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DIETARY SUPPLEMENTATION OF WHEAT SPROUTS EXTRACT AND OXIDATIVE STATUS OF GROWING RABBIT

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ABSTRACT

The aim of this trial was to investigate the effect of ethanol Extract of Wheat Sprouts (EWS), on *in vivo* oxidative status and meat quality of growing rabbit. Forty New Zealand White mixed-sex rabbits were weaned at 30 days of age, divided into two homogeneous groups and fed the same standard diet with a drinking supplementation of 1.5 mL/day of EWS for 50 days. The wheat sprout extracts significantly improved the lipid oxidation of rabbit plasma (43.2 vs 59.5 nmol MDA/mL, respectively), whereas the oxidation level of protein was not affected. The cholesterol concentration was lower in plasma of EWS group. The lipid and protein oxidation of *Longissimus lumborum* muscle of the EWS rabbits showed lower values than control. The retinol, α -tocopherol and γ -tocotrienol amount were higher in meat of EWS than control group; whereas the γ -, δ -tocopherols, and especially α -tocotrienol were lower. The antioxidant compounds of EWS can also have an effect in reducing cholesterol in rabbit meat (47.0 vs 42.1 mg/100g). Even the thiols content was lower in meat of EWS. The addition of EWS to commercial diet positively influenced the rabbit health status and modified the phytochemical profile of meat (antioxidants, cholesterol), improving its quality.

Key words: Rabbit, Meat, Wheat sprouts extract, Cholesterol, Antioxidants.

INTRODUCTION

Reactive oxygen species are continuously produced in many *in vivo* reactions and can cause oxidative damage of several biological molecules such as lipids, proteins, and nucleic acids, which leads to injury of cells and tissues associated with degenerative diseases (Zalatnai et al., 2001; Liu, 2004). Thus, antioxidants have considerable importance as therapeutic agents against diseases in which oxidative damages are involved. During the last decades, there has been an emerging interest in the development of food products with higher antioxidant activity than traditional foods (Ge et al., 2000).

Wheat sprouts, is reported to be an excellent sources of vitamins, minerals, dietary fiber, calories, proteins, and some functional microingredients at a relatively low cost. Due to its high nutritive value and palatability, wheat sprouts could be utilized in animal feeding, in order to transfer bioactive compounds to livestock products and in turn to humans. We have already investigated the effect of some vegetable sprouts (flax and alfalfa) on rabbit meat quality (Dal Bosco et al., 2015); however, the administration of fresh sprouts requires high amount of wet matter to concentrate their phytochemicals. Therefore, in this trial, we studied the effect of wheat sprouts like ethanol extract supplementation, on *in vivo* oxidative status and meat quality of growing rabbits.

MATERIALS AND METHODS

Animals and experimental design

Forty New Zealand White mixed-sex rabbits were weaned at 30 days of age, split into two homogeneous groups and submitted to same standard diet (40% dehydrated alfalfa meal, 30% wheat bran, 9% sunflower meal 30% crude protein, 9% barley meal, 4% soybean meal 44% crude protein, 3% extruded linseed, 0.5% soybean oil and 5.5% mineral supplements) with a drinking supplementation of 1.5 mL/d of ethanol Extract of Wheat Sprouts (EWS, Calzuola et al., 2004) in the treated group, until 80 days old.

The experimental protocol was devised according to the Italian directives (Gazzetta Ufficiale, 1992) on animal welfare for experimental and other scientific purposes, and the research was carried out at the experimental farm of the Department of Agricultural, Food and Environmental Sciences of the University of Perugia (Italy). All of the rabbits were individually housed in flat-deck cages (600 x 250 x 330 mm). The applied temperature and lighting schedules in the rabbit house were 15-18 °C and 16 L:8 D, respectively. Wheat sprouts extract was added daily in drinkers. The drinking program was adjusted according to previous records of voluntary water intake.

Chemical Analyses

At 80 days, 10 rabbits per group were slaughtered after 12 hours of feed withdrawal. The blood was collected cutting the carotid arteries and jugular veins in heparinized tubes for plasma samples and in the vacutainers for serum and centrifuged at 1,500 x g for 10 min at + 4°C, to measure the *in vivo* parameters. The different isoform of plasma vitamin E and retinol were quantified by HPLC (Zaspel and Csallany, 1983). Serum carbonyls were quantified with a spectrophotometric 2,4-dinitrophenylhydrazine (DNPH) assay (Dalle Donne et al., 2003). Cholesterol was extracted and quantified in plasma samples using the method of Folch et al. (1957) with HPLC system. After refrigerating the carcasses (24 h at + 4°C), the two *Longissimus lumborum* (LL) muscles were removed and carefully freed from connective and adipose tissues and stored at -80°C until the analysis. The Thiobarbituric Reactive substances (TBARs) were measured using the modified method of Ke et al. (1977). Tocopherols content (α , β , γ , δ -tocopherol and α -tocotrienol) and retinol of meat were quantified by HPLC according to Hewavitharana et al. (2004). The cholesterol content in rabbit meat was determined according to Naeemi et al. (1995). The protein carbonyls groups and thiols were analyzed with the method reported by Lushchak and Bagnyukova (2006).

Statistical Analysis

Data were analyzed with a linear model of STATA package (2015) with the diet as fixed effect. The level of statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

In Table 1 the *in vivo* oxidative status and the plasma bioactive compounds are reported. The wheat sprout extracts significantly improved the plasma lipid oxidative status, whereas the oxidation level of protein was not affected. The TBARs value was lower in EWS group than in the control one, and at the same time

the α -tocopherol level was higher in such group. Generally, the health-promoting effect of wheat sprouts

was attributed to the high antioxidant content (i.e., polyphenols such as gallic acid, epigallocatechin-3-gallate, epigallocatechin, epicatechin, and catechin; Donkor et al., 2012). However, Lucci et al. (2013) reported that it could be also attributable to phospholipid (PLs), characterized by a high content of essential fatty acids and protein fractions containing both potential antioxidant domains and interaction sites. The cholesterol concentration was lower in the plasma of EWS rabbits. On the basis of what described by several Authors, we can hypothesized that such cholesterol reduction was due to phytochemicals (i.e. phytosterols, isoflavones) present in the wheat sprouts (Ostund et al., 2003). These phytochemicals are biologically active and have a prominent role in cholesterol absorption. In rats, Cara et

al. (1991) reported that soluble protein of wheat germ can reduce the plasma cholesterol, through the inhibition of pancreatic lipase activity, and increases the fecal excretion of bile acids. Such latter finding could have influenced the cholesterol reduction in rabbit meat too (47.0 vs 42.1 mg/100g).

The LL muscle from the rabbits drank EWS had a better oxidative status than control (Table 2). The lipid and protein oxidation showed lower values in such group (TBARs and carbonyls). Wheat sprouts contain a very high level of organic phosphates and a powerful cocktail of antioxidant molecules such as enzymes, reducing glycosides and polyphenols that show a remarkable reducing and radical scavenging activity (Marsili et al., 2004). The retinol, α -tocopherol and γ -tocotrienol amount were higher in meat of EWS rabbits than control ones. On the contrary, the γ -, δ -tocopherols, and especially α -tocotrienol were lower in such group.

Table 1. *In vivo* bioactive compounds and oxidative status of rabbits.

		Control	EWS	<i>p</i> -value	SEM
Retinol	nmol/mL	10.43	7.79	0.062	3.96
α -tocopherol	“	0.37	0.27	0.017	0.09
γ -tocopherol	“	n.d.	n.d.	-	-
δ -tocopherol	“	0.05	0.08	0.082	0.04
α -tocotrienol	“	n.d.	n.d.	-	-
Carbonyls	nmol mg proteins	0.16	0.17	0.258	0.09
TBARs	nmol MDA/mL	59.5	43.2	0.002	4.58
Cholesterol	mg/dL	24.4	18.9	0.020	1.01

(n=10 per group) ; nd = not detected

It is probable that such vitamin E isoforms have been more used in the neutralization of the radical chain. In fact, the antioxidant efficacy of tocotrienols in membranes is higher than that of tocopherols, although their uptake and distribution after oral ingestion are less than that of α -tocopherol (Packer et al., 2001). Serbinova et al. (1991), in rat liver microsome, reported that the activity of α -tocotrienol, in scavenging peroxy radicals is higher than α -tocopherol. In this scenario, tocotrienols may exert protective antioxidant effects exceeding those of tocopherols in EWS group.

The thiols content was lower in EWS meat (6.92 vs 8.10 μ mol SH-group/g meat). It should be noted that glutathione is the major low-weight thiol molecular. Under oxidative stress, the content of glutathione often increases after its *de novo* synthesis (Lushchak et al., 2005), because the free thiols take part in the protection process against ROS, but at the same time, are involved in the regulation of many redox processes (Dröge, 2002).

Table 2. Bioactive compounds and oxidative status of *Longissimus lumborum* muscle

		Control	EWS	<i>p</i> -value	SEM
Retinol	ng/g	140.7	179.0	0.001	21.9
α -tocopherol	“	96.9	125.7	0.007	12.5
γ -tocopherol	“	1.19	0.85	0.028	0.14
δ -tocopherol	“	1.94	1.00	0.035	0.18
α -tocotrienol	“	2.07	0.30	0.003	0.20
γ -tocotrienol	“	9.33	13.4	0.015	2.21
Thiols	μ mol SH-group/g wet tissue	8.10	6.92	0.047	1.05
Carbonyls	nmol mg proteins	0.49	0.25	0.027	0.12
TBARs	μ g MDA/g	0.12	0.10	0.020	0.01
Cholesterol	mg/100g	47.0	42.1	0.013	2.84

(n=10 per group).

CONCLUSIONS

In this study the positive effect of EWS supplementation as sources of bioactive compounds was confirmed. The administration of EWS in growing rabbit improved their health status (reducing plasma TBARs) and the meat quality (increasing the antioxidant content of meat). The meat cholesterol

concentration was reduced with the consumption of EWS both in plasma and meat; however, further studies should be conducted to understand which phytochemicals can be involved in the cholesterol reduction (e.g. isoflavones, lignans, polyphenols) and their metabolic effect on rabbit.

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