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EFFECT OF EXTRUDED LINSEED INTEGRATION IN RABBITS' DIET, ON MEAT'S FATTY ACID PROFILE AND MICROBIOLOGICAL QUALITY

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ABSTRACT

The effects of extruded linseeds (LN) intake on fatty acid profile and microbiological quality of rabbits' meat were investigated. A total of 69 rabbits were weaned at 35 days of age, and equally divided into three experimental groups: Control group (C), receiving a commercial diet; LN2.5 group, were fed diet containing 2.5% LN and LN5 group where rabbits were given 5% LN. At the end of the fattening period (70 days), rabbits were slaughtered. Extruded linseeds' used had a significant effect ($p < 0.05$) on meat fatty acids profiles; rabbits from LN5 group showed had higher polyunsaturated fatty acids (PUFAs) n-3 content ($P < 0.001$), particularly linolenic acid C18:3n3, and lower SFA ($P < 0.05$) compared to LN2.5 and C groups. Hence, LN5 and LN2.5 groups had lower ($P < 0.001$) n-6/n-3 ratio. For microbial count meat was safe up to 14 days of storage without exceeding the threshold. LN5 group showed significantly lower total viable counts (TVC) and *Escherichia Coli* (*E.coli*) count, throughout storage period, thus LN supplementation inhibited microbial growth. Therefore, LN could be used in rabbit feeding in order to improve meat products' nutritional value, with better fatty acids content and microbiological quality.

Key words: Rabbit, linseed, meat, fatty acids, microbiological quality

INTRODUCTION

Worldwide, there is an increased demand for safer meat product with higher nutritional values, and recently there has been increasing interest in PUFA-enriched meats due to their beneficial impact on heart health and potential chemo-protective purposes (Huang and Ziboh, 2001). Rabbit meat is characterised by low lipid and cholesterol levels and high polyunsaturated fatty acid (PUFA) content (Dalle Zotte, 2002; Hernandez, 2008); therefore, rabbit meat consumption could be healthy for consumers. Numerous researches have been undertaken aiming to produce dietetic rabbit meat, with a better nutritional value through dietary supplementation. The most used ingredient to increase PUFA level and to balance the n-6/n-3 ratio of feed and animal products is linseed. Many studies have proposed the dietary use of linseed to obtain meat with raised n-3 PUFA in rabbits (Dal Bosco *et al.*, 2014; Peiretti and Meineri, 2010). The purpose of this study, therefore, was to investigate the effect of the dietary extruded linseed supplementation on meat quality (fatty acids profile and microbiological quality) in rabbit.

MATERIALS AND METHODS

Animals and experimental design

Sixty-nine New Zealand white rabbits weaned at 35 days of age, were assigned to three homogeneous groups of 23 rabbits and were given three different diets: Control (C) diet with no extruded linseed (LN) added; LN2.5 diet containing commercial diet with 2.5% of LN supplementation and LN5 where animal were fed a commercial diet with 5% of LN supplementation. For each group, rabbits were housed in eight cages: seven cages containing three and one cage containing two rabbits. Cages were wired (L×W×H: 390 × 370 × 290 mm) and the feeding program provided a daily administration of pelleted feed. Water and feed were supplied *ad libitum*. The temperature in the rabbitry was 20 ± 3 °C and the lighting schedules were 14 to 16 hours of light per day. At the end of the trial (70 days), rabbits were slaughtered and the dissection procedures were performed according to the recommendation of the World Rabbit Science Association (Blasco and Ouhayoun, 1996). For each treatment, the *Longissimus thoracis et lumborum* (LTL) were excised from both sides of carcasses, trimmed of all external fat and epimysium connective tissue, then, meat samples were stringed and stored in a permeable plastic box at 4°C for 14 days, for microbiological analysis. Ground meat mixes from thorax, loin, forearm and leg frozen at -80 °C for analyses of fatty acid (FA) profile.

Microbiological analysis

The microbiological parameters were measured on days 0, 4, 7 and 14 of storage. Plate Count Agar (PCA), Violet red bile lactose (VRBL), Sorbitol MacConkey agar (SMA), Hektoen Enteric agar (HEA) and Chapman agar (CA) were used to enumerate total viable counts (TVC), *Escherichia Coli*, *Pseudomonas*, *Enterobacteriaceae* and *Staphylococcus aureus* counts, respectively. The microbial counts were expressed as log₁₀ of colony forming units/g (log CFU/g).

Fatty acids measurement

For fatty acid methyl esters (FAME) quantification, meat lipids were extracted in duplicate according to Folch *et al.* (1957) method and the methyl fatty acid esters (FAME) were then quantified using a gas-chromatograph (GC 6890N Agilent, Inc., Santa Clara, CA, USA) with C19:0 as an Internal standard. Fatty acids methyl esters were identified by comparing the peaks retention time of each compound with standard peaks and were expressed as a percentage of the total FAME (% of total FAME).

Statistical analysis

The effect of dietary supplementation on rabbits' meat FA profile was processed by a one-way analysis of variance ANOVA method, using the GLM procedure (SAS, 2003). Rabbits' meat microbiological quality was assessed using the mixed procedure considering dietary treatment as fixed effect and time as a repeated measure. The individual rabbit was used as the experimental unit. Differences among groups were tested by the Tukey test. A P-value of P<0.05 was considered significant for all measurements.

RESULTS AND DISCUSSION

Effect of extruded linseed (LN) incorporation on rabbit's meat microbiological quality

The results of microbiological counts of meat samples are reported in Table 1. The results show that the microbial population significantly (p<0.001) increased during the storage days. Meat samples from all groups had lower microbiological counts at day 0 and increased with storage time from day 4 to 14. No microbial growth was detected for *E.coli* until the end of storage period where the lowest count was recorded in LN5. In fact, LN use, at a dose of 5%, significantly reduced *E.coli* growth. *E. coli* presence is generally an indicator of faecal contamination resulting mostly from defects occurring during skinning and evisceration. For, *Staphylococcus aureus* growth, meat was safe up to 4 days, then the count increased to reach values higher than 2 log CFU/g, a limit established by European legislation (EC, 2005), however, LN groups had lower count than C. *Pseudomonas* and *Enterobacteria* count were

not affected by LN dietary incorporation. Our results are in agreement with previous study in which supplementation in animal diet did not affect microbiological quality of animal food products (Ranucci *et al.* 2015). Total viable count increased with storage time ($p < 0.001$), from 1.36 on day 0 in LN5, to reach the highest value of 3.40 log CFU/g on day 14, recorded in C group. For all groups, the count remained lower than the threshold of 5.69 log CFU/g (Regulation EC 2073/2005). In meat processing, contamination of the product depends on several factors such as the slaughter methods used, a bad carcass and meat handling, the equipment and facilities used or to the personnel involved in the operation (Cerveny *et al.*, 2009).

Table 1 : Effect of extruded linseed (LN) dietary incorporation on rabbit's meat microbiological quality during storage (Log CFU/g)

	Treatment	Storage day				p-time	p-LN
		0	4	7	14		
<i>E. Coli</i>	C	0	0	0	1.90	***	*
	LN2.5	0	0	0	1.97 ^a		
	LN5	0	0	0	1.31 ^b		
<i>Staphylococcus aureus</i>	C	1.96 ^b	2.21 ^a	3.21 ^a	3.20 ^{ab}	***	*
	LN2.5	1.63 ^b	2.08 ^b	2.92 ^b	3.13 ^a		
	LN5	1.52 ^a	1.86 ^c	2.62 ^c	3.10 ^{ab}		
<i>Pseudomonas</i>	C	0	1.81	2.24	2.34	***	ns
	LN2.5	0	1.68	2.30	2.37		
	LN5	0	1.19	2.23	2.44		
<i>Enterobacteria</i>	C	0	0	1.56	3.51	***	ns
	LN2.5	0	0	1.41	3.03		
	LN5	0	0	1.32	2.55		
TVC	C	2.05 ^a	2.00 ^a	2.33 ^a	3.40 ^a	***	*
	LN2.5	1.49 ^b	2.08 ^a	2.14 ^b	3.32 ^{ab}		
	LN5	1.36 ^b	1.89 ^b	2.17 ^b	3.17 ^b		

C: Standard diet; LN2.5: Standard diet +2.5%LN; LN5: Standard diet +5% LN , TVC: total viable counts ; E. Coli : *Escherichia coli* ; ns: no significant difference ($P > 0.05$) ; *: $P < 0.05$; ; **: $P < 0.01$; ***: $P < 0.001$; p-time : effect of storage day ; p-LN : effect of extruded linseed supplementation

Effect of extruded linseed (LN) incorporation on rabbit's meat fatty acids profile

The effect of LN on the fatty acid composition of rabbit meat is presented in Table 2. Total SFA content was significantly higher in C group compared to LN groups ($p < 0.05$). This results may be due to higher individual SFA content recorded in C group, especially, palmitic acid (C16:0) which was the major SFA found. The total MUFA and PUFA were not affected by dietary supplementation. In contrast, a study about the effect of linseed incorporation in rabbit diet on fatty acid proportions showed that MUFA and PUFA were similar for all diets (Mattioli *et al.*, 2020). Individual PUFA n-3 content was significantly ($p < 0.001$) affected by linseed supplementation, mostly linolenic acid C18:3n3 where the highest value was recorded in LN5 group. In fact, several studies have shown that the use of linseed as dietary supplementation can increase α -linolenic acid (C18:3 n-3) content in rabbit meat (Dal Bosco *et al.*, 2004; Peiretti, and Meineri, 2010). For linoleic acid C18:2n6, even though no significant difference was recorded, C18:2n6 concentration was lower in LN5 group. Therefore, these results may explain the decreased n-6/n3 ratio, particularly in LN5. Thus, linseed may be used as dietary supplementation in order to improve rabbit meat fatty acid profile by increasing PUFA n-3 levels.

CONCLUSIONS

The present study showed that extruded linseed supplementation improved rabbit's meat fatty acid profile by increasing PUFA n-3 content; hence, it inhibited TVC and *E.coli* growth during refrigerated storage leading to longer shelf life. In conclusion, LN supplementation, up to 5%, in rabbit diet could be an interesting alternative to improve meat nutritional and microbiological quality and increase its shelf life.

Table 2 : Effect of extruded linseed (LN) dietary incorporation on rabbit's meat fatty acids profile (% of total FAME)

Item	Treatment			P-values
	C	LN2.5	LN5	
ΣSFA	35.78 ^a	35.19 ^{ab}	34.05 ^b	*
ΣMUFA	22.98	22.13	23.73	ns
ΣPUFA	37.44	38.68	38.91	ns
C18:3n3 ALA	2.18 ^b	3.17 ^{ab}	6.05 ^a	***
C18:2n6 LA	28.01	28.75	26.97	Ns
Σn3 PUFA	3.25 ^c	4.36 ^b	7.31 ^a	***
Σn6/Σn3	10.41 ^a	8.09 ^b	4.36 ^c	***

C: Standard diet; LN2.5: Standard diet +2.5%LN; LN5: Standard diet +5% LN; ns: no significant difference (P >0.05); *: P<0.05; **: P<0.01; ***: P<0.001

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