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EFFECT OF MATERNAL SUPPLEMENTATION WITH BETAINE DURING EWE GESTATION ON THE QUALITY OF LAMB MEAT

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I. INTRODUCTION

Maternal nutrition during pregnancy modulates DNA methylation, thus affecting gene expression and driving the phenotype of the offspring (e.g., health, feed efficiency or meat production) throughout the whole life. For example, maternal undernutrition reduces the number of myofibers in the offspring and their sensibility to insulin because fetal skeletal muscle matures during late-gestation; therefore, postnatal growth rate, tenderness, fatty acid metabolism and oxidative stability of the meat produced might be counteracted [1,2]. Moreover, during pregnancy there is a sharp increase in methyl-group donor requirements (e.g., folate, betaine, choline, methionine), the precursors of S-Adenosylmethionine (SAM), the universal methyl-donor for DNA and protein methyl-transferases that is central in the methylation of all biological molecules, including DNA. A reduced availability of methyl donors during pregnancy is hypothesized to result in low SAM synthesis and global DNA hypomethylation. Therefore, the present study was designed to test the hypothesis that betaine supplementation during pregnancy of Merino ewes (F0) induces a nutritional programming effect on their offspring (F1), thus increasing meat quality traits of the F1 male fattening lambs.

II. MATERIALS AND METHODS

Experimental conditions and the handling of the animals followed the recommendations of the European Parliament (2010; Directive 2010/63/EU) on the protection of animals used for experimental and other scientific purposes, as approved by the CSIC Animal Experimentation Committee and the competent authority (protocol number 100102/2021-6). The commercial slaughtering or killing process was carried out according to the European Council (2009), Council Regulation (EC) No 1099/2009 on the protection of animals at the time of killing.

Twenty-eight pregnant Merino ewes (F0) were assigned to either a BETAINE group, being fed *ad libitum* a complete pelleted diet (CPD) formulated with betaine during the last two months of gestation (4 g of betaine/kg of diet), or a CONTROL group, being fed the same CPD with no betaine added. Both CPDs were isoenergetic and isoproteic, avoiding the inclusion of ingredients rich in betaine. After lambing, eighteen male lambs (F1-CTRL and F1-BET) were raised exactly in the same way under natural rearing conditions until weaning, and then fed a CPD (the same for both groups, no betaine added) during the fattening period. Dietary treatment of F0 dams was the only source of variation for F1 male fattening lambs. The lambs were slaughtered at 30 kg live weight.

At 24 h postmortem, longissimus thoracis (LT) et lumborum (LL) muscle were dissected from each carcass. A portion of LT was weighed, freeze-dried and used for proximate composition [3]. In addition, three 6-cm portions from LL muscles were cut transversely, one was analyzed immediately for cooking losses and hardness, and the other two were vacuum packed and aged at 4°C for 4 to 7 days prior to analysis. For analysis, muscle portions were cooked into vacuum bags in a water bath at 70 ºC for 40 min and weigh differences before and after cooking were registered; then, hardness was determined on 1 cm x 1 cm x 3 cm prisms with a Warner-Braztler probe attached to a TA-XT2i analyser following Honikel [4]. Analysis of variance was carried out to assess the effect of dietary treatment or ageing time on the traits studied. For cooking loss and shear force, this analysis was followed by a Tukey post hoc test to obtain pairwise significance between ageing times.

III. RESULTS AND DISCUSSION

Maternal supplementation with betaine did not significantly change the composition of LT muscle (Table 1).

Table 1. Proximal composition (g/100 g raw meat) of *longissimus thoracis* muscle for control (CTRL) and betaine (BET) dietary treatments

	CTRL	BET	SEM	*P*-value
Moisture	76.32	75.30	0.679	0.304
Protein	20.63	21.56	0.516	0.223

Fat	1.76	1.84	0.161	0.746
Ash	1.11	1.62	0.026	0.089

SEM: standard error of the mean

However, cooking loss was lower while shear force was higher in meat from lambs whose dams were supplemented with betaine (Table 2; $P = 0.049$). As for the effect of ageing, as expected, the cooking loss increased with time and the shear strength decreased for both dietary treatments, which is explained by the postmortem proteolytic degradation of muscle proteins [5]. The interaction treatment x ageing time was non-significant suggesting a similar rate of proteolysis.
Overall, at a specific ageing time the shear force should tend to be higher in LL meat that loses more water during cooking, but in this study the opposite was found to be the case. The differences in cooking losses and hardness between meat groups would be due to differential DNA methylation in lambs responsible for changes in muscle structure aspects [6], such as fiber type distribution or collagen amount and characteristics. However, the difference in hardness (3 N) may not be very noticeable for consumers since 10 N is considered to be the sensory detection threshold in the degree of toughness of the meat [7].

Table 2. Changes in cooking losses (CL, %) and shear force (SF, N) of l*ongissimus lumborum* muscle for control (CTRL) and betaine (BET) treatments during ageing

	Dietary treatments (Trt)			Ageing time (day)				*P*-value		
	CTRL	BET	SEM1	1	4	7	SEM2	Trt	Day	Trt x Day
CL	15.74	14.65	0.373	12.95 ^{c}	14.74 ^{b}	17.77 ^{a}	0.463	0.049	<0.001	0.251
SF	50.93	53.97	1.060	58.40^{a}	50.46^{b}	48.50^{b}	1.298	0.049	<0.001	0.867

SEM1: standard error of the mean to compare dietary treatments; SEM2 to compare ageing times

^{a,b,c}: superscripts mean significant differences between treatments among means, Tukey test ($P < 0.05$).

IV. CONCLUSION

Maternal betaine supplementation during gestation may affect the meat quality of the offspring lambs in terms of texture. To clarify the mechanism of action, further studies on the effect on the histological characteristics of muscle and the differential expression of genes responsible for meat texture are needed.

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