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Bilberry pomace in growing rabbit diets: effects on quality traits of hind leg meat

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ABSTRACT

The aim of the present study was to evaluate the effects of dietary bilberry pomace (BP) on colour, cooking losses, proximate composition and fatty acid (FA) profile of hind leg meat in growing rabbits. One hundred and forty-four Grimaud weaned rabbits (35 days old) were randomly divided into four groups of 36 animals each and fed *ad libitum* with a basal diet (C diet) tested against three assay diets developed by substituting 50, 100 and 150 g/kg of the C diet with BP (BP5, BP10 and BP15 diets, respectively). At 83 days of age, the rabbits were slaughtered without fasting. Inclusion of BP in the diet did not significantly affect colour, cooking losses, ash and protein contents, but determined a significant increase of ether extract in the muscle. Increasing dietary inclusion of BP also determined a proportional increase of total polyunsaturated fatty acids (PUFA) and total n-3 FA, as well as a proportional decrease of total saturated fatty acids (SFA), total branched chain fatty acids (BCFA) and total monounsaturated fatty acids (MUFA) in the muscle. Dietary BP significantly improved the PUFA/SFA ratio, the Σ n-6/Σ n-3 FA ratio and the atherogenicity, thrombogenicity and hypocholesterolemic/hypercholesterolemic FA indexes of rabbit meat but it decreased Δ5-desaturase plus Δ6-desaturase index. The obtained results suggest that BP inclusion in growing rabbit diets can improve the fatty acid profile of hind leg meat, with consequent health benefits to consumers.

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Rabbit; *Vaccinium myrtillus*; by-product; thigh; fatty acids

Introduction

Over the last years, there has been a growing interest in the lipid composition of meat from domestic animals because of its relationship to human health (De Smet & Vossen 2016). The positive perception of the nutritional properties of rabbit meat by consumers is related to unique characteristics compared to meat derived by other livestock: lean meat with low fat and cholesterol contents, favourable fatty acid (FA) profile and particularly a high content of unsaturated FA. Moreover, these appreciated properties can be further improved through dietary strategies (Dal Bosco et al. 2004; Trebušak et al. 2014). The manipulation of rabbits' diet is very effective in producing 'enriched meat'; some bioactive compounds such as n-3 polyunsaturated fatty acids (PUFA), conjugated linoleic acid and vitamin E can be easily incorporated into the meat (Dalle Zotte & Szendrő 2011). On the basis of these evidences, scientists have developed feeding strategies that guarantee the sustainability of livestock production systems based on cost-effective alternatives and local feed sources in order to capitalise the potential of rabbit meat as a 'functional food'.

The interest in the use of agro-industrial wastes from healthy plants has recently increased. Fruit and vegetable processing by- and co-products are promising sources of valuable substances such as phytochemicals (carotenoids, phenolics and flavonoids), antioxidants, antimicrobials, vitamins and dietary fats that possess favourable technological activities or nutritional properties (Schieber et al. 2001; Dabbou et al. 2015). They have traditionally been used in animal nutrition as the main feed ingredients (Crawshaw 2001; Bampidis & Robinson 2006; Pfaltzgraff et al.

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2013) and their effect on rabbit performance and meat quality have been extensively studied (Dal Bosco et al. 2012; Peiretti et al. 2013; Dabbou et al. 2014a, 2014b).

Pomace is the residue remaining when fruits are processed for juice, wine or marmalade. Many studies reported that the fruit pomaces contain abundant phenolic compounds (Struck et al. 2016), which indicates that by-products from juice and wine industries might be useful feed additives for improving quality and safety of meat products (Ahmad et al. 2015; Peiretti & Gai 2015).

At this regard, bilberry (*Vaccinium myrtillus* L.) has been reported as a rich source of phenolic compounds in the human diet. Large amounts of pomace by-product are generated from juice processing of bilberries and they still contain an assortment of beneficial phytochemicals including proanthocyanidins, anthocyanins and other flavonoids, being therefore suitable for the development of novel functional food ingredients (Vulić et al. 2011). The antioxidant activity of bilberry pomace (BP) was also recently confirmed by a high percentage of inhibition (about 65%) of 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging activity (Dabbou et al. 2017). In addition, 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, 2012).

To the best of our knowledge, till now no studies have been carried out to determine the effects of dietery BP on rabbit hind leg meat quality. Therefore, the objective of this trial was to evaluate the effect of dietary inclusion of BP on quality traits and FA composition of rabbit hind leg and consequently to assess the related nutritional value for human consumption.

Materials and methods

Animals and experimental design

The study was carried out at the experimental rabbitry of the Department of Agricultural, Forest and Food Sciences (DISAFA) of the University of Torino, located in Carmagnola (TO), Italy. One hundred and forty-four weaned crossbred (Grimaud) rabbits (35 days old) were randomly divided into four groups of 36 animals each with initial weight equal to 938 ± 33 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. The rabbits were fed *ad libitum* with a basal diet not containing BP (C diet; alfalfa meal 300, wheat bran 200, barley 170, dried beet pulp 150, soybean meal 115, molasses 20, wheat straw 20, and soybean oil 5 g/kg fresh matter) tested against three assay diets developed by substituting 50, 100 and 150 g/kg of the C 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 and stored in darkness to prevent auto-oxidation of the lipid sources.

Proximate composition and fatty acid profile of bilberry pomace and experimental diets

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

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 analysed for dry matter (DM, # 930.15), ash (# 923.03), crude protein (CP, # 984.13), ether extract (EE, # 2003.05), acid detergent fibre (ADF, # 973.18) and acid detergent lignin (ADL, # 973.18) according to AOAC procedures (AOAC International 2000, 2003). Neutral detergent fibre (NDF) was determined according to Van Soest et al. (1991); α-amilase (Sigma Aldrich, Saint Louis, MI) but no sodium sulphite was added and results were corrected for residual ash content. Feed proximate composition was expressed as g/kg DM. Gross energy (GE) was measured using an adiabatic calorimetric bomb (C7000, IKA, Staufen, Germany). The digestible energy content of the diets was estimated according to the prediction equation proposed by Villamide et al. (2009). Feed FA composition was assessed as described by Renna et al. (2014). The results are expressed as g/100 g DM and reported as g/100 g of total detected FA.

The proximate and FA compositions of feeds are reported in Tables 1 and 2, respectively.

Growth performance, slaughter procedures and muscle sampling

During the experiment, live weight and feed intake were recorded individually on a fortnightly basis. Average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated.

Table 1. Ingredients (g/kg as fed) and proximate composition (g/kg DM, unless otherwise stated) of bilberry pomace (BP) and experimental diets.

Experimental diets	BP	C	BP5	BP10	BP15
Ingredients					
Basal mixture[a]	–	980	930	880	830
Bilberry pomace	–	0	50	100	150
Vitamin-mineral premix[b]	–	15	15	15	15
Bicalcium phosphate	–	5	5	5	5
Proximate composition					
Dry matter, g/kg	944	882	882	880	885
Ash	18	75	75	71	72
Crude protein (CP)	142	177	177	175	176
Ether extract (EE)	155	26	33	39	42
Neutral detergent fibre (NDF)	626	368	372	391	408
Acid detergent fibre (ADF)	433	198	208	220	233
Acid detergent lignin (ADL)	258	35	46	56	68
Gross energy, MJ/kg DM	22.7	17.9	18.1	18.4	18.6
Digestible energy[c], MJ/kg DM	–	11.5	11.4	11.3	11.1

DM: dry matter.
[a]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.
[b]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.
[c]The digestible energy content of the diets was estimated according to the following prediction equation proposed by Villamide et al. (2009): $DE = 16.43 - 0.0191\ ADF - 0.0208\ Ash + 0.0148\ EE$.

Table 2. Fatty acid composition (g/100 g of total FA) of bilberry pomace (BP) and experimental diets.

Fatty acid	BP	C	BP5	BP10	BP15
C10:0	<0.01	0.03	0.04	0.05	0.02
C12:0	0.02	0.09	0.09	0.10	0.09
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 *t*3	0.03	0.08	0.07	0.05	0.05
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 n-6	36.33	45.33	44.24	42.08	40.61
C18:3 n-3	32.59	8.88	13.47	16.98	19.06
C18:3 n-6	0.10	0.07	0.08	0.09	0.09
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 n-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
Σ SFA	6.61	26.66	21.80	19.39	17.36
Σ MUFA	24.35	18.89	20.28	21.35	22.79
Σ 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

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.

At 83 days of age, 12 rabbits per group were randomly chosen (mean weight 2984 ± 138 g) and slaughtered in a slaughterhouse without fasting. After 24 h chilling at 4 °C, samples of hind legs were taken immediately after dissection, following the procedure described by Blasco and Ouhayoun (1996). Meat colour was measured on the *biceps femoris* (BF) muscle of the right hind leg of each rabbit; the same muscle was then used for cooking losses evaluation. The whole thigh of left hind leg of each rabbit was entirely deboned to separate bones and cartilages from edible meat and the latter was then ground and freeze-dried. The freeze-dried samples were used for the analysis of proximate composition and FA profile.

Quality parameters of hind leg meat

Colour

Meat colour was measured at room temperature (20 °C) in a transversal section of the BF muscle surface using a portable colorimeter Chroma Meter CR-400 Konica Minolta Sensing (Minolta Sensing Inc, Osaka, Japan). Colour measurements were reported in terms of lightness (L*), redness (a*) and yellowness (b*) in the CIELAB colour space model (Commission Internationale de l'Éclairage 1976). The values were recorded for CIE standard illuminant D65 and CIE 2° standard observer. Chroma (C*), which is a measure of the colour intensity, and hue angle (H*), which describes the fundamental colour of a substance, were calculated as: $(a^*2 + b^*2)^{0.5}$ and $\tan^{-1}(b^*/a^*)$, respectively. Negative values of the hue angle were converted to positive values by adding 180° when a* was negative and b* positive and when both a* and b* were negative. The colour values were obtained considering the average of three readings for each sample.

Cooking losses

Samples of BF muscle of each rabbit were weighed, vacuum-packed in plastic bags and cooked at 80 °C for 1 h by immersion in a water bath as described by Dabbou et al. (2014b). Cooked samples were cooled under running water for 30 min. The samples were then removed from the bags, blotted and weighed. Cooking losses were determined by calculating the weight difference in samples before and after cooking and expressed as percentage of initial weight.

Proximate composition

The proximate analyses were carried out according to the AOAC International (2000) methods. Water content in tissue samples was directly determined using the difference in weight before and after drying the sample at 125 °C for 5 h (# 950.46). The samples remaining from water analysis were lyophilised and ground in a blender for the analyses of nitrogen, fat and ash. Nitrogen was determined by Kjeldahl method and CP was calculated by multiplying N × 6.25 (# 960.52). Lipid extraction was determined by Soxhlet method

(# 960.39). Ash determination was determined by a furnace oven at 550 °C for 5 h (# 923.03).

Fatty acid composition

The FA composition of freeze-dried hind leg samples was assessed as reported by Belforti et al. (2015). Briefly, total lipids were extracted with dichloromethane/methanol (2:1, v:v) by homogenisation at room temperature. The solution was filtered in a separating funnel containing 1 ml of a distilled water solution of $MgCl_2$ (2%, w/v) and 20 ml of distilled water. The organic phase was separated and evaporated to dryness. Glycerides were saponified with a methanolic solution of NaOH 0.5 M and then BF_3 (about 10% in methanol) was added for methylation. Peaks were identified by injecting pure FAME standards as detailed by Renna et al. (2012). Quantification was assessed using tridecanoic acid (C13:0) as internal standard. The results are expressed as mg/100 g of hind leg meat and reported as g/100 g of total detected FA.

Statistical analyses

The statistical analyses were performed using the SPSS software package (version 17 for Windows, SPSS Inc., Chicago, IL). One-way ANOVA was used to evaluate the effect of BP dietary inclusion levels on growth performance and meat quality traits. The assumption of equal variances was assessed by Levene's homogeneity of variance test. If such an assumption did not hold, the Brown–Forsythe statistic was performed to test for the equality of group means instead of the F one. Pairwise multiple comparisons were performed to test the difference between each pair of means (Duncan's new multiple range test and Tamhane's T2 multiple comparison test in the cases of equal variances assumed or not assumed, respectively).

Results and discussion

Proximate composition and fatty acid profile of bilberry pomace and experimental diets

Table 1 shows the ingredients and chemical composition of BP and experimental diets. The compositional data showed that the by-product from juice processing was a good source of protein (142 g/kg DM), fat (155 g/kg DM) and fibre (626 g/kg DM). In literature, little information has been reported about the chemical composition of pomace obtained from berry fruit processing and about its suitability as a protein and cellulose source (Borycka & Górecka 2001). The CP content of BP used in this study resulted higher than those detected in an industrial seedless blackcurrant pomace by Sójka and Król (2009), who reported values ranging from 118 to 126 g/kg of DM in seedless size fractions. As far as the fibre fractions values are concerned, our BP samples contained 626, 433 and 258 g/kg DM of NDF, ADF and lignin, respectively. Similar values were reported by Nawirska and Uklańska (2008) in blackcurrant pomace samples, which contained 636 and 477 g/kg DM of NDF and ADF, respectively. On the other hand, similar values of lignin (241 and 246 g/kg of DM) were also found by Nawirska and Kwaśniewska (2005) and Górecka et al. (2010) in dried chokeberry and raspberry pomace, respectively.

According to the chemical composition of BP, the EE and fibre fractions levels of the experimental diets increased while increasing the dietary inclusion level of the by-product. The digestible energy of the diets decreased with increasing dietary levels of BP; this may be ascribed to the high ADF content found in BP. The FA compositions of BP and experimental diets are reported in Table 2. Results showed that BP is rich in unsaturated FA (93.38 g/100 g total FA). The most abundant FA was linoleic acid (C18:2 n-6, LA; 36.33 g/100 g total FA), followed by α-linolenic acid (C18:3 n-3, ALA; 32.59 g/100 g total FA) and oleic acid (C18:1 *c*9, OA; 23.50 g/100 g total FA). BP consists of skins, pulp residue and seeds, with the latter containing the major content of FA and a notable high proportion of unsaturated FA such as OA (21.8%), LA (35.9%) and ALA (36.1%) (Yang et al. 2011). Although studies on the FA composition of bilberries are still scarce, Bunea et al. (2012) also showed that OA, LA and ALA are the most abundant FA in some wild and cultivated Romanian blueberries.

Inclusion of BP increased the total FA content in the experiemental diets. Monounsaturated fatty acid (MUFA) and PUFA represented the major classes of the FAs in BP diets. OA was the most abundant MUFA in the control diet (17.28 g/100 g total FA) and its content in the BP diets increased substantially (up to 18.78, 20.02 and 21.50 g/100 g total FA in BP5, BP10 and BP15 diets, respectively). The content of ALA in the experimental diets proportionally increased following increased BP inclusion. Saturated fatty acids (SFA) decreased mainly due to the reduction of C16:0. The values of the Σ n-6/Σ n-3 ratio were 5.13, 3.30, 2.49 and 2.14 in C, BP5, BP10 and BP15 diets, respectively.

Growth performance

The inclusion of BP in the diet significantly reduced both ADFI (C: 165.30 g; BP5: 159.20 g; BP10: 147.60 g;

Table 3. Effect of dietary bilberry pomace (BP) on the quality traits of hind leg meat.

	C	B5	BP10	BP15	SEM	*p* Value
Colour						
L*	53.53	55.44	54.98	55.08	0.378	.299
a*	−1.02	−1.21	−1.46	−1.55	0.128	.451
b*	3.18	3.58	3.59	3.40	0.136	.683
Hue	106.25	108.37	115.55	115.11	2.293	.380
Chroma	3.51	4.18	4.12	4.00	0.134	.278
Cooking losses, %	23.47	23.73	22.67	23.31	0.343	.742
Proximate composition, % fresh matter						
Water	74.05	74.17	73.56	73.09	0.155	.051
Protein	21.78	21.30	21.82	22.15	0.115	.063
Ether extract	2.22^{c}	2.68^{b}	$2.90^{a,b}$	3.15^{a}	0.086	<.001
Ash	1.33	1.34	1.34	1.33	0.005	.456

[a–c]Different superscripts within a row indicate significant differences.

BP15: 155.90 g) and FCR (C: 3.54; BP5: 3.41; BP10: 3.17; BP15: 3.30) of the growing rabbits ($p < .001$), resulting in a better feed efficiency if compared to the control group.

Colour, cooking losses and proximate composition of hind leg meat

The colour, cooking losses and proximate composition of meat are summarised in Table 3. Colour and cooking losses were not significantly different among groups. Abdel-Khalek (2013) reviewed that dietary supplementation with antioxidants has no clear effects on the physical and chemical characteristics of rabbit meat. The proximate composition of the hind leg meat was not significantly affected by the inclusion of BP in the diet. The only exception was the ether extract content that significantly increased with increasing BP dietary inclusion levels, mirroring the increased ether extract content of the corresponding diets.

Fatty acid composition and nutritional indexes of hind leg meat

The FA composition and nutritional indexes of hind leg meat are shown in Table 4. Several factors (e.g. sex, housing system, genetic type, age, slaughter weight) are able to affect the FA composition of meat, the diet component being considered as a major one in monogastric animals (Dalle Zotte 2000; Pla 2004). The inclusion of BP in the diets induced significant modifications in the proportion of the majority of individual detected FA in the hind leg meat.

Usually in beef, pork and sheep, variations in the intramuscular fat concentration can determine differences in the FA composition of the meat. De Smet et al. (2004) reviewed that meat with higher intramuscular fat content shows higher levels of SFA or a lower PUFA/SFA ratio. For rabbit meat, such relationship is not consistently found (Cavani et al. 2004; Hernández et al. 2008; Dal Bosco et al. 2014b). The results obtained in our trial are in agreement with those reported by Peiretti et al. (2011), who found that rabbit meat with increasing EE content showed decreasing levels of SFA and MUFA and increasing levels of PUFA.

Gigaud and Le Cren (2006) stated that the nutritive value of rabbit meat is strongly correlated with the FA profile of their diet. Our results showed that the FA profile of the meat well reflected the composition of dietary FA, which is in agreement with previous reports on hind leg meat of rabbits (Ramírez et al. 2005; Papadomichelakis et al. 2010a; Hernández et al. 2008).

As expected, palmitic (C16:0), oleic and linoleic acids were the most abundant FA in the hind leg meat. The dietary BP inclusion led to a significantly lower SFA content ($p < .001$) in the hind leg meat, mainly due to a reduction in the contents of C14:0, ($p < .001$), C15:0 ($p < .001$), C16:0 ($p < .001$) and C17:0 ($p = .014$). Branched chain fatty acids (BCFA) are indicative of the rabbit peculiar digestive system, which allows the recycling of large amounts of caecal microorganisms, and consequently microbial FA, via caecotrophy (Papadomichelakis et al. 2010b). The total BCFA amount was significantly affected ($p = .014$) by BP inclusion in the diet, with lower values recorded in the BP15 group if compared to the other groups. Till now very few literature data are published reporting BCFA contents of rabbit meat. Papadomichelakis et al. (2010c) reported unchanged concentrations of BCFA in the caecotrophs of rabbits fed unsaturated lipid supplemented diets when compared to a control diet, which were reflected to unchanged concentrations of BCFA incorporated into BF muscle. In contrast, our results seem to suggest a difference in caecotrophy activity among treatments, which may be related to the lipid composition or the antioxidant properties of

Table 4. Effect of dietary bilberry pomace (BP) on the fatty acid profile (g/100 g of total FA) of hind leg meat.

	C	BP5	BP10	BP15	SEM	*p*-Value
Σ *SFA*	45.80[a]	41.26[b]	40.63[b]	37.74[c]	0.485	<.001
C10:0	0.20	0.20	0.16	0.21	0.011	.351
C12:0	0.23	0.24	0.20	0.21	0.060	.421
C14:0	3.21[a]	2.81[b]	2.62[b]	2.41[c]	0.054	<.001
C15:0	1.08[a]	0.96[b]	0.91[b,c]	0.87[c]	0.018	<.001
C16:0	31.97[a]	28.56[b]	28.00[b]	25.53[c]	0.400	<.001
C17:0	0.73[a]	0.72[a,b]	0.66[c]	0.67[b,c]	0.010	.014
C18:0	7.18	6.55	6.94	6.76	0.084	.050
C20:0	0.07	0.06	0.06	0.06	0.002	.549
C22:0	0.12[b]	0.17[a]	0.14[b]	0.15[a,b]	0.006	.019
Σ *BCFA*	1.01[a]	0.99[a]	0.97[a]	0.86[b]	0.018	.014
C15:0 iso	0.10	0.11	0.13	0.09	0.006	.237
C15:0 aiso	0.10[a,b]	0.11[a]	0.09[b,c]	0.08[c]	0.003	.019
C16:0 iso	0.32	0.31	0.30	0.29	0.006	.366
C17:0 iso	0.09	0.09	0.10	0.09	0.005	.916
C17:0 aiso	0.40[a]	0.38[a]	0.34[b]	0.31[c]	0.007	<.001
Σ *MUFA*	32.94[a]	30.42[b]	29.73[b,c]	27.72[c]	0.470	.001
C16:1 *c*9	5.66[a]	4.54[b]	4.36[b]	3.98[b]	0.187	.008
C17:1 *c*9	0.41[a]	0.37[b]	0.34[c]	0.31[d]	0.007	<.001
C18:1 *t*6-11	0.42	0.34	0.33	0.31	0.016	.078
C18:1 *t*12-14	0.07[b]	0.09[a,b]	0.11[a]	0.12[a]	0.007	.042
C18:1 *c*9	24.56[a]	23.47[a,b]	23.05[a,b]	21.52[b]	0.366	.028
C18:1 *c*11	1.46[a]	1.20[b]	1.14[b,c]	1.06[c]	0.029	<.001
C18:1 *c*12	0.09	0.10	0.10	0.12	0.004	.114
C18:1 *c*14 (+*c*13+*c*15)	0.09[c]	0.15[b]	0.17[a]	0.15[b]	0.005	<.001
C20:1 *c*11	0.18	0.16	0.14	0.14	0.007	.060
Σ *PUFA*	21.27[c]	28.32[b]	29.63[b]	34.55[a]	0.773	<.001
Σ C18:2[e]	0.81	0.83	0.91	0.77	0.041	.654
C18:2 n-6	17.44[c]	21.62[b]	21.43[b]	23.65[a]	0.418	<.001
C18:2 *c*9*t*11 (CLA)	0.08[b]	0.11[a]	0.11[a]	0.11[a]	0.004	.012
C18:3 n-3	2.15[d]	4.77[c]	6.36[b]	9.19[a]	0.383	<.001
C20:3 n-6	0.11[a]	0.12[a]	0.09[b]	0.09[b]	0.005	.009
C20:4 n-6	0.68	0.87	0.74	0.75	0.033	.219
Σ n-3 FA	2.15[d]	4.77[c]	6.36[b]	9.19[a]	0.383	<.001
Σ n-6 FA	18.23[c]	22.61[b]	22.26[b]	24.48[a]	0.433	<.001
Σ n-6 FA/Σn-3 FA	8.55[a]	4.81[b]	3.55[c]	2.68[d]	0.329	<.001
Σ PUFA/Σ SFA	0.46[c]	0.69[b]	0.73[b]	0.92[a]	0.026	<.001
PI[f]	26.33[c]	36.47[b]	38.94[b]	46.66[a]	1.16	<.001
Δ5- plus Δ6-desaturase[f]	3.34[a]	3.20[a,b]	2.61[b,c]	2.21[d]	0.120	.001
AI[g]	0.85[a]	0.69[b]	0.66[b]	0.58[c]	0.016	<.001
TI[g]	1.32[a]	0.93[b]	0.83[c]	0.65[d]	0.037	<.001
HH[h]	1.28[c]	1.63[b]	1.69[b]	1.99[a]	0.44	<.001
TFA, mg/100 g FM	1935.15	2169.02	2318.83	2591.99	113.88	.233

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; HH: hypocholesterolemic/hypercholesterolemic fatty acids; TFA: total fatty acids; FM: fresh matter.

[a–d]Different superscripts within a row indicate significant differences.

[e]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.

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

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

Estimated Δ5-desaturase plus Δ6-desaturase activity = (C20:2 n6 + C20:4 n6 + C20:5 n3 + C22:5 n3 + C22:6 n3)/(C18:2 n6 + C18:3 n3 + C20:2 n6 + C20:4 n6 + C20:5 n3 + C22:5 n3 + C22:6 n3) × 100.

[g]Calculated as reported by Ulbricht and Southgate (1991):

AI = (C12:0 + 4 × C14:0 + C16:0)/[(Σ MUFA + Σ n-6) + Σ n-3];

TI = (C14:0 + C16:0 + C18:0)/[(0.5 × Σ MUFA +0.5 × Σ n-6 + 3 × Σ n-3) + (Σ n-3/Σ n-6)].

[h]Calculated, as reported by Santos-Silva et al. (2002):

HH = (C18:1 c9 + C18:2 n6 + C20:4 n6 + C18:3 n3 + C20:5 n3 + C22:5 n3 + C22:6 n3)/(C14:0 + C16:0).

BP. At this regard, further studies will be needed to ascertain dietary factors able to affect caecotrophy activity and related muscle BCFA contents in rabbits.

Significantly higher PUFA and both total n-3 and total n-6 FA contents ($p < .001$) were observed in the hind leg meat of the rabbits fed the BP diets compared to the rabbits fed the control diet. As already reported for α-tocopherol (Dal Bosco et al. 2004), the antioxidants contained in BP may have inhibited the peroxidation of FA with higher (PUFA) rather than low (MUFA) degree of unsaturation, with consequent proportional increasing contents of PUFA at the expense of SFA and MUFA in the muscle. Moreover, the higher dietary supply of ALA may have affected the activity of enzymes, such as Δ5-desaturase and Δ6-desaturase, implicated in the elongation of ALA and LA to long-chain PUFA (Dal Bosco et al. 2014b). Indeed, the Δ5-desaturase plus Δ6-desaturase index was significantly lower (p=.001) in the rabbits fed the BP10 and BP15 diets if compared to the rabbits fed the control diet.

Regarding the nutritional and human helath-related indexes, the Σ n-6/Σ n-3 ratio and the atherogenicity (AI) and thrombogenicity indexes (TI) were significantly lower in the rabbits fed the BP diets compared to the rabbits fed the control diet. Particularly, the Σ n-6/Σ n-3 ratio in the meat was consistently improved (8.55 *vs*. 4.81 *vs*. 3.55 *vs*. 2.68 for C, BP5, BP10 and BP15 group, respectively). These results are very promising considering that in ordinary dietary conditions, the Σ n-6/Σ n-3 ratio in rabbit meat is set at around 10 (Dalle Zotte 2002).

Decreasing the Σ n-6/Σ n-3 ratio is a useful goal to improve the nutritional value of rabbit meat for human consumption and, at this regard, the BP10 and BP15 diets allowed hind leg meat having this ratio fallen within optimal values (Simopoulos 2011).

The hypocholesterolemic/hypercholesterolemic fatty acids (HH) index is also used to estimate the nutritive attributes of food, being more specifically related to cholesterol metabolism (Herranz et al. 2008); greater HH values indicate better nutritional quality of food (Testi et al. 2006). The HH ratio in the hind leg meat from rabbits fed the BP diets was higher than that of hind leg meat from rabbits fed the control diet ($p<.001$). Such result suggests that the BP diets tend to favour hypocholesterolemic properties of the hind leg meat of growing rabbits.

The peroxidability index (PI) was noticeably higher in the groups fed the BP5, BP10 and BP15 diets if compared to control, due to the higher percentage of unsaturated FA, mainly polyunsaturated ones, which are more susceptible to oxidation. This may arise problems, particularly in processed products (such as sausages or balls) that are nowadays gaining consideration by the producers with the purpose of reviving rabbit meat market. However, as shown by Dabbou et al. (2017), BP is a nutrient-rich agro-industrial by-product characterised by high-antioxidant activity due to its noticeable content of total phenols and tannins. Dabbou et al. (2017) demonstrated that in the *longissimus thoracis et lumborum* muscle of rabbits, such antioxidant activity of BP effectively prevented the oxidation of unsaturated lipids, contributing to the preservation of the dietetic-nutritional value and the shelf-life of the meat.

Conclusions

Till now there is no real market for bilberry pomace and therefore this by-product does not have its own price. The inclusion of bilberry pomace in diets destined to livestock animals could be of interest for both the by-product producers, who can save the money usually spent for waste discharge, and the farmers, who can save money by replacing other more expensive feed ingredients with this by-product.

Specifically, regarding rabbit nutrition, the obtained results demonstrate that the inclusion of bilberry pomace in diets for growing rabbits can be considered as an effective feeding strategy to ameliorate the nutritional attributes of rabbit meat. Particularly, bilberry pomace can be successfully used to produce favourable changes in the meat fatty acid composition. Our results show that bilberry pomace is able to significantly increase the PUFA and n-3 FA contents, and contemporarily decrease the Σ n-6/Σ n-3 FA ratio and the AI, TI and HH indexes in the meat, which may provide nutritional benefits to consumers. Undesirable increases in the ether extract content of the meat should, however, be taken into consideration when formulating rabbit diets which include bilberry pomace as feed ingredient.

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