Simulated disease process during late pregnancy compromises developmental outcomes of lambs independently of the weaning method applied


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ABSTRACT

Stress during pregnancy negatively affects fetal development, and artificial weaning can negatively affect animal health and welfare; however, maternal care can reverse the possible consequences of stress on the offspring. Our study aims to determine the combined effect of a prenatal disease challenge and artificial weaning on welfare and productive performance of lambs. During pregnancy, 43 ewes were distributed into three experimental groups, and at 70th and 120th days of pregnancy (Ig; n = 14; Fg = 14;), the ewes were administered with Escherichia coli Lipopolysaccharides (LPS). Fifteen ewes were included in the control group (Cg = 15). Cortisol and rectal temperature measurements were done subsequently to LPS or saline injection. Forty-six lambs (21 males; 25 females) born in a six-day interval from Ig, Fg, and Cg ewes were subjected to two types of weaning, namely progressive (from 39 to 45 days) and abrupt (at 45 days of age). Lamb data, including plasma cortisol levels, rectal temperature, weaning weight, and performance in feedlot were analyzed and compared through F test and Student’s t-test (PDIFF; P = 0.05). The injection of LPS resulted in a 619% increase in cortisol levels after two hours, and rectal temperature reached 39.48 ± 0.134 °C after four hours of LPS administration in a pregnant sheep. Both male and female lambs from the Fg group had lower birth weight (P < 0.05) as compared to other groups. Cortisol levels and rectal temperature decreased during progressive weaning (P < 0.05), in which a higher weaning weight was observed than in abrupt weaning (P < 0.05). On the first day at feedlot, cortisol level was reduced after 60 min upon entrance (P < 0.05), and higher cortisol values were observed during abrupt weaning (P < 0.05). Lower values of dry mater intake and average daily gain were observed for Fg males (P < 0.05). LPS challenge during late pregnancy compromised the lambs’ indicators of productive performance. Albeit progressive weaning was less stressful during feedlot entrance and total bond separation, more days of maternal care during weaning had no relation with stress during pregnancy.

1. Introduction

Stressful situations during pregnancy, which were represented as a single challenge with lipopolysaccharide (LPS), compromise fetal development of lambs, particularly in the final third of gestation, wherein it can interfere with fetal growth and lead to life-long consequences (Iwasa et al., 2009; Hild et al., 2011; Coulon et al., 2012; Petit et al., 2015). Similarly, artificial weaning process is a well-documented source of stress in lambs even if they were not born from stressed ewes (Freitas de Melo and Ungerfeld, 2016). This phase can be firstly attributed to maternal separation and secondly to the changes in the lamb’s access to food (Orgeur et al., 1998). Weaning stress causes the release of cortisol, which may decrease the level of growth hormone (GH), thus influencing the lamb’s development (Kuhn et al., 1990; Orgeur et al., 1998).

On the other hand, maternal care can reverse the possible consequences of stress on offspring (Nowak, 1996; Hood et al., 2003; Hild, 2011). In this context, progressive weaning can be an alternative to modulate stress response (Napolitano et al., 2008); however, it may also...
cause an increased stress response due to repetitive separations (Alexander, 1977; Torres-Hernandez and Hohenboken, 1979; Orgeur et al., 1998). Moreover, abrupt weaning is reported to be more stressful than progressive weaning, because it disrupts the bond between an ewe and a lamb (Orihuela et al., 2004; Freitas-de-Melo and Ungerfeld, 2016).

The development outcomes of lambs can be below their genetically programmed potential due to the inhibition of cell division generated by several factors, including stress during late pregnancy (Owens et al., 1993). Moreover, after birth, lamb growth rate is influenced by several factors directly linked to stress responses. Furthermore, the activation or inhibition of endocrine responses is affected by environmental and nutritional factors (Widdowson, 1980; Gluckman, 1986).

The objective of our study is to determine the combined effects of prenatal diseases and weaning methods on the stress responses and productive performances of lambs.

2. Material and methods

2.1. Location and animals

The experiment was carried out in the campus of University of São Paulo in Pirassununga-SP under the coordinates of 21° 59’ south latitude and 47° 26’ west longitude (W.Gr), with an average altitude of 635 m. The climate was classified as Cwa under the Koppen climate classification; and in summers, it is predominantly mesothermal with rainfall and has dry winter and hot summers, with an average annual temperature of 22 °C, and an average annual rainfall that is approximately 1363 mm.

All procedures were approved by the Research Ethics Committee of Faculdade de Zootecnia e Engenharia de Alimentos of Universidade de São Paulo, protocol number 13.1.2109.74.8.

Forty-three multiparous Santa Ines ewes, which is a Brazilian native sheep breed, were inseminated with White Dorper semen on the same day, and their offsprings were used in this study (Paiva et al., 2005). Lambing was concentrated in six days in March 2014. Ewes and lambs were housed in a maternity paddock until they were seven days of age and were later moved to a rotational grazing system. All animals had access to concentrated diets, which were composed of ground corn (63.1%), soybean meal (31.1%), limestone (0.8%), and mineral supplement (5%) in creep feeding from seven days of age.

2.2. Experimental treatments

Forty-three ewes were included in the study through a 3 × 2 factorial system in which ewes were randomly grouped. Fourteen ewes were challenged with IV LPS injection during pregnancy (LPS, 0.01 g/ml, 0.8 mg/kg body weight; Escherichia coli 0127: B8, Sigma Aldrich, USA) at 70 days of gestation (Ig; n = 14). Fourteen ewes were injected with the same LPS doses at 120 days of gestation (Fg; n = 14). Fifteen pregnant ewes, which were kept as the control group (Cg; n = 15), received only saline IV with a volume similar as that of LPS received by other groups. During LPS challenge, blood samples were collected and rectal temperatures (RTs) were measured. For blood collection, all ewes were housed in 1.30 m long by 1.20 m wide individual boxes that allowed visual contact with other companions. The animals went through a five-day habituation period prior to LPS challenge and sampling. The blood samples were collected in vacuum tubes through jugular puncture (serum tubes and needle size: 25 × 8 mm, BD Vacutainer), starting from the basal prechallenge sample (05 h 00 min).

After LPS or saline injection, two more blood collections were carried out (06 h 00 min and 07 h 00 min). Further samplings were taken at 120-min interval until the end of 24 h (05 h 00 min). RT was concomitantly measured with blood sampling using a digital thermometer (V966F/V965F Vicks’ ComfortFlex® Digital).

Sixty-one lambs were born from 43 ewes during an interval of six days: 24 from Cg (2 still birth, 1 dead after birth, 1 dead after weaning), 17 from Ig (1 stillbirth), 20 from Fg (3 still birth, 7 dead after birth). A total of 46 lambs were used in the rest of our study: 27 females, 19 males, 13 twins, and 20 single births. Birth weight was collected after the lamb suckled colostrum. Afterwards, they were identified with ear tags.

2.3. Weaning

Forty-six lambs were weaned using two management strategies during the experimental period: a) progressive weaning (Pw) and b) abrupt weaning (Aw). Definitive ewe and lamb separations were accomplished for both approaches when the lambs reached 45 ± 6 days of life. The animals were randomly distributed to either abrupt or progressive weaning, with the goal of attaining an equal number of lambs from each prenatally treated group (Cg, Ig, and Fg).

Progressive weaning was carried out for six days, and the ewes were removed daily from the paddock at 7 h 00 min; and the lambs were kept separated from their mothers until 17 h 00 min.

RT was measured ensuing blood sampling. Three blood samples were collected daily, from the 39th to 44th days of the lamb’s life. The samples were collected after maternal separation: first collection was done when lambs and ewes still had auditory contact with each other (T0); second collection was done 60 min after ewe and lamb separation (T60); and the third collection was made 180 min after separation (T180). When the lambs were 45 days old, weaning was completed, and the animals were introduced into the feedlot. In the feedlot, blood samples were collected at three different periods. The first collection was made during the last contact with the mother (T0), the second at 60 min after entering the feedlot (T60), and the third at 180 min after entering the feedlot (T180). During the 46th and 47th days of life, blood samples were taken once a day for 24 and 48 h after introduction into the feedlot.

In abrupt weaning, blood samples and RT measures were obtained at the 45th, 46th, and 47th day of the lamb’s life in a similar manner as in progressive weaning.

For blood and RT measures, the sampling order was randomly established on the first evaluation day. This selection method followed the same previously used order of animals in all sample collections of different weaning types (ordered samples). All measurements were done inside the corral, with minimal disturbance to minimize stress associated with restraint and sample collection.

The blood samples, 10 ml each, were collected through jugular venipuncture (serum tubes and needle size: 25 × 8 mm, BD Vacutainer) to evaluate cortisol levels. The tubes containing serum were stored at −20 °C until cortisol determination using an Electrochemiluminescence immunoassay kit (Roche Cobas Cortisol assay, Roche Diagnostics). All samples were re-assayed if the duplicates yielded a difference of more than 10%. Inter-assay coefficient of variance (CV) was 9.25%, and intra-assay CV was 12.1%. The kits were validated through the demonstration of parallel curves between standard concentrations and serially diluted samples.

The combination of stress during pregnancy and weaning resulted in six groups: CgPw (n = 9), CgAw (n = 11), IgPw (n = 8), IgAw (n = 8), FgPw (n = 5), and FgAw (n = 5).

2.4. Feedlot productive performance

When the experimental animals reached 45 days of age, they were housed in individual pens, measuring 1.20 m by 1.30 m with external view, where they stayed for 73 days preceded by seven days of adaptation. The diet was uniform for all groups, with corn silage as forage food, 30% dry matter (DM) basis proportion, and concentrate composed of ground corn (49.6%), soybean meal (17.7%), mineral salts (1.8%), and limestone (0.9%) in the proportion of 70% dry matter basis (DM).

Food was given twice a day at 07 h 00 min and 16 h 00 min.
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