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# Maternal nutrition and antioxidant supplementation: Effects on mother–young behaviors in a Patagonian sheep extensive grazing system

Francisco Sales[a], Victor H. Parraguez[b,c], Aline Freitas-de-Melo[d], Rodolfo Ungerfeld[d,*]

[a] INIA Kampenaike, Punta Arenas 6212707, Chile
[b] Facultad De Ciencias Veterinarias y Pecuarias, Universidad De Chile, Santa Rosa 11735, Santiago 8820808, Chile
[c] Facultad De Ciencias Agronómicas, Universidad De Chile, Santa Rosa 11315, Santiago 8820808, Chile
[d] Departamento de Biociencias Veterinarias, Facultad de Veterinaria, Universidad de la República, Lasplaces 1620, Montevideo 11600, Uruguay

A R T I C L E I N F O

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A B S T R A C T

Undernourishment is a frequent condition in sheep pregnancies under extensive systems, since most of the pregnancies occur when the prairie has the lowest forage allowance. Underfed sheep pregnancies are associated with maternal-fetal oxidative stress, low birth weight and deteriorated ewe–lamb bond, which negatively affects the probability of postnatal survival. The aims of the present study were to determine if daily supplementation of underfed single-bearing grazing ewes during pregnancy (starting at Day 44) with concentrate food and/or vitamins C and E: 1) improves maternal body weight and body condition, and increases udder size and skin temperature of the udder at parturition, 2) increases lamb' body weight and both surface and rectal temperatures, and 3) modifies ewe–lamb behavior at lambing. A complementary goal was to determine if those effects differ between male and female lambs. The experiment was carried out under field conditions representative of Chilean Patagonia. Maternal supplementation with concentrate resulted in enhanced ewes' body weight (59.4 ± 0.9 kg vs 55.1 ± 0.9 kg; $P$ = 0.0007) and body condition during gestation (2.21 ± 0.05 vs 1.69 ± 0.05; $P$ < 0.0001), enhanced udder volume ($P$ < 0.0001), increased newborn lambs' body weight (5.0 ± 0.1 kg vs 4.7 ± 0.1 kg P = 0.03), and strengthened maternal bonding with its lamb during a handling test, without effects on lamb' rectal temperature. Supplementation with vitamins C and E, did not produce a clear effect on the studied variables, and did not improve the response to supplementation with concentrate. In conclusion, improvement of nutritional plane in underfed single-bearing ewes it is not only relevant to improve the pregnancy outcomes, but also to ensure a better ewe–lamb bonding, which may improve lamb's survival and performance during lactation.

## 1. Introduction

Sheep nutrition in extensive production systems of southern Chilean Patagonia depend mainly on the natural pasture availability and quality, which varies widely throughout the year. Similar to other latitudes, the availability and quality of the pasture decreases markedly during winter (Covacevich and Ruz, 1996), concomitant with most of the gestational period of the ewes. This environmental constraint results in an inadequate level of nutrition during most pregnancy, with even stronger effects during the last third, when the greatest fetal and maternal mammary gland growth occurs (Kenyon and Webby, 2007). Thus, pregnant ewes mobilize tissue and reserves to support fetal growth, losing body weight (BW). Despite the above, under Patagonian normal conditions, this is still insufficient to cover fetal demands, leading to a restricted fetal growth (Sales et al., 2018) and thus, reduced BW of newborn lambs (Roca Fraga et al., 2018). In this sense, in extensive productive systems it was reported that undernutrition during pregnancy results in lower maternal udder volume (Freitas-de-Melo et al., 2018) and lower colostrum production (Mellor and Murray, 1985). In addition to deteriorating the newborn lamb immune response (Nowak and Poindron, 2006), it may also impair its thermoregulatory abilities as it reduces body reserves (Mellor and Murray, 1985) and the amount of brown adipose tissue (Budge et al., 2000). Taken together, all these aspects reduce the probability of lamb survival, affecting animal welfare and the efficiency of the productive system.

Undernutrition during pregnancy also alters both maternal and newborn lamb behavior. Dwyer et al. (2003) showed that nutrient restricted ewes spend less time licking their lambs and are more reactive when their lambs are handled by humans. In this sense, lambs born from nutritionally restricted ewes are more demanding from their

* Corresponding author.
*E-mail address:* rungerfeld@gmail.com (R. Ungerfeld).

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mothers than those born from ewes that had access to more resources (Corner et al., 2010). Interestingly, the negative consequences of undernutrition of ewes during pregnancy on lamb behavior is greater in male than female lambs (Freitas-de-Melo et al., 2015). This difference may be related to the greater lamb birth BW of males, and consequently greater energetic investment from the mother.

A common practice to overcome the negative impacts of maternal nutrient restriction is to supplement the ewes during the last stages of pregnancy. Providing adequate supplementation to pregnant ewes increases their BW and that of lambs at birth, colostrum production and lamb rate survival (Banchero et al., 2002; Mukasa-Mugerwa et al., 1994; Pedernera et al., 2018). Moreover, supplementation during the last stage of gestation with concentrate influences maternal behavior, advancing the lamb' first suckling (Pedernera et al., 2018). However, the negative effects of maternal undernutrition on fetal development are not only consequence of the decreased transfer of nutrients to the fetus, but also of a state of fetal hypoxemia and oxidative stress (Sales et al., 2018). The oxidative stress status may be prevented by maternal administration of vitamins C and E during pregnancy, partially reducing the restricted intrauterine growth and improving fetal and newborn BW (Parraguez et al., 2011; Sales et al., 2019). In this sense, administration of vitamin E during late gestation increases newborn lambs' BW, vigor score and rectal temperature at birth, as well as survival rates (Ali et al., 2004; Dafoe et al., 2008; Kott et al., 1998). However, the effect of maternal supplementation during gestation with the aforementioned antioxidant vitamins on mammary gland growth and metabolic status (reflected in the skin surface temperature), as well as the effect on lambs' thermoregulation capacity and ewe–lamb postpartum behavior have not yet been studied.

Considering all this information, we hypothesized that supplementation of underfed single-bearing grazing ewes during pregnancy with concentrate food and/or with vitamins C and E: 1) increases maternal BW and body condition score (BCS), udder size and skin temperature of the udder at parturition, 2) lamb' BW and both, surface and rectal temperatures, and 3) favors the establishment of the ewe–lamb bond at lambing. We predicted that supplementation of undernourished grazing ewes during pregnancy with concentrate food and/or antioxidant would increase maternal BW and BCS, udder size and skin temperature of the udder at parturition, lamb' BW and both, surface and rectal temperatures, and favors the ewe–lamb bonding behaviors at lambing. Thus, the goal of the present study was to establish the effects of maternal supplementation with concentrate and/or vitamins C and E in underfed grazing pregnant ewes, on ewes' BW, BCS and udder characteristics, lambs' BW and temperatures and the ewe–lamb behavior at lambing. A complementary goal was to determine if those effects differ between male and female lambs.

## 2. Materials and methods

The experimental procedure was approved by the Bioethics Committee of the INIA and the Bioethics Review Committee of the Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile (Protocol # 11-2016), as well as by the Bioethics Advisory Committee of the Chilean National Commission for Scientific and Technological Research (CONICYT, Chile), as funder of the study. The study followed the recommendations from the Handbook of Laboratory Animal Management and Welfare (Wolfensohn and Lloyd, 2008), including a 15 cutting point evaluation protocol considered as criteria for intervention and final point, including appearance, feeding behavior, weight and BCS, behavior and clinical signs. As all animals were daily observed, all those parameters were daily controlled by trained workers. No interventions were necessary during any stage of the protocol.

### 2.1. Animals management and treatments

The study was carried out at the INIA research farm, 65 km north from Punta Arenas, in the Magellan Region, Chilean Patagonia (52° 36′ S; 70° 56′ W). It involved 99 multiparous Corriedale ewes, 4–6 years old. Estrous cycles were synchronized inserting to all ewes an intravaginal controlled internal drug release device (CIDR G, 0.3 g of progesterone, Pfizer, Santiago, Chile), which remained in situ for 12 days. After CIDR withdrawal, ewes were mated using 10 proved fertile rams, with colored-paint chest. The exact day in which ewes came into estrus (Day 0) was identified by daily visual inspection of the colored rumps of the ewes mated every day.

Pregnancy was determined by transrectal ultrasound exam, 40 days after mating, and only ewes bearing a single fetus (70% of the synchronized ewes) were considered for the study. Ewes were allocated to 4 experimental treatments in a 2 × 2 factorial arrangement, with access to concentrate or to vitamins as the main effects. The treatments, which began on Day 44, were: only grazing natural pastures (group OP; $n$ = 22); grazing natural pastures and supplemented with concentrate (group PC; $n$ = 21); grazing natural pastures and supplemented with vitamins C and E (group PV; $n$ = 25); and grazing natural pastures and supplemented with concentrate and vitamins C and E (group PCV; $n$ = 31). The final sample size in each group was not balanced as some animals spontaneously changed their group, and this was not noticed during the first five days of treatment, forcing to leave the groups unbalanced in size. In addition, one female was death for unknown cause in the PC group.

During the entire gestation, the animals were maintained on natural pasture [*Festuca gracillima–Chiliotrichium diffusum*; crude protein (CP): 3.3%, metabolizable energy (ME): 1.9 Mcal/kg, total digestible nutrients (TDN): 45%], with a stocking rate of 0.9 ewes per hectare, where ewes experience a nutritional restriction of approximately 30% of their requirements (Lira, 2012). Pelleted concentrate was offered daily in yards, where animals were separated by treatment groups and feeding was offered in common feeders with enough space to allow all the ewes to access simultaneously. All the amount of offered concentrate was eaten every day. Ewes from PC group received incremental amounts (400–600 g) of concentrate per ewe (CP: 22.0%; ME: 3.0 Mcal/kg), aiming to ensure a total dam BW increase in pregnancy at a level similar to that of the expected conceptus mass (Van der Linden et al., 2011). Each ewe from the PV group received daily 650 mg of vitamin C (ascorbic acid) and 450 IU of vitamin E (alpha-tocopherol) added to 50 g of a concentrate of low nutritional quality (CP: 17.0%; ME: 1.6 Mcal/kg). Each ewe from the group supplemented with concentrate and vitamins (PCV) received daily 50 g of concentrate with vitamins C and E added to the concentrate amount consumed by ewes from the PC group. These doses of vitamins increase their concentration in fetal cord blood (Sales et al., 2019). Finally, the group OP received 50 g daily of low nutritional quality concentrate, to mimic the same nutrient intake of the PV group.

### 2.2. Body weight and body condition score

Ewes were weighted on an electronic scale and their BCS (1–5 scale; (Russel et al., 1969) was evaluated on Days −24, 55, 84, 106 and 145.

### 2.3. Skin temperature and volume of the udder

On Day 145, the volume and surface skin temperature of the udders of 51 ewes randomly selected from each group (OP = 13, PC = 14, PV = 12 and PCV = 12) were evaluated. The mean air temperature and humidity at the time of measurements was 11.9 °C and 56.5%, respectively. The surface skin temperature was determined by infrared thermography. For this, ewes were positioned in a ventral position to take a thermographic picture of the posterior udder area, using a FLIR C2 infrared camera (FLIR Systems, Wilsonville, Oregon, USA). All pictures were taken without touching the udders and from the same distance (0.2 m). Three regions of interest with no wool were selected in the infrared images: dorsally, ventrally and medially to the teats. The regions of interest were chosen using the Flir Tools free software. The emissivity was set at 0.98 as previously described for lambs (Labeur et al., 2017), and the camera was calibrated to ambient temperature and humidity. The min, max and average temperatures of each udder region were considered for statistical analysis.

After taking the pictures, the circumference (circumference of the udder near the belly), and depth (from udder base to nipple base) were determined using a measuring tape. Each semi-axis, and then the udder volume ($V$), were calculated assuming that the udder was a semi-sphere: $V = (2/3)(\pi.R1.R2)$, where each $R$ corresponds to each semi-axis (horizontal and depth).

### 2.4. Recordings at birth

Behavioral recordings at birth were made by 5 experienced and previously trained observers, between 08:00 h and 10:00 h, in 10-ha paddocks where ewes grazed natural pasture. Each behavior was recorded by the same observer in all the tests. The observers detected ewes that lambed during the night (all recorded lambs were dry, and thus not covered with amniotic fluid). Thereafter, the ewes and their lambs were moved together to a pen (16m × 20m), where the sex of the newborn lamb and its' BW (digital scale) were recorded. Moreover, newborn rectal temperature was measured with a digital thermometer, and the surface interscapular and dorsal neck temperatures were measured with a laser thermometer (IR-102 Infrared Thermometer, Super Elec. Equip. Co, Guangdong, China).

Thereafter, the ewe and the lamb remained together without any interference during at least 2 h before doing a lamb handling test, modified from the Maternal Behavior Score (MBS) (O'Connor et al., 1985). The lamb was taken up and restrained by a person that remained immobile to minimize the interference on the ewe' or lamb' behavioral response. The lamb was restrained during 3 min, and the distance at which the ewe was 60 s after beginning the test was recorded with a laser distance measurer (GLM250VF, Bosch, Solothurn, Switzerland). The exact distance was recorded if the ewe stayed closer less than 10 m from her lamb, or considered as > 10 m when it was far from this distance according to the precision of the equipment. Two observers recorded the total number of bleats of the ewe and the lamb during those 3 min, staying at a distance that avoided any interference in the test. Thereafter, the lamb was released, and the time required to join its mother (less than approximately 0.5 m) and from this reunion until suckling (during at least 3 s) was recorded during 3 min (Corner et al., 2010; Everett-Hincks et al., 2005). If the lamb did not suckle until this period, the lapse was assumed to be 3 min. Again, the number of bleats from the ewe and the lamb were recorded during these two periods, and the numbers of vocalizations during these two periods were calculated for both the ewe and the lamb.

### 2.5. Statistical analysis

Normal distribution of the data was analyzed with the Shapiro–Wilk test, and in the case that data were not normally distributed (lapse from ewe–lamb reunion until the lamb suckle after handling the lamb), data were root-squared transformed. Udder volume and udder temperatures were compared with proc mixed using SAS University Edition (SAS Institute Inc., http://www.sas.com), including the treatments (concentrate and vitamin) and their interaction as main factors of the model. Ewes' BW and BCS, were compared with the same mixed model, but including the time and the corresponding interactions between ration and vitamins, ration and time, vitamins and time, and ration, vitamins and time, as fixed effects. Lamb birth weight, rectal and surface temperatures, and all the behavioral variables were compared with the same mixed models, including the treatments (concentrate and vitamin), the sex of the lamb, and the interactions as fixed effects, and the day of birth as a random effect.

Due to the extensive management and practical limitations, not all data were collected in all the animals in all the recordings. Data are presented as LS means ± SEM. Significant effects were considered when $P \leq 0.05$ and a tendency when $0.05 < P \leq 0.1$.

## 3. Results

Overall, there were 9 female and 13 male lambs in group OP, 10 female and 11 male lambs in group PC, 13 female and 11 males and one lamb whose sex was not recorded in group PV; and 15 female and 16 male lambs in group PCV.

### 3.1. Ewe's body weight and body condition score

Ewes supplemented with concentrate presented greater BW and BCS than non-supplemented ewes (BW: df = 95; $F$ = 12.21; 59.4 ± 0.9 kg vs 55.1 ± 0.9 respectively; $P$ = 0.0007; BCS: df = 95; F = 60.7; 2.21 ± 0.05 vs 1.69 ± 0.05, respectively; $P < 0.0001$). No effect of vitamin supplementation or interaction between both treatments were observed. In both variables, BW and BCS, there was also a significant effect of time (BW: df = 351; $F$ = 66.4; $P < 0.0001$; BCS: df = 352; $F$ = 23.4; $P < 0.0001$), and an interaction between supplementation with concentrate and time (BW: df = 351; $F$ = 20.1; $P < 0.0001$; BSC: df = 352; $F$ = 18.4; $P < 0.0001$). There was also a triple interaction (df = 351; $F$ = 2.47; $P$ = 0.045) in BW. Ewes supplemented with concentrate were heavier and had a greater BCS than those not supplemented on Days 84 (BW: df = 351; $F$=-3.39; $P < 0.0008$; BCS: df = 352; $F$ = −6.27; $P < 0.0001$), 106 (BW: df = 351; $F$ = −5.57; $P < 0.0001$; BCS: df = 352; $F$ = −8.13; $P < 0.0001$) and 145 (BW: df = 351; $F$ = −6.49; $P < 0.0001$; BCS: df = 352; $F$ = −11.5; $P < 0.0001$). The BW and BCS along the study in ewes receiving concentrate or not is presented in Fig. 1A and 1B respectively.

### 3.2. Skin temperature and volume of the udder

There were no significant effects on the dorsal or medial udder temperatures. In ventral udder, the minimum temperature showed a significant interaction between supplementation with concentrate and

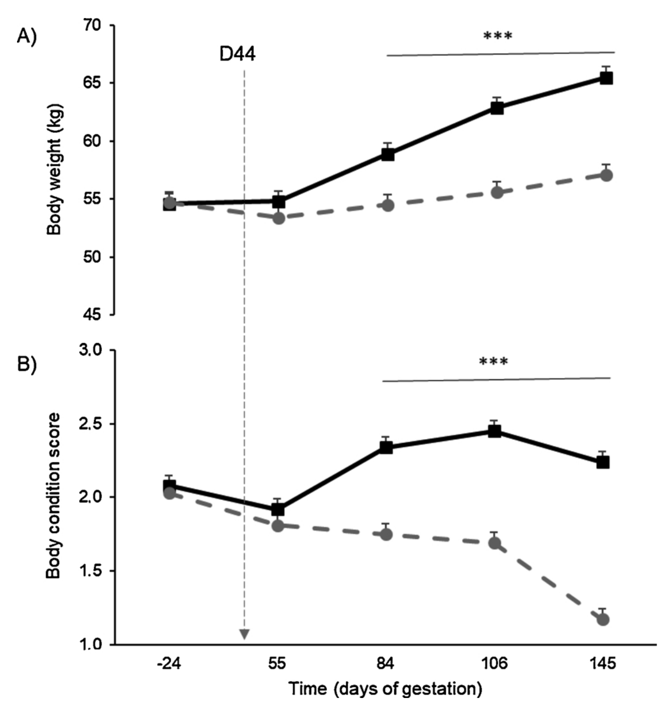


**Fig. 1.** (A) Body weight and (B) Body condition score in ewes that were supplemented (–■–) or not (--●--) with a commercial supplement beginning on Day 44 of the gestation.

supplementation with vitamins (df = 22; $F$ = 5.95; $P$ = 0.023). In this sense, while the minimum temperature was greater if the ewe received concentrate (31.0 ± 0.8 °C vs 27.8 ± 1.2 °C; ewes with or without concentrate supplementation, respectively; df = 22; $F$ = 2.31; $P$ = 0.03), the inclusion of vitamins only tended to increase the minimum temperature (31.2 ± 1.3 °C vs 29.4 ± 0.7 °C; ewes with or without vitamins supplementation, respectively; df = 22; $F$ = −1.94; $P$ = 0.066). The maximum temperature in ventral udder was not affected by either treatment. Interactions between treatments receiving supplementation with concentrate and with vitamins tended to be significant for the ventral and medial (df = 22; $F$ = 3.47; $P$ = 0.076), and maximum temperatures (df = 22; $F$ = 2.94; $P$ = 0.1).

Udder volume was greater in ewes supplemented with concentrate than in those not supplemented (root square (vol) = 24.4 ± 0.9 vs 17.9 ± 1.0, respectively; df = 47; $F$ = 23.8; $P$ < 0.0001), with no effect of supplementation with vitamins or interaction between supplementation with concentrate and vitamins.

### 3.3. Newborn lamb body weight and rectal and surface temperatures

The $P$-values of the main effects studied on lamb BW, rectal and surface temperatures are presented in Table 1.

Lambs from ewes supplemented with concentrate were heavier than those from non-supplemented ewes (5.0 ± 0.1 kg vs 4.7 ± 0.1 kg respectively; df = 80; $F$ = 4.8; $P$ = 0.03). Male lambs were heavier than female lambs (5.0 ± 0.1 kg vs 4.7 ± 0.1 kg respectively; df = 80; $F$ = 5.91; $P$ = 0.017). There were no other significant effects on lamb BW.

Lamb's rectal temperature was not affected by the treatments, their sex, or the interactions, observing only a tendency for an interaction between supplementation with concentrate and lamb sex (df = 79; $F$: 2.72; $P$ = 0.1) (LSmeans of lambs born from ewes that received or not concentrate and received or not vitamins were 39.3 ± 0.1 °C, 39.4 ± 0.1 °C, 39.4 ± 0.1 °C and 39.3 ± 0.1 °C, respectively). The surface temperatures were greater in male than female lambs (interscapular: 24.5 ± 1.0 °C vs 23.2 ± 1.0 °C respectively; df = 72; $F$ = 4.60; $P$ = 0.04; dorsal neck: 23.7 ± 1.1 °C vs 21.9 ± 1.1 °C respectively; df = 72; $F$ = 4.73; $P$ = 0.03).

### 3.4. Ewe–lamb behaviors

The $P$-values of the main effects on ewe–lamb behaviors are also presented in Table 1. One minute after the lamb handling started, ewes that were supplemented with concentrate were closer to the handler than non-supplemented ewes (406.1 ± 82.1 cm vs 541.7 ± 83.1 cm; df = 80; $F$ = 4.84; $P$ = 0.03). There was also a significant interaction between maternal concentrate supplementation and lamb sex (df = 80; $F$ = 4.13; $P$ = 0.046): while there was no difference in the distance from the handler to the ewes that lambed males (534.6 ± 94.0 cm vs 521.2 ± 90.9 cm, respectively), supplemented ewes that lambed females were closer to the handler than non-supplemented mothers (291.1 ± 92.7 cm vs 548.7 ± 93.3 cm; df = 80; $F$ = 2.95; $P$ = 0.004). When the lamb was released, ewes that lambed females tended to be closer to the handler than those that lambed males (420.0 ± 82.2 cm vs 527.9 ± 82.1 cm; df = 80; $F$ = 3.31; $P$ = 0.07).

During the 3 min lamb handling, ewes that were supplemented with concentrate vocalized more than non-supplemented ewes (28.2 ± 2.3 bleats vs 19.7 ± 2.2 bleats; df = 80; $F$ = 14.71; $P$ = 0.0002). There was also a significant interaction between supplementation with vitamins and sex of the lamb (df = 80; $F$ = 4.21; $P$ = 0.04), so that ewes that lambed a female and were supplemented with vitamins vocalized less than ewes with female lambs and receiving no vitamins, while there was no difference when the lamb was a male, regardless of vitamin treatment [ewes that lambed female lambs with and without vitamins: 20.2 ± 2.5 bleats vs 27.7 ± 2.9 bleats (df = 80; $F$ = 2.39; $P$ = 0.019); ewes that lambed male lambs with and without vitamins: 24.7 ± 2.7 bleats vs 23.3 ± 2.7 bleats]. On the other hand, there were no significant differences in the number of bleats emitted by the lambs according to their mothers' treatments, their sex, or the different interactions, except a tendency for a triple interaction (df = 80; $F$ = 3.89; $P$ = 0.052).

When analyzing the time spent between lambs were released and they joined their mother, a significant interaction between maternal concentrate supplementation and sex of the lamb was observed (df = 80; $F$ = 4.61; $P$ = 0.035). Thus, while there were no differences in female lambs according to the supplementation of the mother, time was shorter in male lambs whose mothers were supplemented [root square (x): females with and without supplementation with ration: 2.97 ± 0.40 min vs 2.75 ± 0.39 min; males with and without supplementation with ration: 3.82 ± 0.38 min vs 2.40 ± 0.37 min; df = 80; $F$ = 2.69; $P$ = 0.009). For this variable, there was also a tendency for interaction between receiving supplement and vitamins (df = 80; $F$ = 3.10; $P$ = 0.08).

The time between lambs joining the mother and suckling only tended to be shorter in male than female lambs [root square (x): 8.1 ± 0.8 vs 9.7 ± 0.8; df = 80; $F$ = 2.88; $P$ = 0.09], and there was a tendency for a triple interaction between supplementation with ration, with vitamins and sex of the lamb (df = 80; $F$ = 3.92; $P$ = 0.051).

The frequency of ewes' bleats since their lambs were released and they joined them again, and between release and lamb suckling were not affected by any of the studied factors. In the lambs, the frequency of bleats until joining their mother tended to be greater in males than in females (0.43 ± 0.06 bleats/s vs 0.27 ± 0.06 bleats/s; df = 80; $F$ =

**Table 1**
Mean effects in the different recordings performed after birth in ewes and lambs. Ewes were supplemented or not with commercial supplement (Sup) or with vitamins C and E (Vit) beginning on Day 44 of the gestation. Sex = sex of the lamb.

| | Sup | Vit | Sex | Sup*Vit | Sup*Sex | Sup*Sex | Sup*Vit*Sex |
|---|---|---|---|---|---|---|---|
| Lamb | | | | | | | |
| Body weight | 0.03 | ns | 0.017 | ns | ns | ns | ns |
| Rectal temperature | ns | ns | ns | ns | ns | ns | ns |
| Interscapular temperature | ns | ns | 0.04 | ns | ns | ns | ns |
| Dorsal neck temperature | ns | ns | 0.03 | ns | ns | ns | ns |
| Behaviors | | | | | | | |
| Ewe-lamb distance (60 s) | 0.03 | ns | 0.07 | ns | 0.046 | ns | ns |
| Ewe' bleats during lamb handling | 0.0002 | ns | ns | ns | ns | 0.04 | 0.052 |
| Lamb' bleats during lamb handling | ns | ns | ns | ns | ns | ns | ns |
| Lapse to ewe-lamb reunion | ns | ns | ns | 0.08 | 0.03 | ns | ns |
| Frequency of ewe' bleats from lamb releasing until reunion | ns | ns | ns | ns | ns | ns | ns |
| Frequency of lamb' bleats from lamb releasing until reunion | ns | ns | 0.06 | ns | ns | ns | ns |
| Lapse from reunion until the lamb suckle | ns | ns | 0.09 | ns | ns | ns | 0.051 |
| Frequency of ewe' bleats from lamb releasing until suckling | ns | ns | ns | ns | ns | ns | ns |
| Frequency of lamb' bleats from lamb releasing until suckling | ns | 0.06 | 0.08 | ns | 0.03 | ns | 0.07 |

3.51; $P = 0.06$). Lambs' frequency of bleats from release until suckling was affected by an interaction between sex and supplementation with concentrate of their mothers (df = 79; $F = 4.65$; $P = 0.03$). In this sense, while there was no difference in the females whose mothers were supplemented or not (0.13 ± 0.11 bleats/s vs 0.15 ± 0.11 bleats/s), male lambs born from non-supplemented ewes vocalized more frequently than those born from supplemented ewes (0.50 ± 0.11 bleats/s vs 0.09 ± 0.10 bleats/s; df = 79; $F$: 3.21; $P = 0.002$).

## 4. Discussion

In general, supplementation of single-bearing ewes with a commercial concentrate enhanced ewes' BW and BCS during gestation, increased their udder volume, and resulted in a stronger maternal bonding with its lamb during the handling test. On the other hand, supplementation with vitamins C and E did not result in a clear effect on the studied variables, and did not improve the response to supplementation with concentrate. Overall, and in concordance with the final aim of supplementation, ewes with concentrate enhanced their maternal behavior (supplemented ewes were closer to their lambs and vocalized more frequently); however, supplementation of their mother did not modify lambs' behavior. However, during the immediate postpartum period ewe behavior has greater importance than lamb behavior on the strength of their bond, and lambs become more active in the relationship at 4 weeks of age (Hinch et al., 1987). Therefore, although in this study survival rate was not determined, the improvement in maternal behavior is critical to enhance it, as mother provides immunological protection, food and protection against predators (Nowak, 1996).

The positive effects of supplementing with concentrate differ according to the sex of the lamb. Similar to what was previously reported (Hogg et al., 1992), male lambs imply a higher energy expenditure for ewes than female lambs, as shown with their greater BW. In this sense, as male lambs were heavier than female lambs, ewes invested more energy during gestation to produce them. The greater BW of male lambs may partially explain that they had greater surface temperature, vocalized more and spent less time to join again with their mother, indicating a greater vitality. However, ewes also continue investing more energy after birth, as mothers of male lambs also reacted faster, and thus, joined with male lambs again earlier.

As udder size is positively related with the ability to produce colostrum (Banchero et al., 2007), it is interesting that supplementation with concentrate had also positive effects on udder volume. This expands the previous concept of positive effects of supplementation in non-extreme environmental conditions as of those of this study. In this sense, colostrum availability is essential for establishing a stronger ewe–lamb bonding, and thus increase the probabilities of lamb survival (Nowak, 1996). Considering that the ewes supplemented with concentrate were also more motivated for their lambs, this together reinforces the advantages of supplementation on improving ewe's maternal behavior.

Contrary to what we expected, although maternal supplementation improved lamb BW, there were no positive effects on lamb temperature, which is related to the accumulation of brown adipose tissue, and is thus a trustable indicator of lamb thermoregulatory capacity (Symonds et al., 1992). It is important to highlight that in sheep productive systems as those that are predominant in Magallanes Patagonia, newborn lambs are exposed to extreme cold and windy conditions; thus, lambs' energy depots are crucial for thermoregulation and postnatal survival. The growth of adipose tissue during fetal life is subjected to a careful maternal nutritional balance, so maternal undernutrition at the end of pregnancy leads to a reduction in fatty tissue depots, including brown fat (Symonds et al., 2003). Maternal undernutrition during the entire pregnancy results in less total fetal fatty deposits, but no change in brown fat (Wallace et al., 2015). Therefore, it is possible that the newborns of undernourished and supplemented mothers did not present differences in the amount of brown fat, explaining the absence of differences in the recorded temperatures. However, as in our study, the lambs remained alive, we did not quantify the adipose tissue.

Supplementation with concentrate had a clear effect in increasing ewes' BW, but, after this initial improvement, BCS was only maintained. This was probably consequence of a greater investment of ewes on lamb development, as lambs' BW was also greater in those lambs born from ewes supplemented with concentrate than in those born from non-supplemented ewes. This pattern is similar to that observed in similar conditions of animal maintenance (Sales et al., 2018), and is consistent with previous studies in which we observed that supplementation during late gestation increases ewes' BW but has lighter effects on BCS (Freitas-de-Melo et al., 2015). Overall, these results suggest that the main effect of supplementation is to increase ewes' transference to the fetus, promoting its' development, and thus, decreasing the negative consequences of intrauterine growth restriction, which is reflected in the greater BW of lambs born from ewes supplemented with concentrate.

In the current study there were no clear effects of maternal supplementation with vitamins C and E on the studied traits. The final effects of antioxidant vitamin supplementation during pregnancy depend on the pro-oxidant/antioxidant status of the maternal-fetal unit. In previous experiments carried out in similar conditions, maternal nutritional restriction of about 30% resulted only in a slight but not significant decrease of fetal blood cord antioxidant capacity in single pregnancies, in contrast with those observed in twin pregnancies (Sales et al., 2018). However, under conditions of more severe oxidative stress conditions as those observed in high altitudes, maternal supplementation with antioxidant vitamins showed important effects in newborn lambs from single pregnancies (Parraguez et al., 2011). Thus, the lack of positive effects of maternal vitamins supplementation in our study can be explained by the fact that single-bearing ewes have less demands for nutrients and oxygen, and therefore less probability of suffering oxidative stress (Sales et al., 2018). Consequently, they are less responsive to antioxidants.

In conclusion, supplementation of single-bearing ewes with concentrate from 44 days of gestation onwards improved ewes' physiological status and their ability to rear their lambs, including a greater bond strength. On the other hand, supplementation with vitamins C and E did not show effects on those variables. Some of the positive effects of concentrate maternal supplementation were even greater in male than in female lambs. Therefore, nutritional improvement of underfed ewe pregnancies is not only relevant to improve pregnancy outcomes, but also to ensure a better ewe-lamb bonding, which may improve lamb's performance during lactation. The improvement of ewes' physiological status and of ewe–lamb bond strength is also relevant from a welfare point of view, as it may contribute to ameliorate the negative conditions of the extreme environment.

## Declaration of Competing Interest

None.

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