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EFFECT OF EXTRUDED LINSEED AND ALGAE *PADINA PAVONICA*'S DIETARY SUPPLEMENTATION ON MEAT FATTY ACID PROFILE IN FATTENING RABBITS

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ABSTRACT

Dietary supplementation with n-3 polyunsaturated fatty acid (n-3 PUFA) in livestock animals is an effective strategy to produce functional foods. This study aimed at investigating the effect of dietary supplementation with extruded linseed alone and in combination with the algae *Padina pavonica*, which has boosting effect on DHA (22:6 n-3) assimilation, on the fatty acid profile of fattening rabbit meat. At weaning age (35 days), 120 rabbits were divided into three groups according to the experimental diet (n=40 rabbits/diet) provided *ad libitum* until the slaughter age (82 days): commercial control diet (CNT), CNT diet supplemented with 5% extruded linseed (L5%), and CNT diet supplemented with 3.5% extruded linseed and 0.2% *Padina pavonica* algae (LPP). At slaughter, the *Longissimus thoracis et lumborum* (LTL) from 20 randomly selected carcasses per group (n=60) was collected, and proximate chemical composition and fatty acid profile analyses were performed. No significant differences were found in the carcass characteristics and proximate composition of LTL. Compared to the CNT group, both L5% and LPP LTL showed higher concentrations of n3 PUFAs, with better n6/n3 ratio, and improved atherogenic and thrombogenic indexes. LPP showed higher n-3 very long chain fatty acids, specifically DHA compared to the L5% group. Results from the L5% group were consistent with the literature, whereas there are no previous studies on the benefits of combining extruded linseed and algae. Therefore, these findings show, for the first time, the benefits on the rabbit meat quality of combining extruded linseed and the algae *Padina pavonica* in the diet of fattening rabbits.

Key words: *Oryctolagus cuniculus*, omega-3 polyunsaturated fatty acids, extruded linseed, algae, meat quality.

INTRODUCTION

The imbalance in the n-6/n-3 fatty acids ratio and the low absolute mass of essential fatty acids consumed in the modern diet have been linked to the increased occurrence of

cardiovascular diseases in developed countries. To reduce the health risk of unbalanced diets, research is engaging in the development of novel functional foods. In this regard, n-3 polyunsaturated fatty acids (n-3 PUFAs) dietary supplementation in livestock animals has proven to be an effective strategy (*i.e.*, meat, eggs, milk; Lewis et al. 2000). This dietary supplementation can also improve the productive and reproductive traits as well as the health and welfare of rabbits (Agradi et al., 2023). In rabbit farming, some experiments have already been conducted using various sources of n-3 PUFA, with encouraging results (Agradi et al., 2023). However, several critical issues still need to be resolved such as the dose of n-3 PUFAs sources to be used and the effect of combining it with other sources, such as *Padina pavonica* algae, which has boosting effect on DHA (22:6 n-3) assimilation, on rabbit meat fatty acid composition. In this sense, our study aimed at investigating the effect of dietary supplementation with extruded linseed alone and in combination with the *Padina pavonica* algae on the fatty acid profile of rabbit meat.

MATERIALS AND METHODS

Animals and experimental design

The rabbits were raised in two commercial farms located in Central Italy. The experimental protocol was approved by the Ethical Committee of the Department of Veterinary Medicine of the University of Milano (OPBA_18_2021). At weaning age (35 days), 120 New Zealand White rabbits (n=60/farm) were divided into three groups according to the isoenergetic experimental diet fed (n=40/group): commercial control diet (CNT), CNT diet supplemented with 5% of extruded linseed (L5%), and CNT diet supplemented with 3.5% of extruded linseed and 0.2% *Padina pavonica* algae (LPP) (chemical composition and fatty acid profile were determined for all the diets, data not shown). Animals were individually housed in conventional cages (L×W×H: 75×35×25 cm) under controlled environmental conditions. All animals were slaughtered at 82 days of age. Only 60 carcasses (n=20 randomly selected carcasses/group) were transported to the CREA-ZA laboratory and dissected following the Worlds Rabbit Science Association recommendations (Blasco and Ouhayoun, 1996). The weights and lengths of the three regions (fore legs, trunk, and hind part) were determined, along with the tissue composition of the right thigh.

Chemical Analyses

Twenty four hours after slaughtering, the *Longissimus thoracis et lumborum* (LTL) was excised from each chilled carcass (4°C), and the proximate chemical composition (dry matter, crude protein, total fat and ash) of LTL (AOAC, 2016) was performed. After chloroform:methanol (2:1 vol:vol) fat extraction and methylation with 2 N methanolic potassium hydroxide, the fatty acid methyl esters (FAME) were quantified using a gas-chromatography (GC 6890N Agilent, Inc., Santa Clara, CA, USA) instrument equipped with flame ionization detector and a CP-Sil88 fused silica capillary column. The GC-FID conditions and other details were described in Failla et al. 2021. Fatty acids methyl esters were identified by comparing the peaks retention time of each compound with standard peaks from Supelco mix 37 (Sigma-Aldrich Merck, Darmstadt, Germany); FAME and the different classes of fatty acids as amount of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, are expressed as % of total FAME. From some fatty acid were calculated the atherogenicity index (AI) and thrombogenicity index (TI) following Ulbricht and Southgate (1991) indications.

Statistical Analysis

Data were analyzed with one-way ANOVA using Welch's F when the assumption of homogeneity of variances was violated. Sidak correction was used for multiple comparisons. Kruskal-Wallis tests were used when the transformation did not improve the data distribution. Statistical analyses were performed with SPSS Statistics version 25 (IBM, SPSS Inc., Chicago, IL, USA). Statistical significances were declared at an alpha value of 0.05.

RESULTS AND DISCUSSION

No significant differences were found in the carcass characteristics, tissue composition, and proximate chemical composition of LTL (data not shown). Previous studies confirm the

limited effect of isoenergetic diets supplemented with different n-3 PUFAs sources on carcass characteristics (Agradi et al., 2023). Conversely, the extruded linseed and algae supplementations modified the fatty acid composition of the LTL (Table 1). This demonstrates the effectiveness of incorporating extruded linseed and *Padina pavonica* algae into the diet of fattening rabbits to produce a functional food.

Table 1: Mean contents and standard errors of means (SEM) of fatty acids in *Longissimus thoracis et lumborum*.

Fatty acids (% of total FAME)	Groups						P-value
	CNT		L5%		LPP		
	Mean	SEM	Mean	SEM	Mean	SEM	
14:0	1.35b	0.05	1.25ab	0.05	1.15a	0.04	0.009
16:0	24.24b	0.22	23.53a	0.21	23.48a	0.13	0.010
18:0	8.13	0.17	8.04	0.12	8.32	0.11	0.364
18:1 cis-9 OA	18.74a	0.19	19.65b	0.20	19.14ab	0.21	0.009
18:2 n-6 LA	28.06b	0.31	24.06a	0.27	24.00a	0.20	<0.001
18:3 n-3 ALA [#]	2.05a	0.05	6.71c	0.33	5.33b	0.24	<0.001
20:4 n-6	6.43b	0.25	5.01a	0.24	6.07b	0.20	<0.001
20:5 n-3 EPA [#]	0.19a	0.02	0.55b	0.03	0.61b	0.02	<0.001
22:5 n-3 DPA [#]	1.16a	0.06	2.37b	0.11	2.70b	0.08	<0.001
22:6 n-3 DHA	0.24a	0.01	0.44b	0.03	0.52c	0.03	<0.001
SFA	35.87b	0.19	34.83a	0.25	35.02a	0.17	0.001
MUFA	24.23	0.31	24.49	0.24	24.10	0.25	0.572
n-6	35.19c	0.28	29.72a	0.22	30.78b	0.23	<0.001
n-3*	3.66a	0.12	10.11b	0.22	9.21b	0.17	<0.001
PUFA	39.07a	0.31	40.07b	0.31	40.22b	0.22	0.011
n-6/n-3*	9.77c	0.27	2.97b	0.07	3.37a	0.07	<0.001
VLCFA n-3 [#]	1.61a	0.09	3.40b	0.16	3.88c	0.12	<0.001
AI	0.30b	0.00	0.29a	0.00	0.28a	0.00	<0.001
TI*	1.18b	0.02	0.72a	0.01	0.76a	0.01	<0.001

Values followed by the same letter(s) are not significantly different at $p \leq 0.05$. # Welch's F statistic. * Non-parametric statistics. For brevity, means and SEM are presented but the data were analyzed with nonparametric techniques. FAME=fatty acid methyl ester; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; VLCFA = very long chain PUFA; AI = index of atherogenicity; TI = index of thrombogenicity.

The SFA, particularly C14:0 and C16:0, which are considered unhealthy fatty acids, were significantly higher in the CNT group compared to the others ($P < 0.05$). No significant differences were observed in MUFA except for OA (18:1 cis-9), which constitutes the principal MUFA. It exhibited a higher percentage in the L5% and LPP groups.

The higher intake of ALA (18:3 n-3) in the L5% and LPP group compared to the CNT group primarily resulted in an increase of this fatty acid in their meat ($P < 0.001$). Additionally, due to the ability of rabbit tissues to desaturate and elongate the ALA into n-3 VLCFA, also EPA (20:5 n-3), DPA (22:5n-3) and DHA (22:6 n-3) significantly increased in the meat of rabbits fed with L5% and LPP diets ($P < 0.001$). As a consequence, the n-6/n-3 ratio, as well as the AI and TI indexes also showed significant improvements, when compared to the CNT group ($P < 0.001$). Our result confirms the added value that linseed supplementation brings to rabbit meat fatty acid composition, as evidenced by previous studies (Matics et al. 2017; Tariq et al. 2017).

Regarding the incorporation of *Pavina pavonica* algae into the diet, this is the first experiment to utilize it in fattening rabbits. Other few studies have examined diets with *Spirulina* or *Schizochytrium* spp. to modify the fatty acid profile on rabbit meat but the reported results have been contrasting (Mordenti et al. 2010; Peiretti and Meineri 2011). Here, the combination of *Pavina pavonica* algae with extruded linseed further increased the content of n-3 PUFA in comparison with CNT group ($P < 0.001$). Furthermore, in LPP group the concentration of n-3 VLCFA (i.e., EPA, DPA, and DHA) in the LTL was even higher than in the L5% group ($P < 0.001$). The beneficial properties of n-3 VLCFA against cardiovascular, neurodegenerative, and other chronic diseases are well documented (Saini et al. 2021). In

human medicine, it has been proven that algae oils (as fish oil) are more valuable in improving the body status of EPA plus DHA rather than linseed oil, probably because of the rate-limiting step of bioconversion of ALA to its reaction intermediates (Saini et al. 2021). For these reasons, rabbit meat produced with the supplementation of extruded linseed with the addition of *Padina pavonica* algae in the diet should be considered as a feasible strategy to increase n-3 fatty acid and improve both n6/n3 ratio and the AI and TI indices. This product could offer functional compounds, particularly beneficial for people suffering from cardiovascular diseases, while also providing high biological value proteins and low cholesterol contents, typical of rabbit meat (Dalle Zotte and Szendro 2011).

CONCLUSIONS

Based on the obtained results and the significant increase in ALA, EPA, DPA, and DHA, we can conclude that the dietary incorporation of linseed derivatives and *Padina pavonica* algae for fattening rabbits is an effective strategy to produce a functional food that may contribute to the preservation of human health.

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