The effect of Goji berries (*Lycium barbarum*) dietary supplementation on rabbit meat quality

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Abstract

This study evaluated the effect of different dietary concentrations of Goji berries (GB) on the meat quality of rabbit. At weaning, 60 New Zealand male rabbits were assigned to three groups and fed with a commercial standard diet (C), C supplemented with 1% (LG) or 3% Goji berries (HG) until slaughter. Supplementation did not affect colour, water holding capacity, and tenderness but regression analyses showed linear relationships between pH ($P<.05$), Thiobarbituric Acid Reactive Substances (TBARS; $P<.001$), Oxygen Radical Absorbance Capacity (ORAC; $P<.001$), Redox Index (RI; $P<.001$), and phenolic content ($P<.001$) of *Longissimus thoracis et lumborum* muscle and the rate of GB in the feed. However, by pairwise comparisons emerged that acidification (pH: $P<.05$), antioxidant/oxidant status (TBARS, ORAC, RI; $P<.001$), and phenolic content ($P<.01$) of muscle significantly improved only in HG compared with C group. Then, a dose-dependent relation was found but only the higher dose of GB guaranteed an increase in protection against oxidative phenomena of meat.

Key words: Rabbit; Goji berries; Meat quality; Antioxidant activity; Redox Index; Phenolic content

1. Introduction

Goji berry (*Lycium barbarum*, GB) have been using in traditional Chinese Medicine for over 2,000 years but, recently, have become very popular also in Western countries. Indeed, scientific studies confirm a number of biological effects, including anti-aging, anti-tumoral, and immune-stimulatory activities (Potterat, 2010). This beneficial properties seem mostly mediated by its antioxidant properties involving several mechanisms of action, from the radical scavenging activity and metal ion chelation to the improvement of enzymes activities (Potterat, 2010). In effect, GBs contain antioxidants such as polysaccharides, phenolic compounds, carotenoids, and vitamins (Potterat, 2010). All these substances are widely used as supplemental antioxidants in animal diets, including the rabbit (Abdel-Khalek, 2013). Really, species-specific characteristics, management, and environmental conditions make rabbit meat particularly susceptible to oxidative phenomena (Abdel-Khalek, 2013). The high levels of poly-unsaturated fatty acids (PUFAs) occurring in meat increase the nutritional value but also reduce its oxidative stability (Dal Bosco et al., 2014; Menchetti, Canali, Castellini, Boiti, & Brecchia, 2018). Many researchers proposed several strategies to limit oxidative damage, concentrating their efforts in the use of natural antioxidants (Abdel-Khalek, 2013). For example, spirulina (Dal Bosco et al., 2014), alfalfa extracts (Dabbou et al., 2018), cauliflower (Perna, Simonetti, Grassi, & Gambacorta, 2019), and some herbs used in traditional Chinese Medicine, such as *Zingiber officinale* (Mancini, Secci, Preziuso, Parisi, & Paci, 2018), have been evaluated. However, the influence of a dietary supplementation with *Lycium barbarum* has not been studied yet in rabbit meat quality although it has already produced encouraging results in pigs (Bai et al., 2016). The aim of the study was to determine the effect of a dietary supplementation with two different concentrations of GB, 1% and 3%, on the meat quality of rabbit with a particular focus on its oxidative status.

2. Materials and methods

2.1 Animals and diets

The trial was carried out at the experimental farm of the Department of Agricultural, Food, and Environmental Science of the University of Perugia. Sixty New Zealand White male rabbits were housed in individual cages in a controlled environment according with Legislative Decree No. 146, implementing Directive 98/58/EC.

At 35 days of age, the rabbits were randomly assigned to three dietary groups (20/group): Control (C), Low Goji (LG), and High Goji (HG). Rabbits of C group were fed with a commercial pelleted feed based on wheat bran and alfalfa meal; rabbits of LG and HG were fed the same ingredients mixed with 1% and 3% of GB (Gianluca Bazzica, Foligno, Italy) before pelleting, respectively (Table1SM; Menchetti et al., 2019). Rabbits were fed *ad libitum* and fresh water was always available. At 91 days of age, rabbits were slaughtered by severing the carotid arteries and jugular veins following electro-stunning. Mean slaughter weights (±SE) were 2.0±0.7 kg, 2.3±0.3 kg and 2.3±0.3 kg in C, LG, and HG groups, respectively.

2.2 Rabbit meat quality parameters

After slaughter, the carcasses were stored at 4°C for 24h. At 1 day *post mortem*, *Longissimus thoracis et lumborum* right muscles of 8 rabbits/group randomly chosen were dissected, freed from connective and adipose tissues, divided into steaks, and used for meat quality assessments. pH and colour measurement were performed in triplicates for each sample using a pH meter equipped with an insertion electrode (Crison pH25, Barcelona, Spain) and a Colorimeter (Minolta

CR400 Chromameter, Osaka, Japan; CIE L*a*b* system, CIE, 1986), respectively (Ranucci et al., 2015).

Proximal composition were assessed according to AOAC (2000) using methods 950.46, 960.30, 992.15, and 923.03 for moisture, fat, protein, and ash determinations, respectively. Shear force, 3 times per subject using an Instron 1011 (Norwood, USA) attached to a V-shaped-Warner Bratzler cutting blade (speed: 100 mm/min), drip loss and cooking loss (one sample per subject) were evaluated as described in Ranucci et al. (2015).

Lipid oxidation was assessed using the TBARS test according to Tarladgis, Watts, Younathan, & Dugan (1960) (values in mg malondialdehyde (MDA)/kg meat). The antioxidant capacity of meat and its phenolic content was determined using the oxygen radical absorbance capacity method (ORACFL) and the Folin–Ciocalteu method, respectively. A FLUOstar OPTIMA microplate fluorescence reader (BMG LABTECH, Offenburg, Germany) and Ultrospec 2100 pro UV/visible spectrometer (Amersham Pharmacia Biotech, Buckinghamshire, UK) were, respectively, used (Miraglia et al., 2018).

Redox Index (RI) was obtained as ORAC/TBARS ratio in order to obtain a measure of oxidative stress (Becatti et al., 2018).

2.3. Statistical analysis

Data were analysed by one-way ANOVA evaluating the effect of Group (3 levels: C, LG, and HG), followed by multiple comparisons with Sidak adjustment, and the Pearson's coefficient (r). Regression analyses were performed including percentage of Goji berries inclusion or ORAC levels as predictors. Statistical analyses were performed with SPSS Statistics version 23 (IBM, SPSS Inc., Chicago, USA). A P-value <.05 was considered statistically significant.

3. Results and discussion

Previous studies have shown that the dietary supplementation with GB can improve productive traits of rabbits suggesting that it could be exploited in pet food formulations and as a marketing strategy to revive the rabbit meat production (Brecchia et al., 2014; Menchetti et al., 2019). In the present work some aspects of meat quality, mainly related to the potential antioxidant capacity of GB, were determined.

The pH was lower in HG than control group ($P<.05$; Table 1) but all samples fell in the normal range for rabbit meat (Lo Fiego et al., 2004). Differences in pH could suggest an effect of GB supplementation on glycogen stores and/or enzyme activity in muscle but P-value associated with our statistic test was just below the significance level. Moreover, the regression analysis showed that GB inclusion in the feed accounted for only a small portion of the pH variance ($R^2=0.259$; Table 2). These findings indicate only a marginal effect of the dietary treatment on pH. A reduction of pH has been observed in the meat of rabbits that received high dose of vitamin E supplementation (Lo Fiego et al., 2004) but most of the previous studies did not report a significant influence of dietary antioxidants, such as alfalfa (Dabbou et al., 2018), spirulina and thyme (Dal Bosco et al., 2014), ginger (Mancini et al., 2018), and cauliflower (Perna et al., 2019). Differences in colour between groups failed to attain statistical significance as well as water holding capacity, both drip and cooking losses, and tenderness (Table 1). Conversely, dietary supplementation modulated TBARS, ORAC, and RI values confirming the antioxidant activity of GB. Regression analysis showed that TBARS were reduced while ORAC and RI enhanced as the percentage of GB in the feed increases ($P<.001$; Table 2). However, compared to the control group, the differences in TBARS (-43%), ORAC (+29%), and RI (+127%) values were

significant only for the HG group (P<.001). This finding suggests that a high dose is needed to obtain a significant increase of the oxidative stability.

The TBARS values, measuring lipid peroxidation end-product, could be affected by the lipid content (Table 2SM) and composition. Future research needs to investigate the relation between GB dietary supplementation and muscle lipid composition. Anyway, GBs have already shown their inhibitory effect on lipid peroxidation and different mechanisms of action have been proposed (Potterat, 2010). For example, GB extract administration increases antioxidant enzymes activities in muscle of rodents subjected to exhaustive exercise (Shan, Zhou, Ma, & Chai, 2011). Moreover, dietary supplementation with 1% GB increased the activity of glutathione peroxidase and decreased the concentration of MDA in the serum of pre-slaughter stressed pigs (Bai et al., 2016).

Reductions in the TBARS values have been obtained in rabbit meat by using several dietary supplementations (Dal Bosco et al., 2014; Lo Fiego et al., 2004) while the ORAC assay is a parameter still little used in rabbit meat evaluation. The ORAC test measures the capability to quench free radicals by hydrogen atom donation, one of the antioxidant mechanisms ascribed to GB (Potterat, 2010). In the present study, there was a negative association between TBARS and ORAC values (P<.01; Table 3SM). In terms of regression, ORAC explained more than half of the variability of TBARS (P=.003; Table 2). Then, a reduction in MDA, as observed in the HG group, could be largely attributed to the increased peroxyl radical scavenging capacity. This finding confirmed that the hydrogen-atom transfer is an important mechanism of antilipidic peroxidative action of the goji compounds in meat (Potterat, 2010).

For the first time in the muscle, we calculated the RI as ratio between antioxidants and oxidative stress markers (ORAC/TBARS). As we have already discussed, there were differences in RI according to the experimental group, and a linear relationship between RI and GB concentration

in the feed. Interestingly, dietetic supplementation explained more than 75% of RI variability in meat (Table 2). Thus, in our opinion, the RI could be used as synthetic index of the redox balance also in the meat.

Phenols are well known for their antioxidant properties. Phenols content in muscle increased proportionally to the percentage of GB in the feed (P<.001; Table 2) although significant differences with respect to the control were found only in the HG group (Table 1). Moreover, phenols content negatively correlated with TBARS and positively with ORAC, and RI values (P<.05; Table 3SM). These results confirm that in rabbit the phenolic content in feed could modulate the phenolic content of the muscle and that foods rich in phenolic compounds increase the antioxidant status of tissues (Perna et al., 2019).

4. Conclusions

In conclusion, GB supplementation did not affect colour, water holding capacity, and tenderness of rabbit muscle but its phenolic content and antioxidant properties. These effects were dose-dependent though at least 3% of GB was needed to achieve significant increases of the oxidative stability.

Conflict if interests

None.

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233 Table 1. Effects of the rate of Goji berries inclusion in the diet of rabbits on physical-chemical
234 analysis of *Longissimus thoracis et lumborum* muscle. C = control diet; LG = diet supplemented
235 with 1% of Goji berries; HG = diet supplemented with 3% of Goji berries.
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	Group				
Parameter	**C (n=8)**	**LG (n=8)**	**HG (n=8)**	**SEM[1]**	**P value**
pH	5.72[b]	5.68[ab]	5.58[a]	0.10	**.043**
Colour					
L*	57.84	59.97	60.04	2.11	.081
a*	1.51	1.51	1.35	0.23	.294
b*	3.35	3.86	3.54	0.55	.202
Drip loss (%)	1.42	1.47	1.67	0.25	.144
Cooking loss (%)	24.93	25.58	25.58	0.88	.257
WBSF (N/cm^2)	39.02	42.23	41.72	5.09	.416
TBARS (mg MDA/Kg)	0.3994[b]	0.4193[b]	0.2262[a]	0.040	**<.001**
ORAC (µMTE /g)	17.29[a]	18.98[a]	22.35[b]	1.86	**<.001**
RI[2]	44.64[a]	43.94[a]	101.12[b]	12.27	**<.001**
Total Phenolic content (mgGAE/100g)	17.06[a]	20.91[ab]	24.96[b]	3.60	**.001**

238 L* = lightness; a* = redness; b* = yellowness (calibrated using a white calibration plate: Y=92.8,
239 x=0.3134, y=0.3194). WBSF = Warner Bratzler shear force; TBARS = thiobarbituric acid
240 reactive substances; ORAC= oxygen radical antioxidant capacity.
241 [1] SEM: standard error of means
242 [2] RI= Redox index obtained as ORAC/TBARS ratio
243 Values followed by the same letter in each row do not differ significantly (P≤0.05; multiple
244 comparisons with Sidak correction)
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Table 2. Results of regression analyses including percentage of Goji berries inclusion in feed or ORAC levels in muscle as independent variables.

Independent variable/ predictor	Dependent variable/ outcome*	Constant	B	SE B	R^2	P value
Goji berries concentration in feed	**pH**	5.720	-0.046	0.016	0.259	.011
	TBARS	0.433	-0.063	0.011	0.706	<.001
	ORAC	17.292	1.685	0.297	0.594	<.001
	RI	36.276	20.220	3.162	0.759	<.001
	Total phenolic content	17.580	2.549	0.681	0.464	<.001
ORAC*	**TBARS**	0.794	-0.023	0.006	0.507	.003

* parameters evaluated in *Longissimus thoracis et lumborum* muscle
B= unstandardized b coefficient
SE = standard error.
R^2 = coefficient of determination.
TBARS = thiobarbituric acid reactive substances (mg MDA/Kg).
ORAC= oxygen radical antioxidant capacity (μMTE/g).
RI= Redox index obtained as ORAC/TBARS ratio.