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

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Abstract

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

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

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1. Animals and experimental design

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

2.2. Chemical analyses of feed

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

2.2.1. Determination of BP phenolic compounds

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

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

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

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

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

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

2.2.2. Antioxidant activity of BP

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

% inhibition = [(absorbance of blank – absorbance of BP extracts) / absorbance of blank] × 100

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

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

The BP and the experimental diets were ground with a cutting mill to pass a 1-mm screen sieve (Pulverisette 15 – Fritsch GmbH, Idar-Oberstein, Germany). They were analyzed for DM (# 930.15), ash (# 923.03), crude protein (CP, # 984.13), ether extract (EE, # 2003.05), acid detergent fiber (ADF, # 973.18) and acid detergent lignin (ADL, # 973.18) according to 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* (pH_{24}) 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 D_{65} 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 $MgCl_2$ (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 BF_3 (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

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

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

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

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

2.5. Statistical analyses

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

One-way ANOVA was used to evaluate the effect of BP dietary inclusion levels on carcass 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, pH_{24} 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

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

3.2. Fatty acid profile of diets and LTL muscle

Inclusion of BP increased the total FA content and modified the FA profile of the lipids in the diets (Table 3). Results showed that BP is rich in unsaturated FA (93.38 g / 100 g total FA). The most abundant FA in BP was linoleic acid (C18:2 n6, LA; 36.33 g / 100 g total FA), followed by α-linolenic acid (C18:3 n3, ALA; 32.59 g / 100 g total FA) and oleic acid (C18:1 *c*9, OA; 23.50 g / 100 g total FA). An increasing percentage of MUFA and PUFA (mainly due to increases in C18:1 *c*9 and C18:3 n3) at the expense of SFA was observed following increasing inclusion levels of BP in the diets. The values of the Σ n6 / Σ n3 PUFA ratio were 5.13, 3.30, 2.49 and 2.14 in BP0, BP5, BP10 and BP15 diets, respectively.

In rabbits, dietary FA are directly incorporated into intramuscular lipids (Dalle Zotte et al., 2014). Expectedly, the FA profile of rabbit LTL muscle (Table 6) thus generally followed that of the dietary ingested lipids (Dal Bosco, Castellini, Bianchi, & Mugnai, 2004). Increasing PUFA and decreasing SFA proportions from BP0 to BP15 diets resulted in significantly higher (26.08 to 37.46 g / 100 g total FA) and lower (42.93 to 36.48 g / 100 g total FA) proportions of these FA groups in the muscle ($P < 0.001$), respectively. The observed trend for total MUFA was opposite, as increasing levels in the diets led to decreasing proportions in the LTL lipid fraction ($P < 0.001$). The estimated Δ9-desaturase activity did not differ among treatments (Table 6). We may therefore exclude the hypothesis of a reduced conversion of SFA into *cis*-9 MUFA due to dietary PUFA inhibition of SCD (Papadomichelakis, Karagiannidou, Anastasopoulos, & Fegeros, 2010a). Most probably, as already reported for 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 Σ n6 / Σ 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 ($P < 0.001$), 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

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

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

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

3.3. Lipid oxidation

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

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

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

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

4. Conclusions

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

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

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

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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
o-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.

Table 2

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

Experimental diets (g / kg as fed)	**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
Proximate composition (g / kg DM, unless otherwise stated)					
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

Abbreviations: DM = dry matter.

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

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

Table 3

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 *c*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 *c*9	23.50	17.28	18.78	20.02	21.50
C18:1 *c*11	0.58	0.98	0.92	0.86	0.83
C18:2 n6	36.33	45.33	44.24	42.08	40.61
C18:3 n3	32.59	8.88	13.47	16.98	19.06
C20:0	0.30	0.54	0.47	0.49	0.52
C20:1 *c*11	0.16	0.35	0.34	0.28	0.27
C20:4 n6	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 FA[1]	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

Abbreviations: FA = fatty acids; *t* = *trans*; *c* = *cis*; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, total fatty acids; DM = dry matter.

[1]Other FA (all ≤ 0.10 g / 100 g of total FA in BP and in the experimental diets): C10:0 + C12:0 + C16:1 *t*3 + C18:3 n6.

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 ($P<0.05$).

Table 5

Effect of dietary bilberry pomace (BP) on the quality traits of *longissimus thoracis et 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

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

Table 6

Effect of dietary bilberry pomace (BP) on the fatty acid composition (g / 100 g of total FA) of *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 *iso*	0.41	0.46	0.39	0.44	0.020	0.606
C15:0 *aiso*	0.07	0.09	0.08	0.08	0.002	0.061
C16:0 *iso*	0.36	0.40	0.34	0.39	0.017	0.630
C17:0 *iso*	0.05	0.05	0.05	0.05	0.002	0.952
C17:0 *aiso*	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 *c*9	4.89^{a}	4.02^{ab}	3.58^{bc}	2.99^{c}	0.178	0.001
C17:1 *c*9	0.32^{a}	0.31^{a}	0.28^{b}	0.26^{b}	0.006	<0.001
C18:1 *t*6-11	0.09^{a}	0.06^{bc}	0.07^{b}	0.06^{c}	0.003	<0.001
C18:1 *t*12-14	0.09^{ab}	0.08^{b}	0.11^{a}	0.11^{a}	0.004	0.023
C18:1 *c*9	23.63^{a}	22.12^{b}	21.76^{b}	21.16^{b}	0.237	0.001
C18:1 *c*11	1.51^{a}	1.21^{b}	1.15^{bc}	1.04^{c}	0.031	<0.001
C18:1 *c*12	0.12^{ab}	0.11^{b}	0.14^{a}	0.14^{a}	0.005	0.012
C18:1 *c*14 (+*c*13+*c*15)	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 *c*11	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 n6	19.67^{c}	22.75^{ab}	22.56^{b}	23.99^{a}	0.319	<0.001
CLA *c*9*t*11	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

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

[a-d] Different superscripts within a row indicate significant differences ($P<0.05$).

[1]Sum of octadecadienoic isomers *t*9*t*12, *c*9*t*13, *t*8*c*12, *c*9*t*12, *t*8*c*13, *t*9*c*12, *t*11*c*15.

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

PI = (% monoenoic × 0.025) + (% dienoic × 1) + (% trienoic × 2) + (% tetraenoic × 4) + (% pentaenoic × 6) + (% hexaenoic × 8);

AI = (C12:0 + 4 × C14:0 + C16:0) / [(Σ MUFA + Σ n6) + Σ n3)];

TI = (C14:0 + C16:0 + C18:0) / [(0.5 × Σ MUFA + 0.5 × Σ n6 + 3 × Σ n3) + (Σ n3) / Σ n6)];

632 Estimated Δ5-desaturase plus Δ6-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) × 100.
635 [3]Estimated Δ9-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.

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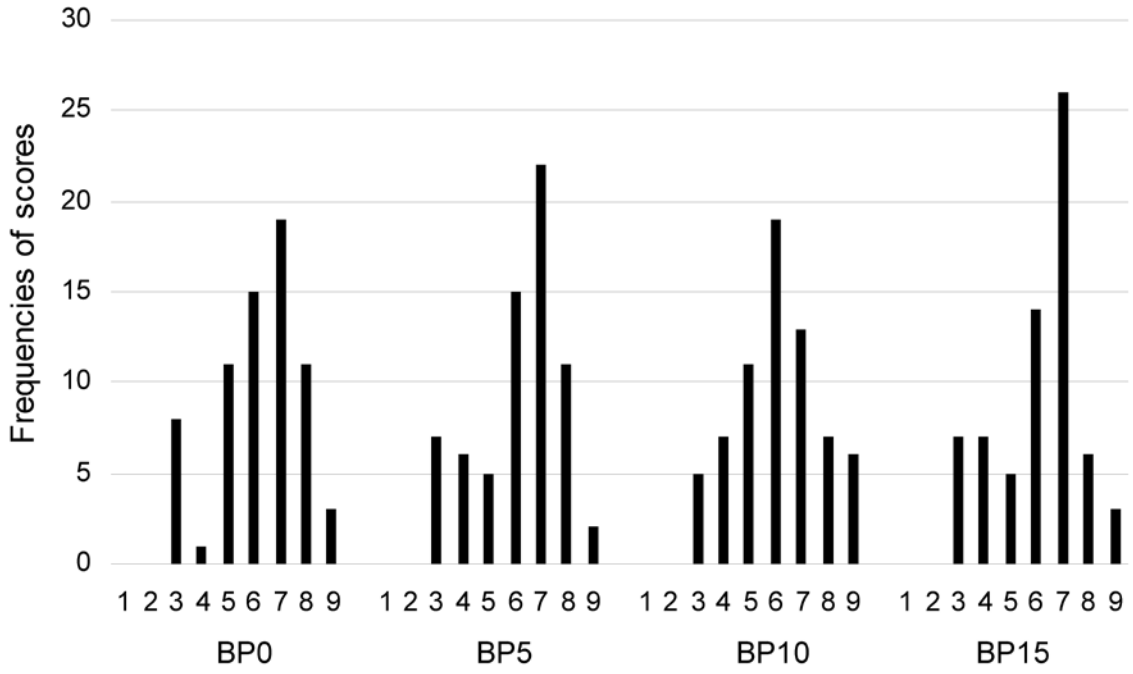

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