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EFFECT OF DIETARY ALFALFA ON FATTY ACID PROFILE AND OXIDATIVE STATUS OF RABBIT MEAT

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ABSTRACT

To verify the effect of fresh alfalfa availability on fatty acid profile and oxidative status of rabbit meat. At 50 days of age rabbits (Martini hybrid, n=40) were divided in two homogeneous groups and fed commercial pelleted feed (Control group) and the same diet plus *ad libitum* fresh alfalfa (Alfalfa group). At 80 days of age rabbits were slaughtered. Lipid content of meat showed significant ($P<0.05$) differences being higher in Control meat, probably due to the lower pellet consumption observed in this group. The same trend was observed by myristic, palmitoleic, oleic and linoleic acid contents. On the contrary, fresh alfalfa feeding increased ($P<0.05$) stearic, linolenic, eicosatrienoic, eicosapentaenoic, docosapentaenoic, docosahexaenoic and total polyunsaturated fatty acids. In the Alfalfa group the total monounsaturated fatty acid content was significantly lower ($P<0.05$) as well as α- and γ-tocopherol. Moreover Alfalfa meat showed lower ($P<0.05$) n-6 contents, n-6/n-3 ratio and trombogenicity index. In conclusion, this specific strategy, accompanied by a good marketing campaign, could improve the economic situation allowing to reach competitive sale price.

Key words: Rabbit, meat, alfalfa, fatty acid, lipid oxidation

INTRODUCTION

Nowadays, the Italian rabbit industry is in a deep crisis for a lot of reasons, mainly represented by the rising prices for feed and energy. Accordingly, the production cost (1.80 €/kg live weight) is under the trade prize (around 1.69 €/kg live weight). This situation arises from several factors, starting with the cultural changes of the people, who are perceiving the rabbit as a pet, to changes in lifestyles that guide consumers towards types of meat faster and easier to cook. Moreover, the rabbit farmers suffer for an ancestral poor negotiation power with respect to the distribution chain.

Being know the close relationship between diet and people health,, consumers demand products that meet their dietary and nutritional preferences. Rabbit meat is very appreciated for its nutritional and dietary properties; it is a lean meat with a low-fat content and less saturated fatty acids and cholesterol than other meats. In addition, manipulation of rabbit's diet is very effective in producing "enriched meat" and some bioactive compounds: n-3 polyunsaturated fatty acids (PUFA), conjugated linoleic acid (CLA), or Vitamin E could be easily incorporated (Dalle Zotte and Szendrő, 2011). Selenium and iron could be also introduced by dietary supplementation (Lynch and Kerry, 2000). It must to promote the nutrition education in order to reconnect people to the consumption of rabbit meat and on the basis of common interests, define common strategies with manufacturers to increase their bargaining power. In this view, the objective of the study was to analyse the nutritional quality of rabbit meat by increasing natural bioactive compounds furnishing fresh alfalfa to rabbit as complementary feed.

MATERIALS AND METHODS

Animals and experimental design

The trial was carried out in a commercial rabbit farm in central Italy. At 50 days of age forty Martini coloured hybrid rabbits were divided in two homogeneous groups and fed commercial pelleted feed (Control group) and commercial pelleted food *ad libitum* plus fresh alfalfa (Alfalfa group). Alfalfa was cut daily and supplied on the top of the cages in quantities that ensured availability *ad libitum*. Rabbits were slaughtered at 80 days of age, and carcasses were immediately transferred to the Laboratory of Department of Applied Biology (Perugia), where, after 24 hours of storage at +4 °C, the *Longissimus lumborum* muscles were accurately dissected. The determination of alfalfa and intramuscular fat content was based on Folch *et al.* (1957) method. Fatty acid composition was determined by gas chromatography. The separation of fatty acid methyl esters (FAME) was carried out on an Agilent capillary column (30 m x 0.25 mm I.D, CPS Analitica, Milan, Italy) coated with a DB-Wax stationary phase (film thickness of 0.25 µm). Individual fatty acid methyl esters were identified with reference to the retention time of tridecanoic acid (C13:0) methyl ester added before extraction as an internal standard. The relative proportion of individual fatty acids was expressed as a percentage. To evaluate the activity of both Δ^5- plus Δ^6-desaturase, the enzymes catalysing the formation of long-chain n-6 and n-3 PUFA, starting from the precursors C18:2n-6 and C18:3n-3, using the equation proposed by Sirri *et al.* (2010). The value of each fatty acid was used to calculate the sum of the saturated (SFA), monounsaturated (MUFA) and PUFA, and calculate the peroxidability index (PI) according to the equation proposed by Arakawa and Sagai (1986). The amount of each fatty acid was used to calculate the indexes of Atherogenicity (AI) and Thrombogenicity (TI), as proposed by Ulbricht and Southgate (1991), and the hypocholesterolaemic/hypercholesterolaemic ratio (HH), as suggested by Santos-Silva *et al.* (2002). The extent of lipid oxidation was evaluated as thio-barbituric acid reactive substances (TBARs) according to the modified method of Ke *et al.* (1977). Oxidation products were quantified as malondialdehyde (MDA) equivalents (mg MDA/kg muscle).

Statistical analysis

Data were analyzed with a linear model (STATA, 2005) with the fixed effect of diet. Least squares means and planned comparisons were used for mean separation when the model was significant ($P < 0.05$).

RESULTS AND DISCUSSION

The fatty acid profile of feed and alfalfa showed great differences in precursors C18:2n-6 and C18:3n-3 levels (38.3 *vs* 13.4 and 0.3 *vs* 49.7 % total fatty acid, respectively; data not shown)). This substantial difference strongly modified all analyzed parameters. Lipid content, acidic profile and Δ^5- plus Δ^6-desaturase activity of *Longissimus lumborum* muscle are presented in Table1. Lipid content showed significant differences being higher in Control meat probably due to the lower pellet consumption (data not shown) observed in Alfalfa group; the same trend was observed by myristic, palmitoleic, oleic and linoleic acid contents. On the contrary, fresh alfalfa feeding increased ($P<0.05$) stearic, eicosatrienoic, linolenic, eicosapentaenoic, docosapentaenoic, docosahexaenoic and total PUFA. In particular, alfalfa group showed higher PUFA concentration and total n-3 in the muscle (35.94 *vs* 31.96% and 12.41 *vs.* 7.12, respectively), whereas MUFA was lower (22.64 *vs* 26.09%). The linolenic acid content of Alfalfa group was almost three times ($P<0.05$) higher than Control meat, whereas linoleic acids were significantly ($P<0.05$) lowered. Capra *et al.* (2010), using similar dietary strategy, found similar results and in particular relevant differences in linolenic value (1.60 *vs* 3.10% in intramuscular fat, respectively for Control and Alfalfa group). Thus, alfalfa supplementation improved the nutritional quality of rabbit meat. Δ^5- plus Δ^6-desaturase activity was doubled ($P<0.05$) in Alfalfa rabbit meat.

Table 1: Lipid content, fatty acid profile and Δ^5- plus Δ^6-desaturase activity of *Longissimus lumborum* muscle.

		Control	Alfalfa	P	SEM
Lipid	% fresh matter	2.15	1.19	0.008	0.72
C14:0	% fatty acid	2.45	1.20	0.004	0.76
C16:0	"	29.61	30.30	0.132	1.68
C18:0	"	7.00	8.33	0.001	1.04
SFA	"	41.75	41.87	0.934	2.53
C14:1n-6	"	0.15	0.07	0.068	0.92
C16:1n-7	"	1.44	0.38	0.021	1.03
C18:1n-9	"	24.37	22.02	0.002	1.42
MUFA	"	26.09	22.64	0.004	1.56
C18:2n-6	"	24.08	22.83	0.041	2.04
C20:2n-6	"	0.19	0.24	0.043	0.08
C20:3n-6	"	0.01	0.05	0.014	0.03
C20:4n-6	"	0.43	0.59	0.106	0.21
C18:3n-3	"	1.00	2.97	0.0002	0.28
C20:3n-3	"	5.38	7.39	0.0001	0.41
C20:5n-3	"	0.14	0.34	0.013	0.02
C21:5n-3	"	0.11	0.24	0.013	0.06
C22:5n-3	"	0.47	1.26	0.0001	0.19
C22:6n-3	"	0.02	0.21	0.0001	0.02
PUFA	"	31.96	35.94	0.002	2.02
Σ n-3		7.12	12.41	0.0001	3.15
Σ n-6		24.84	23.53	0.554	2.26
Δ^5 plus Δ^6 desaturase activity		4.75	9.13	0.001	1.06

n=40.

These findings are in agreement with our previous results in organic-reared rabbits (Dal Bosco *et al.,* 2007; Mugnai *et al.,* 2007, 2008a, 2008b; Cardinali *et al.,* 2008) and those of many other Authors who concluded that grass-based diets can significantly modify fatty acid composition of rabbit meat. Forrester-Anderson *et al.* (2006) suggested that grass-based diets fed to rabbits reared on pasture altered the fatty acid profile, enhancing n-3 fatty acid content. Pla (2008) also found that meat of hindleg of organic source rabbits was poorer in MUFA and richer in PUFA than meat from conventional rabbits. Hernandez (2008), based on studies of Forrester-Anderson *et al.* (2006), suggests that forage-based diets in rabbits reared at pastures increase PUFA n-3 fatty acids. Dalle Zotte and Szendrő (2011), affirm that the dietary lipid composition modifies fatty acid composition of adipose tissues in rabbit. Combes and Cauquil (2006) obtained significant increasing in the content of PUFA in the hind leg intramuscular fat with the inclusion of increasing levels of dehydrated alfalfa.

In Table 2 the main bioactive compounds and the oxidative status of rabbit *Longissimus lumborum* are presented. Meat of Alfalfa rabbits showed higher ($P<0.05$) retinol content, probably due to fresh alfalfa ingestion; on the contrary, according to our previous study (Mugnai *et al.,* 2008a), tocopherols content were lower ($P<0.05$), and this reduction was probably responsible of the containment of the peroxidation processes in the experimental group, that had significantly higher unsaturation levels thus increased the peroxidability index.

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

		Control	Alfalfa	P	SEM
Retinol	ng/g	4.58	7.40	0.0001	0.89
α-tocopherol	"	554.32	400.80	0.047	89.78
γ-tocopherol	"	11.65	5.50	0.004	2.79
TBARs	μgMDA/g	0.09	0.13	0.763	0.05
Peroxidability index		43.91	58.97	0.008	2.56

n=40.

Nutritional characteristics of rabbit *Longissimus lumborum* (Table 3) were affected by dietary fresh alfalfa administration with an improvement of nutritional quality of meat. According to previous findings (Hernandez, 2008; Webb and O'Neill, 2008; Forrester-Anderson *et al.,* 2006; Combes and

Cauquil, 2006; Mugnai *et al.,* 2007 and 2008 b; Capra *et al.,* 2010), grass (derived from pasture or administered in cage) caused an accumulation of n-3 fatty acids and a significant reduction of n-6 fatty acids in the triglyceride fraction of rabbit meat. In this study Alfalfa meat showed lower ($P<0.05$) SFA, MUFA, n-6 contents, and n-6/n-3 ratio and trombogenicity index. It is important to underline that also in Control group the n-6/n-3 value should be considered optimal being lower respect to recommendations. The British Nutritional Foundation, (1999) emphasize the need to eat foods with an n-6/n-3 ratio less than or equal to 6, whereas Simopoulos (2002) indicates 4:1 as the optimal ratio.

Table 3: Nutritional characteristics of *Longissimus lumborum* muscle

		Control	Alfalfa	P	SEM
SFA	mg/100g	617.95	525.99	0.008	65.41
MUFA	"	386.16	284.42	0.015	52.63
∑ n-3	"	105.21	151.63	0.031	14.29
∑ n-6	"	367.66	299.87	0.006	49.81
EPA	"	2.07	4.27	0.003	0.93
DHA	"	0.30	2.64	0.0001	0.21
PUFA	"	473.04	451.50	0.589	42.13
n-6/n-3		3.49	2.00	0.026	0.51
Atherogenicity Index		0.69	0.60	0.091	0.12
Trombogenicity Index		1.29	1.10	0.023	0.16
HH*		1.58	1.59	0.841	0.08

n=40.

CONCLUSIONS

The results of this study suggest that rabbit meat, mainly in the fat profile, has remarkable nutritive attributes that would justify to be considered as a healthy meat (Dalle Zotte and Szendrő, 2011). Both experimental groups of this trial had very lean meat, that facilitate the achievement of this nutritional goal, but the inclusion of fresh alfalfa complementarily a commercial pelleted, under nutritional point of view strongly modified fat content and composition of meat, increasing linolenic, eicosapentaenoic and docosahexaenoic acid and further reduced n-6/n-3 ratio. In the specific case of the farm involved in this trial, this strategy, accompanied by a good promotional campaign, could improve the economic situation allowing to reach a sale price of 2.5 €/kg live weight.

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REFERENCES

British Nutrition Foundation 1999. Meat in the Diet. *London: British Nutrition Foundation*.

Arakawa K., Sagai M. 1986. Species differences in lipid peroxide levels in lung tissue and investigation of their determining factors. *Lipids, 21, 769-775.*

Capra G., Grompone M.A., Urruzola N., Pardo M.J., Martínez R., Fradiletti F., Cozzano S., Repiso L., Adamson R. 2010. Effect of fresh alfalfa in the diet of growing rabbits on growth performance, carcass characteristics and fat composition. *Proc. 4th Congreso de Cunicuiltura de las Americas, Cordoba, Argentina.*

Cardinali R., Rebollar P., Mugnai C., Dal Bosco A., Castellini C. 2008. Pasture availability and genotype effects in rabbit: 3. Ddevelopment of gastro-intestinal tract and immune functions of appendix and Peyer's patch health and welfare. *Proc. 9th World Rabbit Congress, Verona, Italy.*

Combes S., Cauquil L., 2006. Viande de lapin et oméga 3 : Une alimentation riche en luzerne permet d'enrichir la viande des lapins en oméga 3. *Viandes et Produits Carnés, 25, 31-35*

Dal Bosco A., Mugnai C., Mourvaki E., Castellini C. 2007. Effet de la disponibilité du pâturage sur le state oxydatif et sur le profil d'acides grasses de la viande de lapin. *Mem. 12émes Journées de la Recherche Cunicole. Le Mans 27-29/11/07.*

Dalle Zotte A., Szendrő Z. 2011. The role of rabbit meat as functional food. *Meat Sci., 88, 319-331.*

Folch J., Lees M., Sloane-Stanley H.. 1957. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem., 226, 497-509.*

Forrester-Anderson I.T., McNitt J., Way R., Way M. 2006. Fatty acid content of pasture-reared fryer rabbit meat. *J. Food Comp., 19, 715-719.*

Hernández P. 2008. Enhancement of nutritional quality and safety in rabbit meat. *Proc. 9th World Rabbit Congress, 2008 June, 10-13, Verona, Italy, 1287-1300.*

Ke P.J., Ackman R.G., Linke B.H., Nash D.M. 1977. Differential lipid oxidation products in various parts of frozen mackerel. *J. Food Techn., 12, 37-42.*

Lynch P.B., Kerry J.P. 2000. Utilizing diet to incorporate bioactive compounds and improve the nutritional quality of muscle foods. In: Decker E., Faustman F., López-Bote C. (Eds). Antioxidants in muscle foods. Wiley & Sons, Inc. Publication, New York, USA, 455-480.

Mugnai C., Mourvaki E., Dal Bosco A., Castellini C. 2007. Effect of pasture availability on the fatty acid profile and oxidative status of rabbit meat. *Atti 6° Conv. Naz. "Acidi grassi polinsaturi ω-3 ,CLA e Antiossidanti", June 21-23, Ancona, Italy.*

Mugnai C., Finzi A., Zamparini C., Dal Bosco A., Castellini C. 2008a. Pasture availability and genotype effects in rabbit: 2. Productive performance and carcass characteristics. *Proc. 9th World Rabbit Congress, 2008 June, 10-13, Verona, Italy,1405-1410.*

Mugnai C., Moscati L., Battistacci L., Cardinali R., Dal Bosco A., Castellini C. 2008b. Pasture availability and genotype effects in rabbit: 1. Health and welfare. *Proc. 9th World Rabbit Congress, 2008 June, 10-13, Verona, Italy, 1207-1212.*

Pla M. 2008. A comparison of the carcass traits and meat quality of conventionally and organically produced rabbits. *Livest. Sci., 115, 1-12.*

Santos-Silva J., Bessa R.J.B., Santos-Silva F. 2002. Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. *Livest. Prod. Sci., 77, 187-194.*

Simopoulos, A. P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmac., 56, 365−379.*

Sirri, F., Castellini C., Roncarati A., Meluzzi A. 2010. Effect of feeding and genotype on lipid profile of organic chicken meat. *Europ. J. Lipid Sci. Technol., 112, 994-1002.*

StataCorp. 2005. Stata Statistical Software: Release 9.0, College Station, Texas, USA.

Ulbricht T.L., Southgate D.A.T. 1991. Coronary heart disease: seven dietary factors. *The Lancet, 338, 985-992.*

Webb E.C., O'Neill H.A. 2008. The animal fat paradox and meat quality. *Meat Sci., 80, 28–36.*