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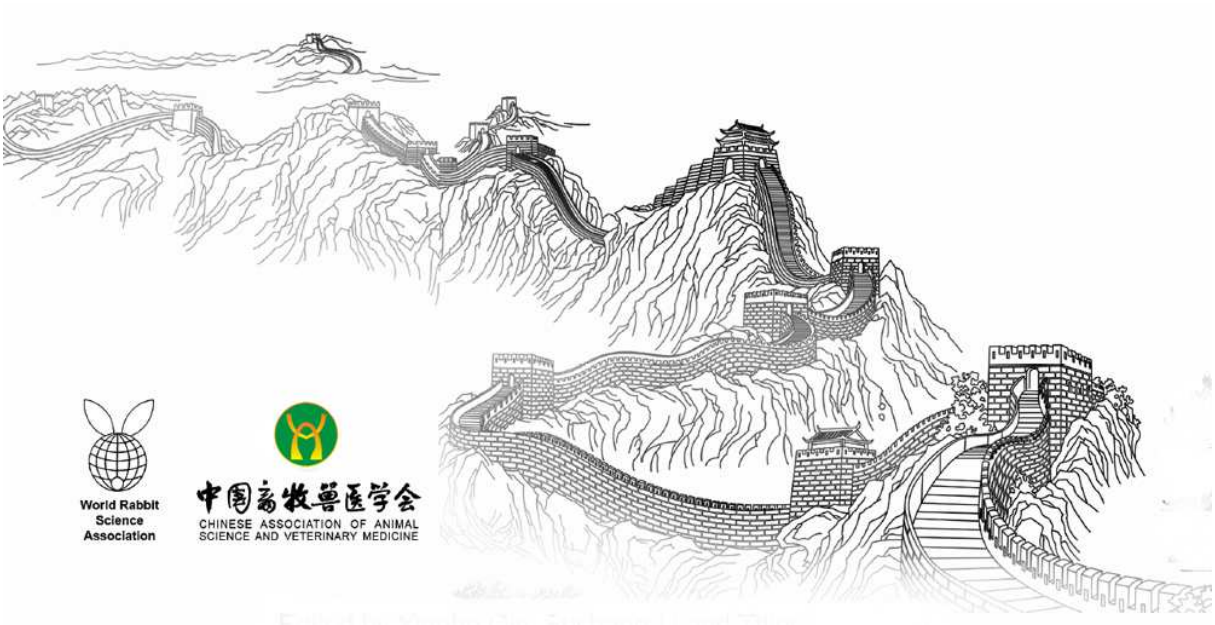

INCLUSION OF BILBERRY POMACE IN GROWING RABBIT DIETS IMPROVES THE NUTRITIONAL QUALITY OF FAT IN THE *BICEPS FEMORIS* MUSCLE

Dabbou S.[1,2]*, Renna M.[2], Lussiana C.[2], Gai F.[3], Rotolo L.[2], Kovitvadhi A.[2], Brugiapaglia A.[2], Helal A. N.[1], Zoccarato I.[2], Gasco L.[2,3]

[1]Laboratory of Bioresources, Integrative Biology and Valorisation, Higher Institute of Biotechnology of Monastir, av. Tahar Hadded, BP 74, 5000, Monastir, Tunisia
[2]Dept. of Agricultural, Forest, and Food Sciences, largo P. Braccini 2, 10095, Grugliasco (TO), Italy
[3]Institute of Science of Food Production, National Research Council, largo P. Braccini 2, 10095, Grugliasco (TO), Italy
*Corresponding author: sihem.dabbou@yahoo.fr

ABSTRACT

A trial was performed to evaluate the effects of dietary bilberry pomace (BP) on pH, color, cooking losses, proximate composition and fatty acid (FA) profile of the *Biceps femoris* muscle in growing rabbits. One hundred 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 (BP0 diet) 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). At 83 days of age, the rabbits were slaughtered without fasting. Inclusion of BP in the diet did not significantly affect pH, color, 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 ω-3 fatty acid, as well as a proportional decrease of total saturated fatty acids (SFA) and total monounsaturated fatty acids in the muscle. Dietary BP significantly improved the PUFA/SFA ratio, the ω-6/ω-3 FA ratio and the atherogenicity and thrombogenicity indexes of rabbit meat.

Key words: rabbit, *Vaccinium myrtillus*, by-product, hind leg, meat quality, fatty acids.

INTRODUCTION

Rabbit meat is often recommended by nutritionists for its low lipid and cholesterol levels and high polyunsaturated fatty acids (PUFA) content compared to other meat (Dalle Zotte, 2002). A lot of research is currently oriented towards the development of feeding strategies aimed tocapitalize the potential of rabbit meat as a 'functional food' (Dalle Zotte & Szendrő, 2011). One strategy to improve the nutritional value of rabbit meat could be the inclusion of antioxidants in rabbit diets. The residual by-products, remaining after the industrial processing of fruits, represent a good source of natural antioxidants (i.e., phenolic compounds).

Bilberry pomace (BP), a by-product of bilberry juice, still contains a wide variety of potentially beneficial phytochemicals including proanthocyanidins, anthocyanins and other flavonoids (Vulić *et al*., 2011). 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 *et al*., 2012).
The goal of this trial was to evaluate the effects of BP inclusion in growing rabbit diets on physic-chemical characteristics and fatty acids (FA) profile of rabbit hind leg meat.

MATERIALS AND METHODS

Animals and experimental design

One hundred forty-four weaned crossbred (Grimaud) rabbits (35 days old; weight: 938 ± 33.4 g) were randomly divided into four groups of 36 animals each. They were allocated to four dietary treatments: a control diet not containing BP (BP0 diet) and three experimental diets developed by substituting 50, 100, and 150 g/kg of the BP0 diet with BP (BP5, BP10, and BP15 diets, respectively). 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. Feed was provided *ad libitum* and the rabbits had free access to clean drinking water.

Table 1: **Proximate composition and FA profile of bilberry pomace and experimental diets.**

	BP	BP0[1]	BP5	BP10	BP15
Dry matter (g/kg)	944	882	882	880	885
Crude protein (g/kg DM)	142	177	177	175	176
Ether extract (g/kg DM)	155	26	33	39	42
Neutral detergent fiber (g/kg DM)	626	368	372	391	408
Saturated fatty acids (g/100 g total FA)	6.6	26.7	21.8	19.4	17.4
Monounsaturated fatty acids (g/100 g total FA)	24.4	18.9	20.3	21.4	22.8
Polyunsaturated fatty acids (g/100 g total FA)	69.0	54.5	57.9	59.3	59.9

Abbreviations: FA = fatty acids; 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.

At 83 days of age, 12 rabbits per group were randomly chosen (mean weight 2984 ± 138 g) and slaughtered in an experimental slaughterhouse without fasting. After 24 h chilling at 4°C, samples of hind leg muscle (*Biceps femoris*, BF) were taken immediately after dissection, following the procedure described by Blasco & Ouhayoun (1996).

Chemical Analyses

The pH of the BF muscle was determined at 24h post mortem using a Crison portable pH-meter (Crison Instruments, S.A., Alella, Spain). Meat color, expressed as L* (lightness), a* (redness) and b* (yellowness), was measured using a portable colorimeter Chroma Meter CR-400 Konica Minolta Sensing (Minolta Sensing Inc, Osaka, Japan) in a transversal section of the BF muscle surface. Cooking losses were determined by calculating the weight difference in samples before and after cooking at 80 °C for 1 h by immersion in water bath (Ramírez *et al*., 2004) and were expressed as percentage of initial weight. The proximate analyses were carried out according to the AOAC International (2000) methods.
The FA composition of freeze-dried BF muscle samples was assessed as reported by Belforti *et al*. (2015). Peaks were identified by injecting pure fatty acid methyl esters standards as detailed by Renna *et al*. (2012). Quantification was assessed using tridecanoic acid (C13:0) as internal standard. The results are expressed as g/100 g of BF muscle and reported as g/100 g of total detected FA.

Statistical Analysis

The data were submitted to one-way ANOVA using the SPSS software (v. 17.0 for Windows). The following model was used: $X_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where: X_{ij} = observation; μ = overall mean; α_i = effect of dietary treatment (1 = BP0; 2 = BP5; 3 = BP10; 4 = BP15); ε_{ij} = residual error.
The significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Meat quality traits of the BF muscle are reported in Table 2. pH, color and cooking losses fell within standard range values for rabbit meat and were not significantly different among groups. The ether extract significantly increased following increasing BP inclusion levels in the diets.

Table 2: Effect of dietary bilberry pomace on the quality traits of the *Biceps femoris* muscle

	BP0	B5	BP10	BP15	SEM	P-value
pH	6.20	6.17	6.19	6.16	0.022	0.855
Color						
L*	53.53	55.44	54.98	55.08	0.378	0.299
a*	-1.02	-1.21	-1.46	-1.55	0.128	0.451
b*	3.18	3.58	3.59	3.40	0.136	0.683
Cooking losses (%)	23.47	23.73	22.67	23.31	0.343	0.742
Proximate composition (% fresh matter)						
Water	74.05	74.17	73.56	73.09	0.155	0.051
Protein	21.78	21.30	21.82	22.15	0.115	0.063
Ether extract	2.22^{c}	2.68^{b}	2.90^{ab}	3.15^{a}	0.086	<0.001
Ash	1.33	1.34	1.34	1.33	0.005	0.456

L*: lightness; a*: redness; b*: yellowness.
$^{a-c}$ Different superscripts within a row indicate significant differences (Duncan's New Multiple Range Test).

The FA composition of BF muscle is shown in Table 3. Increasing dietary inclusion of BP determined a proportional increase of total PUFA and total ω-3 FA, as well as a proportional decrease of total saturated (SFA) and monounsaturated (MUFA) fatty acids in the muscle. The ω-6/ω-3 FA ratio and the atherogenicity and thrombogenicity indexes were significantly lower in the rabbits fed the BP diets if compared to BP0. These results indicate that the inclusion of BP in rabbit diets significantly improves the nutritional quality of fat in the BF muscle, with interesting related health benefits for rabbit meat consumers.

Table 3: Effect of dietary bilberry pomace on the fatty acid profile (g/100 g of total FA) of the *Biceps femoris* muscle

	BP0	BP5	BP10	BP15	SEM	P-value
C10:0	0.21	0.20	0.16	0.21	0.011	0.356
C12:0	0.24	0.24	0.21	0.21	0.009	0.421
C14:0	3.26^{a}	2.84^{b}	2.68^{b}	2.42^{c}	0.057	<0.001
C14:1*t*	0.11^{a}	0.11^{a}	0.09^{b}	0.09^{b}	0.003	0.006
C14:1*c* + C15:0	1.10^{a}	0.98^{b}	0.93^{bc}	0.87^{c}	0.018	<0.001
C16:0	32.55^{a}	28.90^{b}	28.57^{b}	25.59^{c}	0.422	<0.001
C16:1*c*	5.76^{a}	4.78^{b}	4.45^{b}	4.00^{b}	0.188	0.005
C17:0	0.75^{a}	0.73^{ab}	0.68^{b}	0.67^{b}	0.010	0.018
C17:1*c*9	0.42^{a}	0.37^{b}	0.35^{b}	0.31^{c}	0.008	<0.001
C18:0	7.31^{a}	6.65^{b}	7.08^{ab}	6.78^{b}	0.084	0.021
C18:1*t*	0.47	0.41	0.43	0.42	0.019	0.670
C18:1 *c*9	25.02^{a}	24.09^{ab}	23.50^{b}	23.07^{b}	0.201	0.002
C18:1 *c*11	1.47^{a}	1.24^{b}	1.14^{bc}	1.05^{c}	0.031	<0.001
C18:2 ω-6	17.75^{c}	21.94^{b}	21.86^{b}	23.67^{a}	0.414	<0.001
C18:3 ω-3	2.19^{d}	4.91^{c}	6.48^{b}	9.23^{a}	0.389	<0.001
C18:3 ω-6	0.08^{b}	0.08^{b}	0.09^{ab}	0.11^{a}	0.003	0.019
C20:0	0.05^{b}	0.06^{a}	0.05^{b}	0.06^{a}	0.001	0.025
C20:1 *c*9	0.18^{a}	0.17^{a}	0.15^{ab}	0.12^{b}	0.007	0.019
C20:2 ω-6	0.16	0.14	0.15	0.16	0.008	0.851
C20:3 ω-6	0.11^{a}	0.13^{a}	0.09^{b}	0.08^{b}	0.005	0.004
C20:4 ω-6	0.69	0.88	0.76	0.75	0.033	0.207
C22:0	0.12^{b}	0.17^{a}	0.14^{ab}	0.14^{ab}	0.006	0.019
ΣSFA	44.48^{a}	39.77^{b}	39.55^{b}	36.08^{c}	0.493	<0.001
ΣMUFA	34.53^{a}	32.14^{b}	31.03^{bc}	29.93^{c}	0.398	<0.001
ΣPUFA	20.99^{c}	28.09^{b}	29.43^{b}	33.99^{a}	0.775	<0.001
ΣPUFA/ΣSFA	0.47^{c}	0.71^{b}	0.75^{b}	0.95^{a}	0.028	<0.001
Σω-3 FA	2.19^{d}	4.91^{c}	6.48^{b}	9.23^{a}	0.389	<0.001
Σω-6 FA	18.80^{c}	23.18^{ab}	22.94^{b}	24.76^{a}	0.429	<0.001
Σω-6 FA/Σω-3 FA	8.66^{a}	4.79^{b}	3.59^{c}	2.70^{d}	0.341	<0.001
Atherogenicity index[1]	0.83^{a}	0.67^{b}	0.65^{b}	0.56^{c}	0.016	<0.001
Thrombogenicity index[1]	1.30^{a}	0.91^{b}	0.83^{c}	0.63^{d}	0.037	<0.001

Abbreviations: *c*, *cis*; *t*, *trans*; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; FA= fatty acids.
$^{a-d}$ Different superscripts within a row indicate significant differences(Duncan's New Multiple Range Test).
[1] Calculated according to Ulbricht & Southgate (1991).

CONCLUSIONS

The inclusion of BP in growing rabbit diets significantly increases the ether extract content and improves the nutritional quality of fat for human consumption in the *Biceps femoris* muscle.

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