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EFFECT OF CAROB GERM, AS AN ALTERNATIVE PROTEIN SOURCE IN LAMBS, ON MEAT OXIDATIVE STABILITY

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I. INTRODUCTION

Incorporating agro-industrial by-products into animal feeding is a promising strategy to enhance the sustainability of the livestock sector. Since feed production contributes to pollutant emissions in livestock, substituting conventional feed crops with agro-industrial by-products can help minimize the environmental footprint of animal feeding, aligning with a circular economy model [1]. In this context, carob seed germ seems to be a promising alternative feed resource that can be used for small ruminant feeding. In light of the above, this study aimed at investigating the effects of feeding lambs with experimental diets containing two levels of carob germ on meat oxidative stability.

II. MATERIALS AND METHODS

Thirty Valle del Belice × Pinzirita male lambs (60 days old, body weight 12.7 ± 2.09 kg) were assigned to 3 treatments and housed in individual pens. Lambs were fed ad libitum for 56 days with: a conventional diet containing 43% corn and 22% soybean meal (CON) or the same diet containing 25% (C25) or 50% (C50) carob germ as replacement of corn and soybean meal. In particular, for the C25 diet, corn and soybean meal were reduced by 33% and 50%, respectively, whereas in the C50 diet, 65% of the corn and 100% of the soybean meal were replaced. Fatty acid (FA) profile and hydrophilic antioxidant capacity analyses were performed on fresh meat and data were analyzed using one-way ANOVA. Color and lipid oxidative stability were evaluated over 7 days of refrigerated storage. Data were statistically analyzed using a mixed model: dietary treatments, storage time and their interactions were assumed as fixed factors, while individual animal was considered as random factor. The intramuscular fat was extracted from 200 mg of freeze-dried meat according to Folch et al. [2], trans-esterified and analyzed using a gas chromatograph. The hydrophilic antioxidant capacity was determined using five colorimetric assays: Trolox equivalent antioxidant capacity (TEAC) [3], ferric reducing antioxidant power (FRAP) [4], Folin-Ciocalteu [5], ferrous ion chelating activity (FICA) [6] and DPPH assay [7]. Briefly, the extraction was performed using 1 g of minced meat, homogenized for 1 min in water. The samples were centrifuged, filtered and collected in different aliquots for the antioxidant assays. Regarding the shelf-life study, color parameters were taken at days 0, 4 and 7 of refrigerated storage. The reflectance spectrum was recorded for the calculation of metmyoglobin, expressed as a percentage of the total forms of myoglobin [8]. In the same samples, lipid oxidation was assessed by measuring the production of hydroperoxides [9] and thiobarbituric acid reactive substances (TBARS) [10]. Hydroperoxides analysis was conducted by homogenizing 1 g of meat in 15 mL 2:1 chloroform:methanol. After filtering, 7 mL of sample was mixed with 2 mL of 0.5% NaCl and centrifuged. An aliquot of 1.5 mL of lower phase was collected and mixed with 12.5 µL of ammonium thiocyanate and 12.5 µL of iron chloride. After 20 min of incubation, the absorbance at 500 nm was recorded. TBARS analysis was performed with 2.5 g of meat, homogenized in 12.5 mL of water. Then, 12.5 mL of 10% trichloroacetic acid was added to the sample and filtered. Four mL of filtrate was mixed with 1 mL of 0.06 *M* thiobarbituric acid and incubated for 90 min in 80°C water. The absorbance at 532 nm was measured.

III. RESULTS AND DISCUSSION

The content of saturated, monounsaturated, and polyunsaturated FA was not modified by the treatments (P>0.05). Likewise, antioxidant capacity of meat hydrophilic fraction was not affected by treatments, as determined by 5 different assays (Table 1; $P>0.05$)

Regarding the shelf-life study (Table 2), the dietary treatment did not influence neither color and lipid stability of meat or metmyoglobin development over 7 days of refrigerated storage ($P>0.05$). Whereas color parameters and metmyoglobin formation were affected by storage time ($P<0.001$), showing a typical browning pattern of the meat. The hydroperoxide content did not differ among treatments or storage time ($P>0.05$). TBARS values were not affected by dietary treatment or by the interaction between dietary treatment and time of storage ($P>0.05$), but they increased after 7 days of storage ($P<0.001$).

Table 1 Effect of dietary treatment on antioxidant capacity of meat.

	Experimental diets (D)			SED	*P*-value
	C	C25	C50		
Antioxidant capacity (mg/g)					
FICA (EDTA eq)	0.756	0.723	0.740	0.058	0.835
FOLIN (TA eq)	0.624	0.567	0.596	0.033	0.203
FRAP (Fe^{2+} eq)	27.1	25.4	28.1	1.25	0.100
TEAC (Trolox eq)	45.3	48.1	45.8	5.14	0.832
DPPH (Trolox eq)	0.678	0.715	0.614	0.086	0.491

Table 2 Effect of dietary treatment on colour stability and lipid oxidation in lamb over 7 days of refrigerated storage.

	Experimental diets (D)			Time (T), d			SED	*P*-value		
	C	C25	C50	0	4	7		D	T	DxT
Colour stability										
a* (redness)	13.6	12.9	13.9	13,7^{a}	14.0^{a}	12.8^{b}	0.707	0.217	<0.001	0.824
b* (yellowness)	12.0	11.1	12.1	9,67^{c}	13.5^{a}	12.0^{b}	0.716	0.213	<0.001	0.215
h_{ab} (Hue angle;deg)	40.9	40.5	40.7	35,0^{b}	43.9^{a}	43.2^{a}	0.840	0.825	<0.001	0.210
Lipid oxidation, mg/kg meat										
Hydroperoxide	7.18	5.89	5.01	6.28	6.99	4.81	3.340	0.648	0.406	0.448
TBARS[1]	0.66	0.60	0.83	0.304^{c}	0.678^{b}	1.11^{a}	0.204	0.376	<0.001	0.592

[1] TBARS: thiobarbituric acid reactive substances (mg of malondialdehyde per kg of meat).

IV. CONCLUSION

The inclusion of both levels of carob germ as a substitute of corn and soybean meal in the lamb diet did not worsen the FA profile, the antioxidant capacity and the stability of color and lipid oxidation. In light of this, the carob germ could partially substitute corn and soybean meal in lambs' diet without any detrimental effects on FA profile, color stability and lipid oxidation of meat. Further analyses will be conducted to evaluate the content of fat-soluble vitamins with antioxidant power.

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