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Inclusion of bilberry pomace in rabbit diets: Effects on carcass characteristics and meat quality

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(Article begins on next page)

1 **Inclusion of bilberry pomace in rabbit diets: effects on carcass characteristics and meat**
2 **quality**

3

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27

28 **Abstract**

29 A trial was carried out to evaluate bilberry pomace (BP) as an alternative source of nutrients
30 for rabbits. **One hundred and forty-four** Grimaud weaned rabbits were divided into 4 groups
31 of 36 animals each and fed *ad libitum* with a basal diet (BP0) tested against three assay diets
32 developed by substituting 50, 100 and 150 g / kg of the BP0 diet with BP (BP5, BP10 and
33 BP15 diets, respectively). Carcass characteristics of rabbits were not affected by treatment,
34 with the exception of liver weight. Dietary inclusion of BP did not significantly affect the
35 proximate composition and **the** oxidative status of *longissimus thoracis et lumborum* muscle.
36 However, dietary BP significantly ameliorated, in the same muscle, the nutritional quality and
37 health properties of fat. Consumer acceptance of cooked rabbit meat was not significantly
38 affected by treatment. **In conclusion, BP can be included in rabbit diets to improve the lipid**
39 **composition of meat, without adverse effects on carcass characteristics, other physico-**
40 **chemical properties, oxidative status and meat sensorial traits.**

41

42 Keywords: rabbit, *Vaccinium myrtillus*, by-product, fatty acids, TBARS, sensory analysis

43

44 **1. Introduction**

45 Agricultural wastes obtained by processing fruits and vegetables are **inexpensive, easy to store**
46 **and then available for an acceptable length of time during the year.** They may contain
47 bioactive substances, such as antioxidants, characterized by health promoting properties and
48 potential technological applications (Lee & Wrolstad, 2004). **These wastes could be**

49 consequently explored as useful feed sources for the production of animal derived food
50 products with functional nutritional value.

51 In rabbits, some studies have been carried out to evaluate performance, meat quality or health
52 status of animals fed different antioxidants derived from olive pomace (Dal Bosco et al.,
53 2012), artichoke bracts (Dabbou et al., 2014), grape pomace (Eid, 2008), chestnut
54 hydrolysable tannins (Liu et al., 2009), microalgae (Peiretti & Meineri, 2011) and green tea
55 (Eid, Zeweil, Ahmed, Basyony, & Farok, 2011).

56 Bilberry (*Vaccinium myrtillus* L.) is one of the most important sources of phenolic
57 compounds in the human diet. Phenolic compounds, being characterized by various biological
58 activities, may confer bilberries the capability of improving human health conditions (Lee &
59 Wrolstad, 2004; Khanal, Howard, Brownmiller, & Prior, 2009). Juice processing of bilberries
60 generates large amounts of pomace, a by-product which still contains an assortment of
61 beneficial phytochemicals including proanthocyanidins, anthocyanins and other flavonoids,
62 suitable for the development of novel functional food ingredients (Vulić et al., 2011). Bilberry
63 pomace (BP) has already been used as ingredient in extruded products which have been
64 associated with *in vivo* health benefits in animal models, such as reduced plasma cholesterol
65 and abdominal fat (Khanal et al., 2009; Khanal, Howard, Wilkes, Rogers, & Prior, 2012).
66 Kim, Bartley, Rimando, and Yokoyama (2010) suggested that hepatic modulation of bile acid
67 and cholesterol synthesis primarily contributes to the cholesterol-lowering effect of BP.

68 Based on the current knowledge, no studies have been performed on the use of BP either in
69 rabbit or other livestock animals' diets. Therefore, the aim of the present investigation was to
70 evaluate the effects of BP inclusion in rabbit diets on carcass characteristics, and on physico-
71 chemical characteristics, fatty acid (FA) profile, oxidative stability and sensorial
72 characteristics of rabbit meat.

73

74 **2. Materials and methods**

75 *2.1. Animals and experimental design*

76 The trial was carried out at the experimental rabbitry of the Department of Agricultural,
77 Forest, and Food Sciences (DISAFA; University of Turin), located in Carmagnola, Turin,
78 Italy. The experimental protocol was designed according to the guidelines of the current
79 European and Italian laws on the care and use of experimental animals (European Directive
80 86 609/EEC, put into law in Italy with D.L. 116/92). The experimental protocol was approved
81 by the Ethical Committee of the Department of Agricultural, Forest, and Food Sciences of the
82 University of Turin (Italy). One hundred forty-four weaned crossbred (Grimaud) rabbits (35
83 days old) were randomly divided into 4 groups of 36 animals each; the average initial weight
84 was 938 ± 33.4 g. The animals were housed individually in wire cages (41 cm \times 0.30 cm \times 28
85 cm height) and had free access to clean drinking water. The temperature and photoperiod in
86 the rabbitry were 22 ± 2 °C and 16L:8D, respectively. Rabbits were fed *ad libitum* with a
87 basal diet not containing BP (BP0 diet) (ingredients, g / kg fresh matter: alfalfa meal 300,
88 wheat bran 200, barley 170, dried beet pulp 150, soybean meal 115, molasses 20, wheat straw
89 20, and soybean oil 5) tested against three assay diets developed by substituting 50, 100 and
90 150 g / kg of the BP0 diet with BP (BP5, BP10, and BP15 diets, respectively) according to
91 Goby and Gidenne (2008). All diets also contained a vitamin-mineral premix and bicalcium
92 phosphate (15 and 5 g / kg fresh matter, respectively). BP was included in the treated diets
93 during the raw material mixing process. All diets were pelleted fresh, stored in dark bags and
94 kept at environmental temperature in the feed facility to prevent auto-oxidation of the lipid
95 sources.

96

97 *2.2. Chemical analyses of feed*

98 All **chemical** analyses were carried out on three replicates of each feed sample, according to
99 the recommendations of the European Group on Rabbit Nutrition (2001).

100

101 *2.2.1. Determination of BP phenolic compounds*

102 BP generated during fruit juice production was obtained from a private fruit processing
103 company ("Arc en Ciel" Soc. Agr. Coop., Cafasse, Turin, Italy). The bilberries had been
104 harvested during July and August 2013 in wild forests with an organic certification. BP was
105 dried in an oven at 60 °C until constant weight was reached and then finely ground.

106 Total phenols and *o*-diphenols were extracted from about 0.20 g of BP powder using a
107 methanol / water solution (4:1, v:v). The solution was mixed for 30 min at 37 °C under
108 continuous agitation and centrifuged at **3075 g** for 20 min. The methanolic fraction was
109 recovered and stored at -20 °C until analysis. Total phenols and *o*-diphenols were determined
110 colorimetrically at 765 nm and 500 nm respectively and expressed as mg hydroxytyrosol
111 equivalents / g dry matter (DM), as reported by Montedoro, Servili, Baldioli, and Miniati
112 (1992).

113 The same methanolic extract was used for total flavonoids determination at 510 nm (Zhishen,
114 Mengcheng, & Jianming, 1999). The results are expressed as mg catechin equivalents / g DM.

115 Condensed tannins were determined from 50 µl of the same methanolic extract using the
116 vanillin method (Julkenen-Tiitto, 1985). The results are expressed as mg of catechin
117 equivalents / g DM.

118 For anthocyanins determination, acidified methanol (1%) was used to prepare extracts. The
119 absorbance was measured at 530 and 657 nm and results are expressed as mg cyanidin 3-
120 glucoside equivalents (CyE) / g DM (Mancinelli, Huang Yang, Lindquist, Anderson, &
121 Rabine, 1975).

122 All colorimetric determinations were performed using a UV–VIS spectrophotometer (UV–
123 VIS Beckman spectrophotometer DU 650, Beckman Instruments Inc., Fullerton, CA, USA).
124 The concentrations of BP phenolic compounds are reported in Table 1.

125

126 *2.2.2. Antioxidant activity of BP*

127 To evaluate the antioxidant activity of the BP extract, the free radical scavenging activity was
128 determined by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay according to the method
129 described by Kontogiorgis and Hadjipavlou-Litina (2005). The loss of DPPH color caused by
130 consumption of DPPH radical by antioxidant species present in the sample was measured
131 using a UV–VIS spectrophotometer (UV–VIS Beckman spectrophotometer DU 650,
132 Beckman Instruments Inc., Fullerton, CA, USA). Briefly, a solution of 20 µl of BP sample
133 dissolved in absolute ethanol to a final volume of 1 ml was added to 1 ml of DPPH (0.1 mM,
134 in absolute ethanol) and allowed to stand in the dark for 20 min (reaction time) before the
135 measurement of the absorbance at 517 nm. The inhibition of DPPH radical scavenging
136 activity was calculated as follows:

$$137 \text{ \% inhibition} = [(\text{absorbance of blank} - \text{absorbance of BP extracts}) / \text{absorbance of blank}] \times$$
$$138 100$$

139 where the blank is the control solution containing all reagents except the sample.

140

141 *2.2.3. Proximate composition and fatty acid profile of BP and experimental diets*

142 The BP and the experimental diets were ground with a cutting mill to pass a 1-mm screen
143 sieve (Pulverisette 15 – Fritsch GmbH, Idar-Oberstein, Germany). They were analyzed for
144 DM (# 930.15), ash (# 923.03), crude protein (CP, # 984.13), ether extract (EE, # 2003.05),
145 acid detergent fiber (ADF, # 973.18) and acid detergent lignin (ADL, # 973.18) according to
146 AOAC procedures (AOAC International, 2000; 2003). Neutral detergent fiber (NDF) was

147 determined according to Van Soest, Robertson, and Lewis (1991). Starch content was
148 determined using the Ewer's polarimetric method (European Economic Community, 1972).

149 Gross energy (GE) was measured using an adiabatic calorimetric bomb (C7000, IKA,
150 Staufen, Germany).

151 The FA composition of feedstuffs was assessed using a combined direct *trans*-esterification
152 and solid-phase extraction method as described by Alves, Cabrita, Fonseca, and Bessa (2008).
153 Separation, identification, and quantification of fatty acid methyl esters (FAME) were
154 performed as reported by Renna et al. (2014). The results are expressed as g / 100 g DM and
155 reported as g / 100 g of total detected FA.

156 The proximate and FA compositions of feeds are reported in Tables 2 and 3, respectively.

157

158 2.3. Slaughter procedures and muscle sampling

159 At 83 days of age, 12 rabbits per group (mean weight 2984 ± 138.0 g) were slaughtered in an
160 experimental slaughterhouse without fasting. The slaughtered rabbits were bled and the skin,
161 genitals, urinary bladder, gastrointestinal tract and distal part of the legs were removed as
162 recommended by Blasco, Ouhayoun, and Masoero (1993). The carcass was weighed; the skin
163 and full gastrointestinal tract weights were recorded and expressed as a percentage of
164 slaughter weight (SW). Carcasses (with head, thoracic cage organs, liver and kidneys) were
165 chilled at 4 °C for 24 h in a refrigerated room. The chilled carcass weight (CCW) was
166 recorded and the dressing out percentage was calculated as the ratio between CCW and SW.
167 Head and liver weight were expressed as a percentage of CCW. The head, thymus, trachea,
168 esophagus, heart, lungs, liver and kidneys weights were removed from the CCW to obtain the
169 reference carcass weight (RCW). For meat quality analyses, *Longissimus thoracis et*
170 *lumborum* (LTL) muscle was removed from both left and right side. Meat quality parameters
171 were measured on the left loin while sensory analysis was performed on the right loin.

172

173 *2.4. Meat quality parameters*

174 *2.4.1. pH*

175 Meat pH of the LTL muscle (at the level of the 7th lumbar vertebra) was measured at 24 h
176 *postmortem* (pH₂₄) in duplicate using a Crison portable pH-meter (Crison Instruments, S.A.,
177 Alella, Spain) fitted with a spear-type electrode and an automatic temperature compensation
178 probe.

179

180 *2.4.2. Color*

181 Meat color was measured at room temperature (20°C) on a freshly cut surface of the loin at
182 the level of the 7th lumbar vertebra using a portable colorimeter Chroma Meter CR-400
183 Konica Minolta Sensing (Minolta Sensing Inc, Osaka, Japan). Color measurements were
184 reported in terms of lightness (L*), redness (a*) and yellowness (b*) in the CIELAB color
185 space model (Commission Internationale de l'Éclairage, 1976). The values were recorded for
186 CIE standard illuminant D₆₅ and the CIE 2° standard observer. The color values were
187 obtained considering the average of three readings per sample.

188

189 *2.4.3. Proximate composition*

190 The proximate analyses were carried out according to the AOAC International (2000)
191 methods. Tissue samples were weighed, dried at 125°C for 5 h and reweighed to determine
192 the water content. The samples remaining from water analysis were placed into a furnace
193 oven at 525°C for 6 h for ash determination. The meat was further lyophilized and ground in a
194 blender for analyses of protein and intramuscular fat. Nitrogen was determined by Kjeldahl
195 method and CP was calculated by multiplying N × 6.25. Lipid extraction of intramuscular fat
196 was determined by Soxhlet method.

197

198 *2.4.4. Fatty acid composition*

199 The FA composition of freeze-dried LTL muscle samples was assessed as reported by Belforti
200 et al. (2015). Briefly, total lipids were extracted with dichloromethane / methanol (2:1, v:v) by
201 homogenization at room temperature. The solution was filtered in a separating funnel
202 containing 1 ml of a water solution of MgCl₂ (2%, w/v) and 20 ml of water. The organic
203 phase was separated and evaporated to dryness. Glycerides were saponified with a solution of
204 NaOH 0.5 M in methanol and then BF₃ (about 10% in methanol) were added for methylation.
205 Peaks were identified by injecting pure FAME standards as detailed by Renna et al. (2012).
206 Quantification was assessed using tridecanoic acid (C13:0) as internal standard. The results
207 are expressed as g / 100 g of LTL muscle and reported as g / 100 g of total detected FA.

208

209 *2.4.5. Lipid oxidation*

210 Lipid oxidation was determined on meat samples (10 g) at 30 and 60 days of frozen storage,
211 by thiobarbituric acid reactive substances (TBARS) assay as described by Dabbou et al.
212 (2014). The samples were analyzed in duplicate and the absorbance was read at 532 nm with a
213 Helios spectrophotometer (Unicam Limited, Cambridge, UK). TBARS values were calculated
214 from a standard curve of 1,1,3,3-tetramethoxypropane (TMP; Sigma–Aldrich, Steinheim,
215 Germany) and expressed as mg TMP / kg of meat.

216

217 *2.4.6. Sensory evaluation*

218 A sensory panel of 68 untrained assessors, 29 males and 39 females, ranging in age from 21 to
219 60 years, was recruited among the students and staff members of the University of Turin.
220 Participants were regular consumers of rabbit meat and were already involved in surveys on
221 meat acceptability / preference tests. Affective tests were carried out in 8 distinct evaluation

222 sessions during 4 days and performed in the sensory laboratory of DISAFA under controlled
223 conditions with panelists placed in individual tasting booths. In each day, an acceptance test
224 was performed to assess the oxidative stability of meat. Muscles were kept unpackaged in a
225 dark cooler set at 4 °C for 4 days. After aging, the samples were vacuum-packed and stored at
226 -20 °C until analysis. The loins, from rabbits of the 4 groups, were thawed at 4 °C / 24 h,
227 packages were opened and the samples were simultaneously cooked without salt or spice on a
228 double plate grill, preheated at 250 °C, to a final temperature of 70 °C. Cooking temperature
229 was monitored by an iron/constantan thermocouple placed in the geometric centre of each
230 loin. After grilling, the loins were immediately cut into equal portions. The latter were labeled
231 with three-digit numbers and offered using a Williams design to balance the order of
232 presentation (MacFie, Bratchell, Greenhoff, & Vallis, 1989).

233 Immediately before the sensory tasting sessions, the panelists were asked to sign an informed
234 consent form. Each panelist received 4 warm samples corresponding to the 4 experimental
235 diets.

236 The panelists were asked to measure the degree of liking or disliking of rabbit meat by the use
237 of the 9-point hedonic scale (1 = “dislike extremely”, 2 = “dislike very much”, 3 = “dislike
238 moderately”, 4 = “dislike slightly”, 5 = “neither like nor dislike”, 6 = “like slightly”, 7 = “like
239 moderately”, 8 = “like very much”, 9 = “like extremely”; Peryam & Pilgrim, 1957).

240 Tap water was offered to the panelists to rinse their mouths between samples.

241

242 2.5. Statistical analyses

243 The statistical analyses were performed using the SPSS software package (version 17 for
244 Windows, SPSS Inc., Chicago, IL, USA).

245 One-way ANOVA was used to evaluate the effect of BP dietary inclusion levels on carcass
246 characteristics and meat quality traits. The assumption of equal variances was assessed by

247 Levene's homogeneity of variance test. If such an assumption did not hold, the Brown-
248 Forsythe statistic was performed to test for the equality of group means instead of the F one.
249 Pairwise multiple comparisons were performed to test the difference between each pair of
250 means (Duncan test and Tamhane's T2 in the cases of equal variances assumed or not
251 assumed, respectively).

252 The effects of dietary treatment and storage time on lipid oxidation were statistically analyzed
253 with a mixed between-within subjects model (GLM for Repeated Measures).

254 Sensory data from the acceptance test were analyzed using the GLM procedure with overall
255 liking as dependent variable, dietary treatment as fixed effect and panelist as random effect
256 (Naes, Brockhoff, & Tomic, 2010). The scores of acceptability obtained for the 4 samples
257 from each consumer were converted into ranked data by assigning rank order numbers to the
258 evaluations. Ties received average rank scores. Ranking data were analyzed with the
259 Friedman's test (Meilgaard, Civille, & Carr, 1991).

260 For all statistical analyses **significance** was declared at $P < 0.05$.

261

262 **3. Results and discussion**

263 *3.1. Carcass characteristics and meat quality traits*

264 Carcass characteristics are reported in Table 4. **Differences were found in liver weight that**
265 **was lower in the animals fed BP containing diets; all other parameters were not affected by**
266 **BP dietary inclusion.** Similar results were obtained by Heyman et al. (2014) who found a
267 significantly reduced liver mass and liver triacylglycerol lipid accumulation in C57BL/6J
268 mice fed a high-fat diet supplemented (20%) with different berries (lingonberry, blackcurrant,
269 and bilberry) that implies protection against liver steatosis.

270 The proximate composition, pH₂₄ and color of the LTL muscle of rabbits are reported in
271 Table 5. These meat quality traits fell within standard ranges for rabbit meat and were not

272 affected by treatment. Abdel-Khalek (2013) reviewed that dietary supplementation with
273 antioxidants has no clear trend in the physical and chemical characteristics of rabbit meat.

274

275 *3.2. Fatty acid profile of diets and LTL muscle*

276 Inclusion of BP increased the total FA content and modified the FA profile of the lipids in the
277 diets (Table 3). Results showed that BP is rich in unsaturated FA (93.38 g / 100 g total FA).
278 The most abundant FA in BP was linoleic acid (C18:2 n6, LA; 36.33 g / 100 g total FA),
279 followed by α -linolenic acid (C18:3 n3, ALA; 32.59 g / 100 g total FA) and oleic acid (C18:1
280 c9, OA; 23.50 g / 100 g total FA). An increasing percentage of MUFA and PUFA (mainly due
281 to increases in C18:1 c9 and C18:3 n3) at the expense of SFA was observed following
282 increasing inclusion levels of BP in the diets. The values of the Σ n6 / Σ n3 PUFA ratio were
283 5.13, 3.30, 2.49 and 2.14 in BP0, BP5, BP10 and BP15 diets, respectively.

284 In rabbits, dietary FA are directly incorporated into intramuscular lipids (Dalle Zotte et al.,
285 2014). Expectedly, the FA profile of rabbit LTL muscle (Table 6) thus generally followed that
286 of the dietary ingested lipids (Dal Bosco, Castellini, Bianchi, & Mugnai, 2004). Increasing
287 PUFA and decreasing SFA proportions from BP0 to BP15 diets resulted in significantly
288 higher (26.08 to 37.46 g / 100 g total FA) and lower (42.93 to 36.48 g / 100 g total FA)
289 proportions of these FA groups in the muscle ($P < 0.001$), respectively. The observed trend
290 for total MUFA was opposite, as increasing levels in the diets led to decreasing proportions in
291 the LTL lipid fraction ($P < 0.001$). The estimated $\Delta 9$ -desaturase activity did not differ among
292 treatments (Table 6). We may therefore exclude the hypothesis of a reduced conversion of
293 SFA into *cis*-9 MUFA due to dietary PUFA inhibition of SCD (Papadomichelakis,
294 Karagiannidou, Anastasopoulos, & Fegeros, 2010a). Most probably, as already reported for
295 α -tocopherol (Dal Bosco et al., 2004), antioxidants in BP may have inhibited the peroxidation

296 of FA with high (PUFA) rather than low (MUFA) degree of unsaturation, with consequent
297 proportional increasing contents of PUFA at the expense of SFA and MUFA in the muscle.
298 The inclusion of BP in the diets induced significant modifications in the proportion of the
299 majority of individual detected FA in the LTL muscle. The main variations regarded n3
300 PUFA. ALA levels in rabbit meat depend on the level of exogenous fatty acids (Liu et al.,
301 2009; Kouba, Benatmane, Blochet, & Mourot, 2008) as rabbits effectively synthesize
302 endogenous n3 PUFA from its precursor in the liver; the amount produced depends on the
303 dietary $\Sigma n6 / \Sigma n3$ FA ratio (Peiretti & Meineri, 2008). Therefore, the proportional increasing
304 content of ALA in the BP diets (Table 3) expectedly resulted in significant increasing
305 percentages of ALA in the muscle. In particular, a double content of ALA in BP15 if
306 compared to BP0 diets resulted in 4-times higher percentage of ALA in muscles. Rabbits are
307 able to elongate and desaturate ALA, also due to caecal microflora and caecotrophe
308 reingestion (Dal Bosco et al., 2004). As a consequence, the long-chain n3 PUFA (C20:5 n3
309 and C22:5 n3) were also significantly increased following BP inclusion in the diet ($P <$
310 0.001), which is consistent with the results obtained in other nutritional trials using linseed
311 (Dal Bosco et al., 2015; Kouba et al., 2008; Dal Bosco et al., 2004) and fresh forage (Dal
312 Bosco et al., 2014; Forrester-Anderson, McNitt, Way, & Way, 2006).

313 **Probably due to differences in analytical and gas chromatographic conditions as well as lack**
314 **or difficulties in peak identification**, till now very few published trials (Papadomichelakis et
315 al., 2010a; Papadomichelakis Karagiannidou, Anastasopoulos, & Fegeros, 2010b; Leiber et
316 al., 2008) reported information on the odd- (OCFA) and branched-chain fatty acids (BCFA) in
317 rabbit meat. These FA have recently received increasing attention by researchers due to their
318 association with reduced disease risk for coronary heart disease, diabetes and cancer (Jenkins,
319 West, & Koulman, 2015; Oku & Yanagita, 2009). The major part of these FA are produced
320 by bacteria in the caecum and reach the duodenum and the blood via caecotrophs reingestion

321 while, most probably, endogenous synthesis takes place to a lower extent (Leiber et al., 2008).
322 Caecal bacterial response to dietary changes in lipid content and composition has been poorly
323 investigated yet. Papadomichelakis, Anastasopoulos, Karagiannidou, and Fegeros (2010c)
324 reported unchanged concentrations of OCFA and BCFA in the caecotrophs of rabbits fed
325 unsaturated lipid supplemented diets if compared to a control diet, which were reflected to
326 unchanged concentrations of OCFA and BCFA incorporated into *biceps femoris* and
327 *longissimus lumborum* muscles (Papadomichelakis et al., 2010a). In the current study, total
328 OCFA and total BCFA proportions did not differ in LTL muscle of rabbits fed BP0, BP5,
329 BP10 or BP15 diets (Table 6), suggesting lack of differences in caecotrophy activity among
330 treatments.

331 The nutritional quality of fat for human consumption is usually evaluated in terms of the
332 PUFA / SFA ratio (optimal values ≥ 0.45), the $\Sigma n6 / \Sigma n3$ FA ratio (optimal values ≤ 4), the
333 atherogenicity (AI) and thrombogenicity (TI) indexes (both as low as possible) (Lazzaroni,
334 Biagini, & Lussiana, 2009). In all treatments, the PUFA / SFA ratio fell within the
335 recommended values. Anyhow, increasing BP inclusion levels in rabbit diets led to a
336 significant progressive increase of this ratio in the LTL muscle, which was 1.7-fold higher in
337 the BP15 group if compared to BP0 ($P < 0.001$). In ordinary dietary conditions, the $\Sigma n6 / \Sigma$
338 $n3$ PUFA ratio in rabbit meat is set at around 10 (Dalle Zotte, 2002). In the current study, this
339 ratio decreased from 9.30 to 2.88 in muscles of rabbits fed the BP0 and BP15 diets,
340 respectively. BP10 and BP15 diets allowed LTL muscle having the $\Sigma n6 / \Sigma n3$ PUFA ratio
341 within optimal values for human consumption. Optimal values were also obtained in other
342 trials when ALA-rich feedstuffs were fed by rabbits (Peiretti, Gasco, Brugiapaglia, & Gai,
343 2011; Kouba et al., 2008; Peiretti & Meineri, 2008). Significant variations were also observed
344 for the AI and TI, both being lower in the LTL muscle of the rabbits fed BP diets if compared
345 to those fed the control diet. Overall the obtained results on long-chain n-3 PUFA, due to the

346 positive role exerted in the control of cardiovascular diseases (Endo & Arita, 2016), as well as
347 the studied ratios and indexes show that BP inclusion in rabbit diets may be of particular
348 significance for the related nutritional benefits associated to human consumption of rabbit
349 meat.

350

351 *3.3. Lipid oxidation*

352 Rabbit meat, due to its high content of PUFA, is prone to lipid oxidation leading to a reduced
353 shelf life of the product (Dalle Zotte & Szendrő, 2011). Susceptibility of rabbit meat to lipid
354 oxidation, during refrigerated or frozen storage, can be reduced by dietary phenolic
355 compounds supplementation (Dalle Zotte et al. 2014). In our study, the effects of the BP
356 dietary inclusion on the oxidative stability of the frozen LTL muscle were investigated and
357 results are shown in Table 7. In the applied experimental conditions, the dietary treatment did
358 not affect TBARS values. Storage time instead increased significantly the oxidation of frozen
359 muscles, with higher values recorded in the samples stored at -25 °C for 60 days.

360 According to the peroxidability index reported in Table 6, meat samples derived from BP
361 treated rabbits were expected to be more susceptible to lipid peroxidation, while they showed
362 similar TBARS levels compared to the control group. Similar findings were also reported in a
363 study carried out in rabbits fed high dietary fat supplemented with a blackcurrant pomace
364 extract rich in polyphenols (Jurgoński, Juśkiewicz, Zduńczyk, Matusevicius, &
365 Kołodziejczyk, 2014). These authors observed lower concentrations of TBARS in kidneys
366 and serum of rabbits treated with enriched extract diet if compared to control; they concluded
367 that the suppression of lipid peroxidation was due to the blackcurrant polyphenolic
368 compounds that may increase filtration in these organs and inactivate free radicals. Similarly,
369 the results obtained in our study could be related to the antioxidant activity of BP
370 polyphenolic compounds. Such antioxidant activity was confirmed by the high percentage

371 (65.18%) of inhibition obtained with the *in vitro* free radical DPPH assay, which is in
372 agreement with the results obtained by Bunea et al. (2011) on wild and cultivated blueberries.

373

374 3.4. *Sensory evaluation*

375 The results of the affective tests are summarized in Table 8. The data correspond to a sensory
376 analysis where panelists were asked to rate the likeness, in terms of taste and aroma, of 4 meat
377 samples on a 9-point hedonic scale. Both median and mode values of meat samples from the
378 rabbits fed BP5 and BP15 diets were 7, which corresponds to “like moderately” on the
379 hedonic scale. The BP0 and BP10 meat had the same median value (6 = “like slightly”), while
380 the mode value for BP0 meat was higher than that for BP10 meat (7 and 6, respectively). It
381 should be noted that there were no scores in the first two liking categories and that all samples
382 had the majority of scores located in the “liking part” of the hedonic scale (Figure 1). Looking
383 at the distribution of frequencies in Figure 1, it is evident that meat from BP0, BP5 and BP15
384 treatments on one hand, and meat from BP10 treatment on the other hand had more responses
385 in the “like moderately” and in the “like slightly” category, respectively.

386 The average overall liking ratings ranged from 6.1 to 6.2, which correspond to “like slightly”
387 according to the hedonic scale. Although the meat from BP10 and BP15 treatments had
388 different median and mode values, they received the same mean score (Table 8). The
389 ANOVA results showed no significant differences in the average overall liking scores among
390 treatments. The samples were considered acceptable if 50% or more of responses were in the
391 range from 6 to 9 on the hedonic scale; all meats were accepted by panelists (votes \geq 6 from
392 66% to 74%).

393 Although ANOVA can be correctly applied to data that slightly deviate from the Gaussian
394 distribution (Figure 1), we decided also to analyze acceptability data using the non-parametric
395 Friedman’s test by converting the preferences scores given by each consumer into rank order

396 numbers. As the calculated value of the Friedman's statistic (2.93) is lower than 7.81 (the
397 critical value of a chi-square distribution with 3 degrees of freedom), we can confirm the
398 ANOVA results and conclude that there were not significant differences in liking ranking
399 among the 4 treatments.

400 Flavor, which comprises mainly the two sensations of taste and aroma, is an important meat
401 quality attribute affecting consumers' meat-buying habits and preferences. The sensory
402 evaluation indicated that the use of BP **did not affect** the flavor of cooked rabbit meats.

403

404 **4. Conclusions**

405 The obtained results show that BP is a suitable ingredient for growing rabbits' diets. Inclusion
406 of BP up to 15% of the diet does not affect the carcass characteristics of rabbits, nor the
407 proximate composition, lipid oxidation and consumer acceptance of rabbit meat.

408 Dietary inclusion of BP **improved the fatty acid profile of the *longissimus thoracis et***
409 ***lumborum* muscle of rabbits, largely by means of an increase of total n3 PUFA and a**
410 **reduction of the $\Sigma n6 / \Sigma n3$ fatty acids ratio. Such modifications also determined the lowering**
411 **of both the atherogenicity and thrombogenicity indexes of the meat.**

412 Bilberry pomace, being a rich source of **polyunsaturated fatty acids**, bioactive antioxidant
413 compounds and natural colorants (mainly anthocyanins), possesses a good potential for the
414 valorization of bilberry juice by-products through further uses in the feed industry.

415

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422

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590 **Table 1**

591 Mean concentration of the main phenolic compounds of bilberry pomace.

Phytochemical compounds	Content
Total phenols (mg hydroxytyrosol equivalents / g DM)	46.54
<i>o</i> -diphenols (mg hydroxytyrosol equivalents / g DM)	10.55
Total flavonoids (mg catechin equivalents / g DM)	9.45
Total anthocyanins (mg CyE / g DM)	0.14
Condensed tannins (mg catechin equivalents / g DM)	36.38

592 Abbreviations: DM = dry matter; CyE = cyanidin 3-glucoside equivalents.

593 **Table 2**

594 Proximate composition of bilberry pomace (BP) and experimental diets.

<i>Experimental diets (g / kg as fed)</i>	BP	BP0	BP5	BP10	BP15
Basal mixture ¹	-	980	930	880	830
Bilberry pomace	-	0	50	100	150
Vitamin-mineral premix ²	-	15	15	15	15
Bicalcium phosphate	-	5	5	5	5
<i>Proximate composition (g / kg DM, unless otherwise stated)</i>					
Dry matter (g / kg)	944	882	882	880	885
Ash	18	75	75	71	72
Crude protein	142	177	177	175	176
Ether extract	155	26	33	39	42
Neutral detergent fiber	626	368	368	372	391
Acid detergent fiber	433	198	208	220	233
Acid detergent lignin	258	35	46	56	68
Starch	137	199	204	207	205
Gross energy (MJ / kg DM)	22.7	17.9	18.1	18.4	18.6

595 Abbreviations: DM = dry matter.

596 ¹Containing (g / kg fresh matter): alfalfa meal 300, wheat bran 200, barley 170, dried beet
597 pulp 150, soybean meal 115, molasses 20, wheat straw 20, soybean oil 5.

598 ²Containing (per kg of diet): Vitamin A 200 U, α -tocopheryl acetate 16 mg, Niacin 72 mg,
599 Vitamin B6 16 mg, Cholin 0.48 mg, DL-methionin 600 mg, Ca 500 mg, P 920 mg, K 500 mg,
600 Na 1 g, Mg 60 mg, Mn 17 mg, Cu 0.6 mg.

601 **Table 3**

602 Fatty acid composition (g / 100 g of total FA) of bilberry pomace (BP) and experimental diets.

Fatty acid	BP	BP0	BP5	BP10	BP15
C14:0	0.06	0.39	0.32	0.33	0.24
C16:0	4.82	21.21	17.25	15.09	13.37
C16:1 <i>c</i> 9	0.09	0.19	0.16	0.14	0.14
C18:0	1.19	3.41	2.85	2.63	2.48
C18:1 <i>c</i> 9	23.50	17.28	18.78	20.02	21.50
C18:1 <i>c</i> 11	0.58	0.98	0.92	0.86	0.83
C18:2 <i>n</i> 6	36.33	45.33	44.24	42.08	40.61
C18:3 <i>n</i> 3	32.59	8.88	13.47	16.98	19.06
C20:0	0.30	0.54	0.47	0.49	0.52
C20:1 <i>c</i> 11	0.16	0.35	0.34	0.28	0.27
C20:4 <i>n</i> 6	0.02	0.17	0.14	0.12	0.09
C22:0	0.13	0.57	0.44	0.42	0.38
C24:0	0.09	0.42	0.34	0.28	0.26
Other FA¹	0.16	0.27	0.28	0.29	0.25
SFA	6.61	26.66	21.80	19.39	17.36
MUFA	24.35	18.89	20.28	21.35	22.79
PUFA	69.03	54.45	57.92	59.27	59.85
TFA (g / 100 g DM)	15.21	2.13	2.72	3.32	3.98

603 Abbreviations: FA = fatty acids; *t* = *trans*; *c* = *cis*; SFA, saturated fatty acids; MUFA,

604 monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, total fatty acids; DM =

605 dry matter.

606 ¹Other FA (all ≤ 0.10 g / 100 g of total FA in BP and in the experimental diets): C10:0 +607 C12:0 + C16:1 *t*3 + C18:3 *n*6.

608 **Table 4**

609 Effect of dietary bilberry pomace (BP) on the carcass characteristics of rabbits (n = 12).

	BP0	BP5	BP10	BP15	SEM	P-value
SW (g)	3002	2927	2978	3035	19.675	0.256
Skin (% SW)	16.7	17.0	17.2	16.9	0.109	0.516
Full gastrointestinal tract (% SW)	13.7	13.6	13.2	13.2	0.170	0.696
CCW (g)	1878	1844	1872	1927	14.200	0.220
Dressing out (%)	62.5	63.0	62.9	63.5	0.195	0.386
Head (% CCW)	9.19	9.31	9.10	9.17	0.070	0.746
Liver (% CCW)	4.29 ^a	3.68 ^b	3.99 ^{ab}	3.61 ^b	0.080	0.003
RCW (g)	1574	1555	1580	1633	12.306	0.139
Perirenal fat (% RCW)	1.90	1.67	2.13	2.04	0.080	0.196

610 Abbreviations: SW = slaughter weight; CCW = chilled carcass weight; RCW = reference

611 carcass weight.

612 ^{a-b} Different superscripts within a row indicate significant differences ($P < 0.05$).

613 **Table 5**614 Effect of dietary bilberry pomace (BP) on the quality traits of *longissimus thoracis et*615 *lumborum* muscle (n = 12).

	BP0	BP5	BP10	BP15	SEM	P-value
pH ₂₄	5.86	5.79	5.87	5.86	0.021	0.494
Color						
L [*]	52.13	52.67	51.60	51.49	0.491	0.827
a [*]	0.05	-0.08	-0.15	-0.01	0.082	0.862
b [*]	5.52	6.04	5.55	5.99	0.166	0.560
Proximate composition (% fresh matter)						
Water	75.11	74.94	74.68	74.86	0.070	0.158
Protein	22.50	22.53	22.97	22.70	0.070	0.060
Ether extract	0.87	0.95	0.95	1.03	0.043	0.646
Ash	1.21	1.23	1.25	1.22	0.010	0.572

616 Abbreviations: L*: lightness; a*: redness; b*: yellowness.

617 **Table 6**

618 Effect of dietary bilberry pomace (BP) on the fatty acid composition (g / 100 g of total FA) of
 619 *longissimus thoracis et lumborum* muscle of rabbits (n = 12).

	BP0	BP5	BP10	BP15	SEM	P-value
Σ SFA	42.93 ^a	39.89 ^b	38.83 ^c	36.48 ^d	0.376	<0.001
C10:0	0.13	0.11	0.10	0.11	0.006	0.202
C12:0	0.17 ^a	0.15 ^{ab}	0.13 ^b	0.13 ^b	0.005	0.010
C14:0	2.60 ^a	2.37 ^{ab}	2.33 ^b	1.98 ^c	0.052	<0.001
C15:0	1.31	1.29	1.17	1.24	0.026	0.266
C16:0	29.84 ^a	27.66 ^b	26.85 ^b	24.70 ^c	0.302	<0.001
C17:0	0.61	0.60	0.59	0.62	0.009	0.523
C18:0	6.76 ^a	6.16 ^b	6.33 ^b	6.28 ^b	0.080	0.036
C20:0	0.08 ^b	0.09 ^b	0.08 ^b	0.12 ^a	0.004	0.004
C22:0	0.18 ^a	0.11 ^b	0.09 ^c	0.07 ^c	0.007	<0.001
Σ BCFA	1.26	1.35	1.17	1.23	0.036	0.388
C15:0 <i>iso</i>	0.41	0.46	0.39	0.44	0.020	0.606
C15:0 <i>aiso</i>	0.07	0.09	0.08	0.08	0.002	0.061
C16:0 <i>iso</i>	0.36	0.40	0.34	0.39	0.017	0.630
C17:0 <i>iso</i>	0.05	0.05	0.05	0.05	0.002	0.952
C17:0 <i>aiso</i>	0.36 ^a	0.34 ^{ab}	0.31 ^{bc}	0.28 ^c	0.008	0.003
Σ MUFA	30.99 ^a	28.19 ^b	27.40 ^{bc}	25.90 ^c	0.373	<0.001
C16:1 <i>c9</i>	4.89 ^a	4.02 ^{ab}	3.58 ^{bc}	2.99 ^c	0.178	0.001
C17:1 <i>c9</i>	0.32 ^a	0.31 ^a	0.28 ^b	0.26 ^b	0.006	<0.001
C18:1 <i>t6-11</i>	0.09 ^a	0.06 ^{bc}	0.07 ^b	0.06 ^c	0.003	<0.001
C18:1 <i>t12-14</i>	0.09 ^{ab}	0.08 ^b	0.11 ^a	0.11 ^a	0.004	0.023
C18:1 <i>c9</i>	23.63 ^a	22.12 ^b	21.76 ^b	21.16 ^b	0.237	0.001
C18:1 <i>c11</i>	1.51 ^a	1.21 ^b	1.15 ^{bc}	1.04 ^c	0.031	<0.001
C18:1 <i>c12</i>	0.12 ^{ab}	0.11 ^b	0.14 ^a	0.14 ^a	0.005	0.012
C18:1 <i>c14 (+c13+c15)</i>	0.15	0.12	0.16	0.16	0.006	0.116
C18:2 ¹	0.62 ^a	0.48 ^{ab}	0.48 ^{ab}	0.38 ^b	0.028	0.023
C20:1 <i>c11</i>	0.20 ^a	0.16 ^{ab}	0.16 ^{ab}	0.14 ^b	0.006	0.037
Σ PUFA	26.08 ^c	31.92 ^b	33.77 ^b	37.46 ^a	0.670	<0.001
C18:2 <i>n6</i>	19.67 ^c	22.75 ^{ab}	22.56 ^b	23.99 ^a	0.319	<0.001
CLA <i>c9t11</i>	0.05	0.04	0.05	0.04	0.002	0.406

C18:3 n3	2.10 ^d	4.65 ^c	6.28 ^b	8.65 ^a	0.362	<0.001
C20:3 n6	0.35	0.34	0.34	0.33	0.012	0.957
C20:4 n6	2.92	3.04	3.09	3.09	0.105	0.939
C20:5 n3	0.12 ^b	0.16 ^b	0.25 ^a	0.29 ^a	0.013	<0.001
C22:5 n3	0.25 ^c	0.46 ^b	0.73 ^a	0.70 ^a	0.036	<0.001
Σ n3	2.47 ^d	5.27 ^c	7.26 ^b	9.64 ^a	0.393	<0.001
Σ n6	22.94 ^b	26.13 ^a	25.99 ^a	27.40 ^a	0.365	<0.001
Σ n6 / Σ n3	9.30 ^a	5.02 ^b	3.61 ^c	2.88 ^d	0.366	<0.001
Σ PUFA / Σ SFA	0.61 ^c	0.80 ^b	0.87 ^b	1.03 ^a	0.025	<0.001
PI ²	39.86 ^d	49.79 ^c	55.16 ^b	61.24 ^a	0.261	<0.001
AI ²	0.72 ^a	0.63 ^b	0.60 ^b	0.52 ^c	0.011	<0.001
TI ²	1.14 ^a	0.84 ^b	0.73 ^c	0.59 ^d	0.030	<0.001
Δ5- plus Δ6-desaturase ²	13.04	11.79	12.37	11.11	0.356	0.273
Δ9-desaturase ³	0.78	0.77	0.76	0.78	0.009	0.905
TFA (mg / 100 g FM)	732.38	866.78	862.61	905.40	41.268	0.494

620 Abbreviations: FA = fatty acids; SFA = saturated fatty acids; BCFA = branched-chain fatty
621 acids; MUFA = monounsaturated fatty acids; *c*, *cis*; *t*, *trans*; PUFA = polyunsaturated fatty
622 acids; CLA = conjugated linoleic acid; PI = peroxidability index; AI = atherogenicity index;
623 TI = thrombogenicity index; TFA = total fatty acids; FM = fresh matter.

624 ^{a-d} Different superscripts within a row indicate significant differences (**P<0.05**).

625 ¹Sum of octadecadienoic isomers *t9t12*, *c9t13*, *t8c12*, *c9t12*, *t8c13*, *t9c12*, *t11c15*.

626 ²Calculated as reported by Dal Bosco et al. (2014):

627 $PI = (\% \text{ monoenoic} \times 0.025) + (\% \text{ dienoic} \times 1) + (\% \text{ trienoic} \times 2) + (\% \text{ tetraenoic} \times 4) +$
628 $(\% \text{ pentaenoic} \times 6) + (\% \text{ hexaenoic} \times 8);$

629 $AI = (C12:0 + 4 \times C14:0 + C16:0) / [(\Sigma \text{ MUFA} + \Sigma \text{ n6}) + \Sigma \text{ n3}];$

630 $TI = (C14:0 + C16:0 + C18:0) / [(0.5 \times \Sigma \text{ MUFA} + 0.5 \times \Sigma \text{ n6} + 3 \times \Sigma \text{ n3}) + (\Sigma \text{ n3}) / \Sigma$
631 $\text{n6}];$

632 Estimated $\Delta 5$ -desaturase plus $\Delta 6$ -desaturase activity = (C20:2 n6 + C20:4 n6 + C20:5 n3 +
633 C22:5 n3 + C22:6 n3) / (C18:2 n6 + C18:3 n3 + C20:2 n6 + C20:4 n6 + C20:5 n3 + C22:5
634 n3 + C22:6 n3) \times 100.

635 ³Estimated $\Delta 9$ -desaturase activity = (C16:1 c9 + C18:1 c9) / (C16:0 + C18:0).

636 **Table 7**

637 Effect of dietary bilberry pomace (BP) on the oxidative status (TBARS, mg TMP / kg of
 638 meat) of *longissimus thoracis et lumborum* muscle of rabbits during frozen storage at -25 °C
 639 (n = 12).

	30 days				60 days				P-value		
	BP0	BP5	BP10	BP15	BP0	BP5	BP10	BP15	Diet	Time	Interaction
TBARS	0.30	0.30	0.33	0.28	0.34	0.46	0.35	0.35	0.151	0.013	0.269

640 **Table 8**

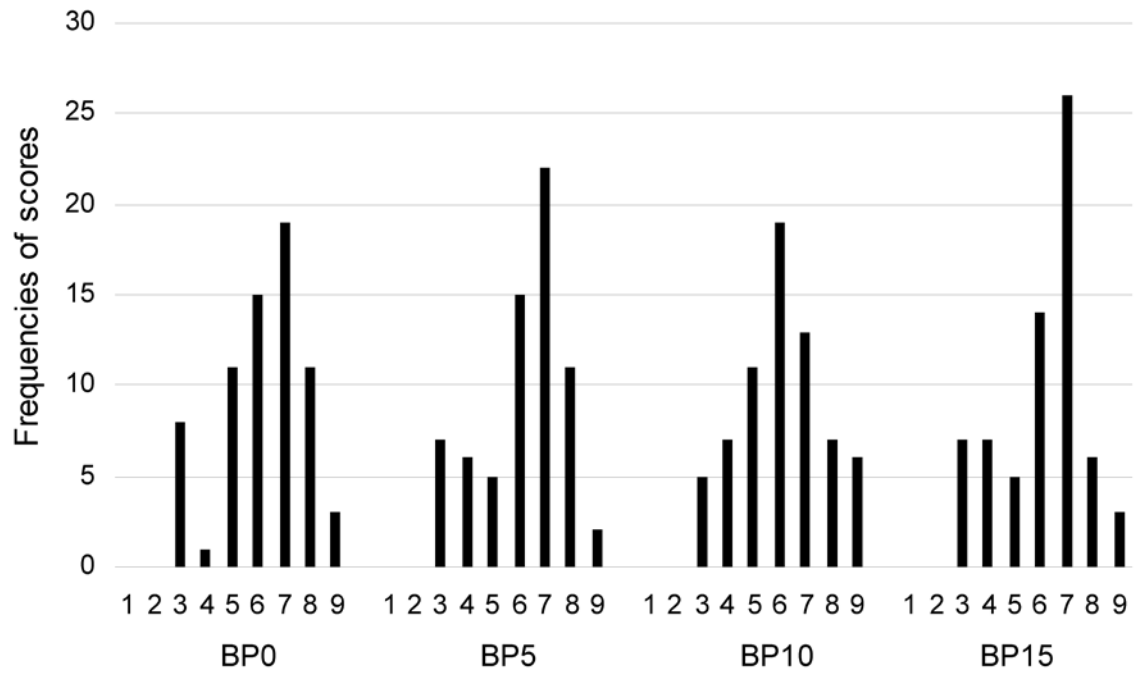
641 Sensory acceptance test of rabbit meat: descriptive statistics, mean score and rank sums.

	BP0	BP5	BP10	BP15	P-value
Median	6	7	6	7	-
Mode	7	7	6	7	-
Mean	6.2	6.2	6.1	6.1	0.958
Rank Sum	161	169	185	165	0.317

642 **Figure 1**

643 Frequency of acceptability scores for each meat sample.

644



645

646