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FOCUSED HIGH-PROTEIN SUPPLEMENTATION TO EWES GRAZING CAMPOS GRASSLAND DURING MID GESTATION: EFFECTS ON MUSCLE GENE EXPRESSION OF THE PROGENY

Ana Laura Astessiano[1], Ana Inés Trujillo[1], Ana Clara Panissa[1], Alejandro Bielli[2], Patricia Genovese[2], Emiliano Herrera[2], Martin Duque[2], Raquel Pérez Clariget[1], Javier Ithurralde*[1]

[1] Agronomy Faculty, Universidad de la República, Montevideo, Uruguay.

[2] Veterinary Faculty, Universidad de la República, Montevideo, Uruguay.

*Corresponding author email: javiithu@gmail.com

I. INTRODUCTION

The concept of fetal programming suggests that certain events occurring during critical periods of pregnancy may cause permanent effects on the offspring [1]. In sheep, prenatal muscle development and meat characteristics can be affected by fetal programming particularly during mid gestation, since skeletal muscle hyperplasia occurs approximately between days 40 and 80 of gestation [2]. We have previously demonstrated that maternal undernutrition in ewes grazing on natural pasture negatively affects prenatal myogenesis [3], altering muscle gene expression in newborn lambs [4], and negatively affecting growth performance, carcass quality, and meat characteristics in heavy lambs [5, 6]. Nevertheless, as far as we know, no previous research has evaluated the effect of focused high protein supplementation offered to *ad libitum* grazing pregnant ewes during the key period for skeletal muscle hyperplasia on muscle mass and gene expression of their progeny. Thus, the aim of the present study was to evaluate the effect of a focused high-protein supplementation to ewes grazing *Campos* grassland during mid gestation on muscle weight and specific gene expression related to muscle growth, structure and function (*MyHC-I*, *PAX7*, *IGF1* and *PPARG*) of their lambs.

II. MATERIALS AND METHODS

All procedures were approved by the Comisión de Ética en el Uso de Animales (CEUA) of the Universidad de la República (UdelaR), Uruguay, (Res. Nº1804). The experiment was performed at Estación Experimental Bernardo Rosengurtt (Facultad de Agronomía, UdelaR, Uruguay, 32º S, 54º W) using 37 single-bearing multiparous Corriedale ewes grazing Campos grassland *ad libitum*. Ewes were randomly assigned in two nutritional treatments between 55 and 80 days of gestation: supplemented ewes (SUP; n= 22, 12 carrying female and ten carrying male fetuses) received daily individidual supplementation with soybean meal at 1 % of their body weight (BW), and control ewes (CON; n= 15, nine carrying female and six carrying male fetuses) grazed adjacent sub paddocks without supplementation. After 80 days of pregnancy all ewes were managed as the same flock and continued grazing on the same paddock of Campos grassland until 100 days of pregnancy when they were sheared and transferred to a multi-year pasture until lambing. After lambing all ewes and lambs continued being managed as one flock grazing on a seeded annual pasture of ryegrass and winter oat until lamb slaughtering (90-day-old lambs). Immediately after slaughter, samples were taken from the left side *Longissimus lumborum* (LL) muscle and were frozen in liquid nitrogen. Left side LL muscle was dissected free from visible fat and connective tissue and weighed. Total RNA from muscle samples was isolated using TRIzol® (Invitrogen, Life Technologies Carlsbad, CA, USA). Transcription was conducted with Super-Scripts III Transcriptase (Invitrogen) using random hexamers and 1 µg of total RNA as a template and the cDNA was stored at -20ºC. Primers specifically designed to amplify cDNA for the target genes of interest (*IGF1, PPARG, PAX7, MyHC-I*) and two endogenous control genes (ß- actin; *ACTB* and hypoxanthine phosphoribosyltransferase; *HPRT*) were used for real time RT-PCR. Real time PCR reactions were performed in a total volume of 15 µl using Maxima SYBR Green/ROX qPCR Master Mix 2X (ThermoFisher Scientific), using standard amplification conditions in a 48-well StepOne™ Real-Time PCR System (Applied Biosystems™ from ThermoFischer Scientific). Data were analyzed considering a completely randomized design using the SAS Systems software (SAS 9.0V; SAS Institute, Cary, NC, USA). The models included the treatment (SUP vs. CON), the progeny sex, and their interaction as fixed effects and the rfather as the random effect. Post-hoc comparisons were done with the Tukey–Kramer test. Statistical differences were considered significant when $P \leq 0.05$, and tendencies when $0.05 < P \leq 0.10$. Data are expressed as LS means ± s.e.m.

III. RESULTS AND DISCUSSION

Maternal supplementation did not affect lamb muscle weight. However, maternal supplementation tended to reduce *mRNA* expression *of MyHC-I* and *PAX7* (Table 1). Likewise, mRNA of *MyHC-I* tended to be affected by the interaction between maternal treatment and progeny sex. High-protein supplementation tended to reduce mRNA expression of *MyHC-I* in male (P = 0.07) but not in female lambs (Table 1). The lower mRNA expression of *MyHC-I* in muscles from lambs suggests that the SUP treatment in mothers affected muscle fiber type composition in lambs. This is consistent with previous findings in our research team which showed that lambs born to underfed ewes showed greater expression of mRNA *MyHC-I* at birth [4] and greater proportions of slow-oxidative fibers at slaughter [5].

The trend for a lower muscle gene expression of *PAX7* in lambs born of SUP than CON ewes is consistent with previous research [3, 4, 7] and may suggest an attempt to compensate for the negative effects induced by a poorer maternal nutrition through a higher number of satellite cells. Furthermore, our current results suggest that the effect would be more intense in the male than in the female progeny.

Table 1 –Muscle weight and muscle gene expression (*Longissimus lumborum*) of lambs born to CON and SUP ewes (fold change in gene expression relative to the expression of the housekeeping genes).

	CON		SUP		P *values*		
	Males	Females	Males	Females	Treatment	Sex	Treatment*Sex
Muscle weight (g)	181.4 ± 8.9	168.8 ± 8.3	186.7 ± 7.4	183 ± 6.9	0.17	0.29	0.54
Muscle mRNA							
MyHC-I	2.3 ± 0.6	1.6 ± 0.6	1.3 ± 0.4	1.6 ± 0.4	0.07	0.79	0.08
PAX7	1.7 ± 0.8	2.2 ± 0.9	0.9 ± 0.6	1.9 ± 0.6	0.09	0.32	0.79
IGF1	3.8 ± 1.3	3.8 ± 1.4	2.9 ± 1.0	4.4 ± 0.9	0.31	0.28	0.87
PPARG	1.1 ± 0.6	1.5 ± 0.7	0.7 ± 0.7	1.2 ± 0.6	0.81	0.29	0.83

IV. CONCLUSION

High-protein supplementation offered to ewes grazing *Campos* grassland during the second third of gestation did not affect muscle weight of the progeny. However, our current results suggest that maternal high protein supplementation during mid gestation can affect the contractile profile of skeletal muscles. This is potentially an important finding since changes in the skeletal muscle fiber profile could be linked to changes in key meat quality traits. Differences in meat color or tenderness can be related to changes in fiber type's profiles, which are known to impact these traits through changes in muscle pH decline rate, sarcomere shortening or protolithic enzyme activities.

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