI and TI values in the IMF of the experimental group are decreased of about 20 and 26% (P < .001), respectively, compared to SD group. This was due to both lower contents of C14:0 and C16:0 acids and higher content of PUFAs. Our results are in line with Li, Wang, Wang, Ma, and Li (2012) who reported that PUFA rich diet in rabbits may increased the PUFA contents, in particular n-3 PUFA proportion, and decreased SFA contents in meat.

#### 3.4. Phenolic content and antioxidant activity

To assess the effectiveness of the dietary administration of CLP, total phenolic content, radical-scavenging (against the radicals ABTS•+) and total reducing (FRAP assay) activities of rabbit meat were determined (Table 7). Obviously, CLP supplementation resulted in a significant increase in phenols content (P < .001). This is supported by the findings of Boots, Drent, De Boer, Bast, and Haenen (2011) who reported that phenolic compounds provided through diet are deposited in

#### Table 7

Total phenolic content and antioxidant activity of *Longissiumus lumborum* muscle of rabbits fed according to the diet (SD: standard diet, CLP: cauliflower leaves powder diet).

	Diet		SEM <sup>1</sup>	P-value <sup>2</sup>
N° of rabbits	SD 50	CLP 50		
Total phenolic, mg of gallic acid equivalents/ 100 g	4.85	5.98	0.054	3.79E-02
ABTS <sup>3</sup> , µg of trolox equivalents/g	10.39	11.56	0.126	0.010
FRAP <sup>3</sup> , $\mu g$ of trolox equivalents/g	9.70	10.40	0.081	0.013

<sup>1</sup> SEM: standard error of means.

<sup>2</sup> *P*-value is significant at P < .05.

<sup>3</sup> ABTS = 2,2<sup>-</sup>.azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay; FRAP = ferric-reducing antioxidant power assay.

muscles where inhibit oxidative stress, and Flis, Sobotka, Antoszkiewicz, Lipiński, and Zduńczyk (2010) who reported that phenolic potential of pig meat could be enhanced by increasing the phenolic content in feed. Statistical analysis showed a significant effect of CLP supplementation on antioxidant capacity of rabbit meat (P < .01). The animal dietary fortification with CLP resulted in an increase of the antioxidant activity of meat which, measured by ABTS and FRAP assays, showed a mean increase of 11.3% and 7.2%, respectively, compared to antioxidant activity of SD group. These results are in agreement with other studies reporting an improvement of the antioxidant status of tissues from animals fed with foods rich in phenolic compounds (Placha et al., 2014; Youdim & Deans, 1999). In the present study, both the higher phenolic content and antioxidant activity detected in meat of CLP group is due to the phenolic substances and vitamins contain in CLP which entered the circulatory system, were distributed and retained in different tissues of animal. Today, since an analytical method for identification and quantification of these components at trace levels has not been developed yet, the bioavailability of any of these cannot be directly demonstrated (Soultos, Tzikas, Christaki, Papageorgiou, & Steris, 2009). However, it was shown that an improvement in the muscle antioxidant capacity leads to a greater resistance to oxidative deterioration of meat (Descalzo & Sancho, 2008) by both quenching free radicals and the activation of antioxidant enzymes (Frankic, Volic, Salobir, & Rezar, 2009).

#### 4. Conclusion

The inclusion of cauliflower (*Brassica oleraceae* var. *Botrytis*) leaf powder in the diet of rabbits did not affect growth performance parameters of the carcass and meat quality. A positive effect of CLP supplementation on drip loss, cooking loss and oxidative stability of meat was observed. Moreover, the use of CPL in the diet of rabbits caused a significant decrease in SFA percentage and a significant increase in PUFA percentage in meat with improvements from the health point of view. The significant effect of dietary fortification on phenolic content and antioxidant activity of rabbit meat was due to the high content of polyphenols in CLP. The results presented in this study showed that CLP supplementation is a valid strategy to improve the functional value of rabbit meat that could determine a better impact on consumers at the time of purchase and to limit production costs for farmers as this is a byproduct in fruit and vegetable industry.

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