University of São Paulo
"Luiz de Queiroz" College of Agriculture

Implications of pregnant sheep nutrition on progeny's myofibers and blood parameters

Giuliana Micai de Oliveira

Thesis presented to obtain the degree of Doctor in Science. Area: Animal Science and Pastures

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versão revisada de acordo com a Resolução CoPGr 6018 de 2011

Advisor:
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1. Programação fetal 2. Energia 3. Insulina 4. Glicose 5. Crescimento I. Título

To my parents Ivete e Antônio, for their love and support. Especially my mother, who I know is taking care of me wherever she is.

To my brother Guilherme and my nieces, that I love so much.

And for my great friends.

With much love,

I dedicate

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“No one can make us feel inferior without our consent.”

“You need to do what you think you are not able to do.”

Eleanor Roosevelt

CONTENTS

RESUMO

Implicações da nutrição de ovelhas gestantes sobre as miofibras e parâmetros sanguíneos da progênie

O objetivo do trabalho foi avaliar o efeito de diferentes níveis de energia, bem como diferentes fontes de energia na dieta da ovelha durante a gestação e seu impacto no crescimento e metabolismo dos cordeiros. Setenta e duas ovelhas foram utilizadas e distribuídas aleatoriamente em 5 tratamentos experimentais diferentes: dieta (CTL) com 100% da energia recomendada pelo NRC (2007), dieta de baixa energia (BE) com 90% da energia recomendada, ou ainda dietas de alta energia (AE) com 110% do nível de energia recomendado. As dietas AE eram compostas de três fontes energéticas diferentes: amido (AEA), amido com propionato de cromo (AEAC) ou amido com gordura protegida (AEAG). Essas dietas foram aplicadas no começo e no final da gestação, e durante a lactação. Para avaliar as consequências dessa nutrição na progênie, na primeira etapa (fase lactente) do trabalho o peso dos cordeiros foi avaliado do nascimento aos 60 dias, e foram coletadas amostras de sangue para parâmetros bioquímicos e hemograma desses cordeiros (machos e fêmeas) aos 60 dias. Na segunda etapa (fase ruminante) os cordeiros machos foram desmamados com 90 ± 15 dias e colocados em confinamento, onde permaneceram por 60 dias recebendo a mesma dieta. O peso dos cordeiros foi avaliado a cada 14 dias, e amostras sanguíneas foram coletadas para parâmetros bioquímicos e hemograma antes do abate. Após o abate foram avaliados parâmetros da carcaça e de qualidade de carne. Como resultados da fase lactente, cordeiros provenientes de uma dieta AE, independente da fonte, foram mais pesados e tiveram maior ganho de peso ($P < 0.05$). Cordeiros de gestação simples se destacaram quanto ao peso e ganho de peso ($P < 0.05$), bem como apresentaram maior concentração sanguínea de creatinina, proteína, albumina e globulina do que os cordeiros gêmeos, independete da nutrição materna ($P < 0.05$). Cordeiros de dieta materna AEA, se destacaram quanto ao valor de creatinina ($P < 0.05$), que é usado como indicador de acúmulo de massa muscular. Na fase ruminante em confinamento, cordeiros de dieta materna AEA e de uma gestação gemelar apresentaram uma maior concentração para glicose ($P < 0.05$), já os que vieram da dieta materna BE apresentaram maior concentração de ureia ($P < 0.05$). O peso inicial e final do confinamento, bem como o peso da carcaça quente e fria foram maiores para cordeiros de dietas maternas AEAC e AEAG e aqueles de gestação simples ($P < 0.05$). O rendimento de carcaça foi maior para cordeiros de uma materna CTL ($P < 0.05$). Com exceção das perdas por descongelamento, que foi menor para cordeiros de BE, e perdas por cocção, que foi maior para cordeiros de dieta materna AEAG ($P < 0.05$), nenhum parâmetro de qualidade de carne foi afetado. Os resultados mostram impactos da alteração na nutrição durante a prenhês e lactação, sobre parâmetros de produção e fisiológicos associados à saúde da progênie. Fontes alternativas de energia ou suplementos em dieta materna de alta energia, como o cromo, tem potencial para melhorar a produção.

Palavras-chave: Programação fetal; Energia; Insulina; Glicose; Crescimento

ABSTRACT

Implications of pregnant sheep nutrition on progeny's myofibers and blood parameters

The objective of the work was to evaluate the effect of different energy levels, as well as different energy sources in the diet of the ewes during pregnancy and its impact on growth and metabolism of lambs. Seventy-two ewes were used and randomly distributed in 5 different experimental treatments: diet (CTL) with 100% of the energy recommended by the NRC (2007), low energy (LE) diet with 90% of the recommended energy, or even high energy diets (HE) with 110% of the recommended energy level. HE diets were composed of three different energy sources: starch (ST), starch with chromium propionate (STCR) ou starch with fat protected (STFP). These diets were applied at the beginning and at the end of pregnancy, and during lactation. The impact of the plane of ewe nutrition on progeny, weight was evaluated from birth at 60 days, and blood samples were collected for biochemical parameters and blood count of these lambs (males and females) at 60 days of age in the first stage (infant stage) of the work. In the second stage (ruminant stage), male lambs were weaned at 90 ± 15 days and placed in a feedlot, where they remained for 60 days receiving the same diet. The lambs' weight was evaluated every 14 days, and blood samples were collected for biochemical parameters and blood count before slaughter. After slaughter, carcass parameters and meat quality were evaluated. As a result of the infant stage, lambs from ewes diet HE, regardless of the source, were heavier and had greater weight gain ($P < 0.05$). Lambs from a single gestation stood out in terms of weight and weight gain ($P < 0.05$), as well as having a higher blood concentration of creatinine, protein, albumin and globulin than twin lambs, regardless of maternal nutrition ($P < 0.05$). Lambs from ST diet, stood out in terms of creatinine concentration ($P < 0.05$), which is used as an indicator of muscle mass accumulation. In the ruminant stage in feedlot, lambs from ewe diet ST and a twin pregnancy showed a higher concentration of glucose ($P < 0.05$), whereas those coming from the LE maternal diet showed a higher concentration of urea ($P < 0.05$). The initial and final weight in the feedlot, as well as the weight of the hot and cold carcass, were higher for lambs from STCR and STFP maternal diets and those from a single gestation ($P < 0.05$). The dressing was higher for lambs from CTL maternal diet ($P < 0.05$). With the exception of thaw losses, which was lower for lambs from LE diet, and cooking losses, which was higher for lambs from a ewe diet STFP ($P < 0.05$), no meat quality parameters were affected. The results show the impact of changes in nutrition during pregnancy and lactation, on production and physiological parameters associated with progeny health. Alternative sources of energy or supplements in a high energy maternal diet, such as chromium, have the potential to improve production.

Keywords: Fetal programming; Energy; Insulin; Glucose; Growth

1. INTRODUCTION

Sheep comprises almost 19 million heads throughout Brazil, which 66.7% concentrated in the Northeast and 21.2% in the South. There has been a progressive growth in national sheep farming, raising 12.3% in the total herd of sheep across the country in the period from 2008 to 2018, according to the latest data published by IBGE (2019). Despite the increase in production, according to FAO (2019) sheep meat consumption per capita for 2019 was 550 grams, with the perspective for 2020 being that this consumption will drop to 548 grams, and brazilian consumption of sheep meat has been considered still very low in relation to other meats (Firetti et al., 2010). On the other hand, even with low consumption values, according to Sousa (2020) from Brazilian Association of Sheep Breeders (A.R.C.O), to supply the domestic market in 2019, it was still necessary to import at least 10% of the volume consumed, mostly coming from Uruguay.

Furthermore, according to a 2018 survey by EMBRAPA (Brazilian Agricultural Research Corporation) about 12% of the Brazilian population (25 million people) have never consumed sheep meat. This may be due to some factors such as low standardization of cuts for daily consumption, high prices and a recurrent lack of this product on the market (Andrade et al., 2016; Firetti et al., 2010). Therefore, according to EMBRAPA survey, sheep production still needs many adjustments to improve production efficiency. One of the strategies that can be adopted is called “fetal programming”.

The concept of “fetal programming” originated when human epidemiological data were analyzed, where it was observed that maternal weight and malnutrition increased the incidence of some types of diseases in their children in adulthood, such as heart disease, stroke, diabetes and hypertension (Barker et al., 2002). It can be defined as a mammal's response to a specific challenge at a critical stage of pregnancy, which can affect the development of the offspring quantitatively and qualitatively, and can have permanent effects (Du et al., 2010).

Thinking about meat production, fetal programming is very important since there is no hyperplasia of muscle fibers after the birth of the animal, only hypertrophy (Du et al., 2010, 2015; Zhu et al., 2004). In the embryonic phase the striated muscle begins to develop, when the primary myofibers are formed during the first myogenic wave, already in the fetal stage, and in the middle of pregnancy, the second myogenic wave occurs, where the secondary myofibers will be formed, that are responsible for the formation of most skeletal muscle fibers and satellite cells (Du et al., 2010, 2015). In the final period of pregnancy, skeletal muscle hypertrophy occurs, where there is an increase in the size of muscle fibers (Du et al., 2010, 2015). Adipogenesis and fribrinogenesis are initiated around the middle of gestation in ruminants, concomitantly with the second myogenic wave, and extends to the post-birth period (Du et al., 2010, 2015; Gnanalingham et al., 2005). It is extremely important for marbling, at the stage of hyperplasia of intramuscular adipocytes (Du et al., 2015; Tong et al., 2009) and formation of the endomysium, perimysium and epimysium (Du et al., 2010). All the fetal phases are illustrated in Figure 1.

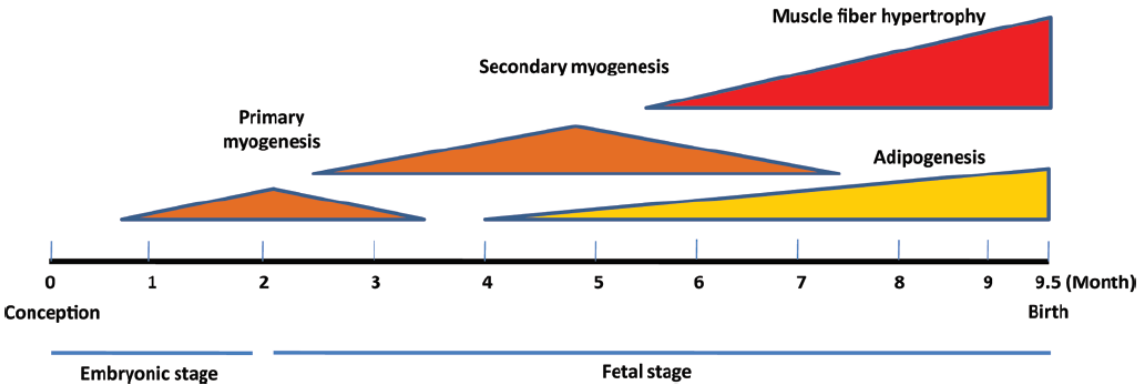

Figure 1. Stages of fetal muscle development in cattle during pregnancy (adapted: Du etal., 2010).

It is important to remember that both food restriction and overnutrition can be considered maternal malnutrition. Knowing this, it has been observed that ewes' food restriction during pregnancy can cause; 1) type I toxemia in the ewe (Ortolani et al., 2002), 2) can cause low birth weight in the progeny (Dong et al, 2008; Fahey et al., 2005; Ford et al., 2007; Husted et al., 2007; Peine et al., 2018; Reed et al., 2007), 3) changes in the pancreas in lambs, mainly a reduction of

beta cells (Bell, 2006; Gardner et al., 2005; Greenwood and Bell 2003; Jones and Ozanne 2009; Kongsted et al., 2014), 4) hyperglycemia (Bell, 2006; Ford et al., 2007; Gardner et al., 2005), 5) increased collagen in the muscle (Alvarenga et al ., 2016; Karunaratne et al., 2005), 6) decreased muscle fiber size (Du et al., 2010; Greenwood et al., 1999; Underwood et al., 2010) and 7) increased visceral fat and decreased lean mass (Ford et al., 2007; Gardner et al., 2005; Karunaratne et al., 2005). It is worth mentioning that a twin pregnancy is considered a type of food restriction, since lambs from this type of pregnancy are born lighter (Casellas and Cajas, 2014; Combellas et al., 1980; Daniel et al., 2007; Ford et al., 2009; Greenwood et al., 1998, 2000; Hammond, 1944; Junior et al., 2012; McCoard et al., 1997, 2000; Van der Linden et al., 2013) due to the total mass of the placenta and the nutrient exchange capacity is reduced causing fetal growth restriction (Casellas and Caja, 2014; Cleal et al., 2007; Gootwine et al., 2007; Greenwood et al., 2000; McCoard et al., 2000; Rhind et al., 1980; Sales et al., 2018).

Overnutrition of the ewes during pregnancy can cause type 2 toxemia in the ewe (Ortolani et al., 2002) and in the offspring can cause inflammatory responses in the fetal muscle, which can deregulate their myogenesis, resulting in smaller diameter primary fibers (Tong et al., 2009; Zhu et al., 2010), type II diabetes (Tong et al., 2009; Yan et al., 2011), problems in the regulation of leptin, the hormone that controls satiety (Hoffman et al., 2016; Long et al., 2010, McMillen et al., 2006), high stress on pancreatic beta cells (Kongsted et al. 2014; McLean et al., 2018) and favoring adipogenesis and visceral fat accumulation, and consequently obesity (Du et al., 2015; Yan et al., 2010).

It is also worth mentioning that the quality and quantity of colostrum and milk are highly dependent on maternal nutrition (Boland et al., 2008; Moretti et al., 2019). As such maternal under nutrition can cause a loss of nutrients and production throughout lactation, including colostrum synthesis (Banchero et al., 2004) and overnutrition, especially in a high energy diet, can affect the

composition of milk, for example by increasing fat, and this may have a long-term effect on offspring (Du et al., 2015).

Therefore, in addition to genetics and adequate nutrition for the lamb after birth, it should be remembered that maternal nutrition, before, during and after pregnancy (especially during lactation), is essential for fetal development and the performance of postnatal offspring (Barker, 1995; Godfrey and Barker, 2000; Drake and Walker, 2004; Du et al., 2015; Van Emon, 2017). However, as the pregnancy progresses, the nutritional requirement increases, for the growth of the fetus, especially in the final weeks, however consumption decreases, due to a displacement of the rumen and reticulum, with no space for their expansion, since there is occupation of space by the fetus (Ingvartsen and Andersen, 2000). So, to try to supply this high demand with low consumption, some supplementation measures for mothers can be adopted, such as using energy supplements. Energy is important for the functioning of vital organs, cell activity and renewal and nutrient utilization processes (Zundt et al., 2006). The form of dietary energy can also be a relevant factor for altering the mother's metabolic-endocrine status during pregnancy, which can influence placental and fetal development. However, as stated above, care must be taken, as nutrition above what is necessary can cause maternal and offspring problems.

Thus, if you have starch, which is the main source of glucose for ruminants and is considered highly insulinogenic (Cabrita et al., 2007; Radunz et al., 2011), this can favor fetal development in the middle and end of pregnancy (Radunz et al., 2011), but high levels of starch can also reduce the consumption of dry matter, reducing the nutritional supply during pregnancy (Silva et al. 2015).

Another way to improve the energy intake of the animals' diet is to use protected fat. The fats that carry the rumen-protected label are those that have been specifically designed to resist biohydrogenation by microorganisms and increase the post-ruminal flow of one or more unsaturated fatty acids (Jenkins and Bridges, 2007) and when lipids are in protected form they

increase its intestinal absorption potential, and in females, there is an increase in the synthesis of steroid hormones and growth factors (Gressler and Souza, 2009).

Chromium is an additive that acts on tissue metabolism by increasing its sensitivity to insulin, helping in the intracellular absorption of glucose, that is, it improves the use of energy in the diet (Davis and Vicent, 1997; Sumner et al., 2007; Hayirli 2001). Chromium acts in conjunction with insulin receptors on cells sensitive to this hormone, increasing glucose intake, improving the use of the energy supplied (Tang et al., 2015; Vincent, 2000). It can also act as a modulator of the body fat of the offspring during pregnancy and lactation (Padmavathi et al., 2010) and for high-energy diets that can cause decreased insulin sensitivity, negatively affecting milk production in dairy cows (LeBlanc, 2010), chromium propionate supplementation in high-energy diets affected insulin and glucose concentrations, avoiding decreased insulin sensitivity (Leiva et al., 2015, 2018).

Thus, the objective of the work reported in this thesis was to observe whether different energy levels of the maternal diet, as well as different energy sources, can affect growth, metabolic profile, as well as the performance of lambs subsequently fed in a feedlot, carcass parameters and attributes of meat quality.

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2. IMPLICATIONS OF FEED ENERGY LEVEL IN PREGNANT EWES ON BLOOD PARAMETERS AND GROWTH OF PROGENY BEFORE MATURITY

Paper according guidelines of Revista *Brasileira de Zootecnia* – submission version

ABSTRACT

Impacts on progeny development, metabolism, and postnatal growth may be due to maternal over- or under-nutrition during pregnancy and lactation, described as fetal programming. Thus, the objective of the work reported herein was to evaluate the effect of different metabolizable energy levels, as well as different sources of energy in ewes' diets during pregnancy and its impact on lamb growth and metabolism. Seventy-two ewes were used and randomly assigned to 5 different experimental treatments: 1) diet with 100% energy recommended by the NRC (2007), diet with 110% energy of the recommended level using three different sources, 2) starch, 3) starch with propionate chromium, 4) starch with protected fat, or 5) diet with 90% of the recommended energy. These diets were applied at the beginning and end of pregnancy, as well as during lactation. The impact of ewe nutrition plane on progeny was evaluated by lamb (male and female) weight from birth until 60 days of age, and blood biochemical parameters and count at 60 days of age. Lambs from ewes fed more energy, regardless of source, were heavier and had greater weight gain. Lambs from a single birth type also stood out for weight and weight gain. Lambs from a high-energy maternal diet based on starch stood out for creatinine values, which is used as an indicator of muscle mass accumulation. Single lambs had higher blood levels of creatinine, protein, albumin and globulin than twin lambs, regardless of maternal nutrition. The results show the impact that even small changes in nutrition during pregnancy and lactation can have on production. As well as indicate alternative energy sources that can improve production.

Keywords: chrome, lambs, nutrition, single, twins, weight

2.1. Introduction

Fetal programming is a strategy that aims to improve animal production through the adequate nutrition of the mother from conception to the birth of their progeny (Du et al., 2015), guarantee adequate fetal development and optimal performance of the mother in the lactation phase, leading to enhanced progeny performance. If there are disturbances in these phases, not only will the mother be liable to experience type I (maternal undernutrition) or type II (maternal overnutrition) toxemia (Ortolani, 2002) and obesity (Zhu et al., 2010), but there may also be changes in the birth weight of the offspring (Ford et al., 2007; Osgerby et al., 2004; Reed et al., 2007; Roca Fraga et al., 2018), as well as physiological changes and future metabolic

disturbances such as obesity and diabetes mellitus type II (Glavas et al., 2010; Jones and Ozanne, 2009; Kongsted et al., 2014; Quigley et al., 2008; Tong et al., 2009) in the progeny. Further lamb growth in the first weeks after birth is also related to the amount of colostrum and milk ingested, and their quality may be influenced by sheep nutrition before and after lambing (Moretti et al., 2019b).

Thus, in addition to genetics and adequate postnatal lamb nutrition, maternal nutrition before, during and after pregnancy is essential for fetus and postnatal growth (Barker, 1995; Drake and Walker, 2004; Van Emon et al., 2017). Among the main nutrients to be supplied, energy has been highlighted to be very important for the activity and renewal of cells and nutrient utilization (Zundt et al., 2006).

Non-fibrous carbohydrate-based diets with high starch levels as an energy source help increase propionate in the blood, thereby increasing glucose production and insulin release, favoring fetal development (Radunz et al., 2011). Protected fat is also an alternative, as it increases the animal's energy intake, since it is protected from rumen biohydrogenation and has an increased intestinal absorption (Gressler and Souza, 2009). Chromium is an additive that acts on tissue metabolism, increasing insulin sensitivity, helping in the intracellular absorption of glucose, and as such it improves the utilization of dietary energy (Davis and Vincent, 1997; Hayirli et al., 2001; Sumner et al., 2007).

Thus, the objective of the study reported herein was to establish the effect of different energy levels as well as different sources of energy on sheep nutrition during pregnancy, on the growth, metabolism and blood parameters of the offspring.

2.2. Material and Methods

2.2.1. Experiment place and animals

The experiment was carried out in Pirassununga, São Paulo, Brazil (latitude: 21.98/21°59’46’ S, longitude: 47.425/47°25’33’ W, altitude: 627 m).Research on animals was conducted according to the institutional committee on animal use (nº 2072131218).

Seventy-two crossbred (Dorper x Santa Inês) ewes were used, aged 2 to 4 years, randomly distributed in five paddocks, subjected to different experimental treatments, with each group having the same number of primiparous and multiparous ewes. All sheep were synchronized for oestrous and the mating success was recorded.

The 5 experimental diets were calculated and adjusted according to gestation phaseThe experimental diets were offered for 50 days at the beginning of pregnancy, right after mating, at the end of pregnancy (last 50 days) and during lactation (90 days). The animals were fed twice a day at 8.00 hours and 16.00 hours, and received water and mineral salt *ad libitum*. In the intermediate period of gestation (after 50 days) the sheep received the same diet, according to the NRC (2007) recommendation. Data on food composition and total diets are shown in Tables 1 to 4.

1) Low metabolizable energy (LE): below the requirement (90%) in energy according to the NRC (2007) (15 sheep);

2) Control (CTL): within the requirement (15 sheep);

3) High energy starch (ST): above the requirement (10%) with starch as a supplementary energy source (15 sheep);

4) High energy starch plus protected fat (STPF): above the requirement (10%) using starch plus protected fat (calcium salts of palm oil) as a supplementary energy source (12 sheep);

5) High energy starch plus chromium propionate additive (STCR): above the requirement (10%) with starch as a supplementary energy source and chromium propionate as additive (15 sheep).

After birth, the ewes were kept with their lambs, and the progeny had access to the same diet in *creep feeding* system (18% Crude Protein and 2900 Mcal ME) up to 90 ± 15 days. Eight two lambs were born (14 LE, 18 CTL, 20 ST, 13 STFP, 17 STCR).

2.2.1. Metabolic profile and animal performance

At 60 days, blood was collected from all lambs by venipuncture of the jugular vein in sterile tubes specific for each test. Biochemical parameters were analysed in blood collected in vacuum collection tubes with 10 mL clot activator (except for glucose analysis, in which 4mL fluoride vacuum tubes were used), centrifuged for 20 minutes at 2500 rpm to obtain a serum blood aliquot, which was stored in 1.5 ml Eppendorf tubes frozen at −20 °C until analysis. The variables analysed were: glucose, insulin, urea, creatinine, CK (creatine kinase), AST (aspartate aminotransferase), GGT (Gamma-Glutamyl Transferase), total protein, albumin, globulin, calcium and phosphorus. These serum blood samples were submitted to the Mindray BS120 automated analyser (manufacturer) at Pirassununga, São Paulo, Brazil (latitude: 21.98/21°59’46’ S, longitude: 47.425/47°25’33’ W, altitude: 627 m)and specific Labtest kits (manufacturers) were used for each analysis of the biochemical parameters.

For the blood count, a 4 mL EDTA vacuum tube was used to collect the sample, which was analysed on a Mindray BC-2800Vet automated analyser. Differential leukocyte counts and morphological evaluation of the figurative elements of the blood were also performed by means of a Rosenfeld-stained blood smear.

Lamb weight was monitored every 15 days, using an automatic scale, from birth to 60 days of age and the average daily weight gain was calculated based on these measurements.

2.2.2. Statistical analysis

Statistical analyses were performed using PROC MIXED from SAS version 9.4 statistical package for Windows (SAS Institute Cary, NC, USA).A completely randomized experimental design was used in a 5 * 2 * 2 factorial arrangement (5 treatments, 2 genders and 2 types of birth), according to Equation 1. Treatment (ewe diets), gender (male and female) and type of birth (single or twin) were considered fixed effects in the model and all possible interactions included in the model. The original or transformed data were submitted to the 5% Tukey test.

$$Y_{ijkl} = \mu + T_i + G_j + TB_k + T_i \times G_j \times TB_k + e_{ijkl} \qquad (1)$$

in wich Y_{ijkl} = characteristics evaluated in animal l, treatments i, gender j, and type of birth k; μ = inherent constant in the data; T_i = treatments effects i; G_j = gender effects j; TB_k = type of birth effects k; T_i x G_j x TB_k = interaction effects between treatments i, gender j and type of birth k; and e_{ijkl} = random error $Y_{ijkl.}$

2.3. Results

2.3.1. Effect on birthweight and growth rate

There was no effect of treatment on birth weight (*P > 0.05,* Table 5). At 60 days of age, the lambs born from ewes fed diets with greater energy were much heavier and had the highest average daily weight gain (*P < 0.05,* Table 5). Lambs from the STCR treatment were the heaviest at 60 days of age and heavier than those born from ewes of CTL and LE groups (*P > 0.05,* Table 5).

There were no differences between genders for any of the growth parameters (*P > 0.05,* Table 5). On the other hand, the type of birth influenced all growth parameters, where single lambs were heavier (*P < 0.05,* Table 5) at all stages of the growth period. There were no interactions between the fixed effects for any of the growth parameters (*P > 0.05*).

2.3.2. Effect on blood metabolites

Ewe diet energy level or source, lamb gender or birth type had no impact on glucose (*P > 0.05*, Table 6). There was difference between genders for insulin (*P < 0.05*) with male lambs exhibiting greater levels (Table 6). Gender and ewe energy level and source showed an interaction (Table 7), where among male lambs the ones born from ewes fed CTL diet had lower insulin levels than those born from ewes fed STCR diet (*P < 0.05*).

Urea concentration was influenced by the ewe diet energy level or source (*P < 0.05,* Table 6), with lambs from CTL fed ewes having the lowest level. Gender and type of birth had no impact on urea concentration (*P < 0.05,* Table 6). The creatinine concentration was influenced by the ewe diet energy level or source, with lambs from the CTL and ST ewes having the highest values (Table 6); it was also influenced by the type of birth, with single born lambs having a higher concentration (*P < 0.05*).

For the enzymes evaluated, CK, AST and GGT, only GGT was influenced by the ewe diet energy level or source, and the lambs from the low energy in the maternal diet (LE) had the lowest concentration when compared to those lambs suckling ewes with elevated energy diets (*P < 0.05,* Table 6). None of the enzymes were influenced by gender (*P > 0.05*). AST and GGT concentration was affected by birth type, with twins having a greater AST concentration and a lower GGT concentration (*P < 0.05*).

Protein parameters (Total Protein, Albumin and Globulin) were not influenced by the ewe diet energy level or source, or gender (*P > 0.05,* Table 6), but all were influenced by the type of birth, with singles having higher concentrations. Calcium and phosphorus concentration were not influenced by the ewe diet energy level or source, or gender (*P > 0.05,* Table 6), but phosphorus was influenced by the type of birth, where singles had a greater concentration than twins.

2.3.3. Effect on blood count

Neutrophils, red blood cells and hemoglobins were influenced by the ewe diet energy level or source ($P < 0.05$, Table 8). The lambs from the LE diet showed lower values for red blood cells and hemoglobin, while lambs from ewes fed the CTL diet showing greater values for segmented neutrophils. Gender influenced monocyte and hemoglobin concentrations and the MCH level, with females exhibiting greater concentrations ($P < 0.05$, Table 8).

The type of birth influenced the eosinophil, red blood cell, hemoglobin and hematocrit concentrations, MCH and MCHC, with single lambs exhibiting higher values for all these parameters ($P < 0.05$, Table 8).

2.4. Discussion

2.4.1. Effect on birthweight and growth rate

The birth weight results in the present work, are in agreement with Daniel et al. (2007), who observed no difference in birth weight between lambs, even when the ewes were restricted at the beginning of gestation (where it can cause myogenesis disorders) or at the end (where the greatest fetal growth occurs). Daniel et al. (2007) did observe however, that lambs from restricted mothers had slower growth and lower weight gain in a similar way to the results from the current study. Other authors have observed that when ewes are subjected to a 50% nutrient restriction at the end of gestation the lambs are lighter at birth, showing that the lack of nutrients in this phase can reduce fetal muscle growth (Dong et al., 2008; Fahey et al., 2005; Husted et al., 2007; Peine et al., 2018). Feed restriction in the first weeks of life may also cause growth retardation in animals where there are problems in both hyperplasia and hypertrophy, because at this stage, muscle growth is closely linked to the amount of DNA synthesizing protein, but if there is adequate nutrition after this period, lambs can recover weight (Greenwood et al.,

2000a; Hopkins and Tulloh, 1985; Krausgrill et al., 1997). In obesogenic diets it was observed that sheep that received 40% more nutrients had heavier lambs (Hoffman et al., 2016), which did not happen in the present study. However, a difference of 0.5 kg less on average for lambs on the LE diet was observed, which is a big difference, but not statistically significant, potentially reflecting the number of animals in the study per treatment.

Colostrum and milk quality and quantity are known to be highly dependent on maternal nutrition (Boland et al., 2008; Moretti et al., 2019b), and as such maternal undernutrition can cause nutrient losses and lower production throughout lactation, including colostrum synthesis (Banchero et al., 2004) and this may be one of the factors that explains the elevated weight and growth of the lambs born to ewes fed higher energy diets.

Starch is the main source of glucose for ruminants and is considered highly insulinogenic (Cabrita et al., 2007), nevertheless, high-energy diets may lead to decreased insulin sensitivity, and in dairy cows this negatively affects milk production and breast synthesis of milk constituents (LeBlanc, 2010), however, previous research with dairy cows showed that chromium propionate supplementation in high energy diets affected insulin and glucose concentrations, avoiding decreased insulin sensitivity (Leiva et al., 2015, 2018). These factors may be an explanation for the difference in weight between lambs from chromium propionate-supplemented mothers and lambs compared to starch-supplemented mothers, where STCRs were heavier, even though there was no statistical difference.

Another factor that may contribute to lambs from sheep with obesogenic diets gaining more weight is that these animals have problems regulating leptin, a hormone that controls satiety (Hoffman et al., 2016; McMillen et al., 2006), leading to a higher food intake than control treatments (Hoffman et al., 2016; Long et al., 2010; McMillen et al., 2006).

The differences between genders for all growth parameters corroborates with the findings of others (Casellas and Caja, 2014; Daniel et al., 2007 Junior et al., 2012), who do not

find differences in weight or weight gain between the two genders. Other authors have reported differences where females were lighter than males (Fahey et al., 2005).

It is already well established that twin born lambs are lighter (Casellas and Cajas, 2014; Combellas et al., 1980; Daniel et al., 2007; Ford et al., 2009; Greenwood et al., 1998, 2000b; Hammond, 1944; Junior et al., 2012; McCoard et al., 1997, 2000; Van der Linden et al., 2013). This may be due to the size of the placenta per fetus. In twin pregnancies the placenta is smaller than that of a single lamb, consequently restricting nutrient intake, especially at the end of pregnancy, where fetal growth is accelerated and nutrient requirement increases (Cleal et al., 2007; Gootwine et al., 2007; Greenwood et al., 2000b; McCoard et al., 2000; Rhind et al., 1980), and also due to intrauterine competition for nutrients (Gootwine et al., 2007; Greenwood et al., 2000b; Junior et al., 2012; Rattray et al., 1974). Twin lambs also tend to grow more slowly until weaning (Ford et al., 2009; Junior et al., 2012), maybe due to competition for maternal milk (Junior et al., 2012).

2.4.2. Effect on blood metabolites

Disturbances during pregnancy, either by dietary restriction or an obesogenic diet, can cause changes in progeny glucose-insulin homeostasis. Although glucose showed no difference between the analyzed parameters in the current study, they presented values above the ideal range, 50-80 mg/dL (Kaneko et al., 2008), however other authors have reported higher glucose values for lambs are than in adult animals, being the ideal range between 80-120 mg/dL for lambs (Reece and Swenson, 2004).

For insulin Hoffman et al. (2016) observed that lambs from a restricted (60% of recommended nutrients) or obsogenic (140% of recommended nutrients) maternal diet had a value of 0.49 ng/mL of insulin, and that the lambs that came from a group of ewes fed control diet (100% of the recommended nutrients) had values of 0.33 ng/mL on average. There was no

statistical difference between treatments in the present study, but higher insulin values were observed for lambs from ewes fed the STCR or STFP diets compared to those reported by Hoffman et al. (2016). These authors showed that the values obtained for insulin in lambs, where mothers' diets were above or below the recommended levels, indicated a possible development of insulin resistance. ST, LE and CTL presented values below those found by the authors indicative of insulin resistance.

One of the reasons for this glucose-insulin homeostasis disorder is direct changes in the pancreas. Obesogenic diets (150% of recommended nutrients) (Ford et al., 2009), use high fat (Kongsted et al., 2014) or have high starch rates (McLean et al., 2018), and lead to an increase of insulin and glucose in the blood, as well potentially inducing insulin resistance in pregnant animals. Glucose freely crosses the placenta, so when you have high glucose levels in the mother, you have high glucose levels in the fetus, but insulin does not cross the placenta. Therefore, the increase in insulin in the fetal and pancreatic plasma is from the fetal pancreas, and this is accompanied by an increased weight of the pancreas and a larger number of beta cells (Ford et al., 2009). However, this high glucose concentration at this stage may expose beta cells to high stress, impairing their sensitivity in the future (Kongsted et al. 2014; McLean et al., 2018).

Importantly, fetal growth restriction is associated with up-regulation of insulin receptors before birth, and increased abundance of these receptors persists in lambs until post-birth, where nutrient supply is not as restricted as in the fetal environment, and this may explain the accelerated assimilation of nutrients in the skeletal muscle and the accelerated growth rate in lambs that suffered fetal growth restriction, suggesting that compensatory growth originates in fetal adaptation in response to an environment with low substrate and high insulin (Muhlhausler et al., 2009). As we can see in Table 5, where LE lambs had a weight at 60 days and a daily

average weight gain, even if not statistically significant, greater than CTL, it was still not enough to reach the same weights as lambs from high energy treatments.

If a diet has 50% more than the recommended nutrients this supports adipogenesis in the fetal skeletal muscle, thereby impairing glucose metabolism and causing insulin resistance and predisposing offspring to type II diabetes and obesity (Yan et al., 2011). An alternative for better use of high energy would be the use of chromium, as per the STCR treatment (Table 6) a greater amount of circulating insulin may be observed, which is reflected by the lower glucose level, which is closer to the CTL treatment, which demonstrates good utilization of dietary energy. Chromium acts on insulin receptors, increasing the intake of glucose in cells sensitive to this hormone (Tang et al., 2015; Vincent, 2000, 2001). It is a compound whose supplementation has allowed the control of hypoglycemia or protection against diabetes, and is recommended for animals that have some kind of infection (Amata, 2013). Chromium supplementation was effective to improve glycemic level and alter insulin sensitivity of patients with hypoglycemia (Anderson, 1987). Chromium may also act as a modulator of offspring body fat during pregnancy and lactation, since in experiments using rats as a model, restriction of this mineral caused high adiposity in the offspring as well as insulin resistance (Padmavathi et al., 2010).

The results in Table 6 show that there was a difference between genders, as well a treatment by gender interaction (Table 7) for insulin. Kongsted et al. (2014) noted that diet affected both genders in the same way, with no differences in insulin and glucose secretions, but noted that this could change after puberty. In a study with rats that suffered protein restriction during gestation, it was observed that males tended to develop type II diabetes earlier than females, and that they only developed insulin resistance in old age (Fernandez-Twinn et al., 2005). In the present study, there was a difference in insulin concentration only among males for treatments, where STCR presented a higher value than the others and CTL treatment

presented lower value (Table 7), showing the action of chromium on insulin. However, STFP treatment also had a high value, but it was not enough to be significant.

Urea is a good indicator of protein metabolism (Nunes et al., 2011) because it is the primary way mammals excrete nitrogen, and its plasma concentration reflects the use of crude protein in ruminants (Marini and Van Amburgh, 2003; Valadares et al., 1999). The reference value range is 17.2 - 43.1 mg/dL (Kaneko et al., 2008), and as such all values were within the stipulated range in the current study (Table 6). However, lambs from the LE treatment had a higher value than CTL lambs ($P < 0.05$), and the other treatments, but the number was not enough to show this difference. This is explained by the fact that the concentration of urea is increased by prolonged fasting due to the catabolism of body proteins (Braun and Lefebvre, 2008), and this treatment may have mainly affected the sheep's milk production, causing the lambs to be without food for an extended time. Another factor that could explain this difference in the amount of urea is that the diet provided for LE sheep had less crude protein, a lower protein in the diet decreases the amount of protein in milk and can cause low milk production (Cowan et al., 1981; Pulina et al., 2006).

Creatinine is a product of the breakdown of creatine and phosphocreatine and moves from the blood into the urine for excretion (Nelson and Cox, 2014), and because it is a large molecule, it does not exceed the tubular membrane of the kidneys, so all filtered creatinine is not reused and exits in the urine (Guyton and Hall, 2006). Creatinine is used to monitor renal function, and when there is a problem there is a lower glomerular filtration rate, and creatinine values increase above the normal range, 1.2 – 1.9 mg/dL (Kaneko et al., 2008). This did not occur in the present study, since all treatments had lower levels, similar to data presented by Ramos et al. (1994), in which values increased until they stabilized in adulthood.

Creatinine is also related to protein intake, when increasing protein intake also increases the amount of circulating creatinine (Hatfield et al., 1998; Van Niekerk et al., 1963). As stated

earlier LE sheep received less protein than other treatments, consequently lower protein in milk and this may have caused a lower concentration of circulating creatinine in lambs. Protected fat has been found to be the cause of lower protein concentration in milk as it can induce insulin resistance, impairing milk protein production (Pulina et al., 2006), explaining why STFP also had a lower value.

Creatine kinase (CK) catalyzes the exchange of a phosphate fraction between phosphocreatine and ATP. In skeletal muscle, CK allows energy storage as phosphocreatine when demand is low, but when energy is needed for muscle contraction, CK catalyzes the transfer of high energy phosphate from phosphocreatine to ADP to form ATP (Hoffmann and Solter, 2008; Valberg, 2008). In domestic species the increase in CK is related to muscle injuries, nutritional myopathies or muscle strain (Wilson et al., 1990). In the present study, the CK values did not differ for any of the evaluated parameters, but they were above the reference value 106 - 168 U/L (Kaneko et al., 2008); perhaps the management of blood collection of the animals caused a high muscle stress, and consequently increased CK values.

Aspartate aminotransferase (AST) is an enzyme used to identify neuromuscular disorders of domestic animals, and elevations of their serum activity have been reported in numerous muscle disorders (Valberg, 2008). Although AST analysis has been used to aid in the diagnosis of various types of lesions, additional testing is usually required to identify which organ is injured, since the activity of this enzyme appears in similar amounts in skeletal and cardiac muscle, liver and blood cells (Hoffmann and Solter, 2008). The reference value range for AST is 60 - 280 U/L (Kaneko et al., 2008), showing that all parameters evaluated in the present study were within range (Table 6). Only type of birth had an effect ($P < 0.05$), but the values do not suggest any muscular issues.

Gamma glutamyltransferase (GGT) is an enzyme that is mainly produced in the kidneys and liver, its regulation in blood serum is related to the bile ducts, specifically biliary epithelial

cells, so when there is an increased release of bliar acids, GGT concentration in blood serum increases (Hoffmann and Solter, 2008). This enzyme is also produced in large quantities in colostrum and in smaller amounts in milk and can be transferred to progeny during the lactation phase (Hoffmann and Solter, 2008; Thompson and Pauli, 1981). In the present study we saw that GGT levels were above the range in reference values, 20 – 52 U/L (Kaneko et al., 2008). Corroborating with the study in calves, it can be observed that the concentration of GGT in newborns after the first colostrum intake was 60 times higher than the reference value, and that this enzyme concentration could remain high up to 16 times in animals before reaching puberty (Thompson and Pauli, 1981). The transfer of this enzyme by milk may explain why lambs from the ewes fed diet LE had a lower concentration of this enzyme, since the mother's malnutrition may have impaired milk production, and consequently impaired milk passive transfer of this enzyme. There was also a difference due to birth type. Another factor that may explain the low concentration in both lambs from the ewes fed diet LE and twin lambs is the fact that GGT are modulated by bile acids, as stated above, which are released in greater amounts when there is a higher intake, mainly fat, which may not have occurred in both cases.

In the present study, both total proteins and globulins did not differ between treatments (Table 6), but were below the reference values, 6.0 - 7.9 g/dL for total proteins and 3.5 - 5.7 g/dL for globulins (Kaneko et al., 2008). This was contrary to what is described in the literature (Antunović et al., 2012; Bórnez et al., 2009; Madureira et al., 2012), where these parameters tend to increase with age until they stabilize with sexual maturity. However Morretti et al. (2019b), working with Santa Inês lambs from birth to 60 days, observed that total protein and globulins decreased to 60 days, and albumin increased. Albumin was within the reference values, 2.4 - 3.0 g/dL (Kaneko et al., 2008) in the current study. All protein parameters exhibited differences due to birth type, and this difference can be explained, because despite the decrease

in total proteins with the advancement of lactation, they are still present in milk (Moretti et al., 2019a), showing that competition for food, milk, when having twins affects nutrient intake.

For calcium no difference was found between any of the studied parameters (Table 6), but all values were within or very close to the recommended levels 11.5 - 12.8 mg/dL (Kaneko et al., 2008). Although there were no differences between treatments for phosphorus, the values were above the suggested reference level, 5.0 - 7.3 mg/dL (Kaneko et al., 2008), however Meyer and Harvey (1992) explain that growth hormones, which are high in young animals, increase renal phosphate reabsorption. Phosphate can also be provided by food, so perhaps there was a difference between birth types, due to competition for food.

2.4.3. Effect on blood count

Leukocytes are the body's first line of defence against infection (Junqueira and Carneiro, 2013), and are divided into 5 different types according to form and action in the body: neutrophils, lymphocytes, monocytes, eosinophils and basophils (Junqueira and Carneiro, 2013; Reece and Swenson, 2004).

In the current study lambs from the ewes fed the CTL diet showed a very high value compared to the other treatments. In addition to being a higher value than other treatments, it was also a higher value than the suggested reference values, 1.75 - 3.00 $x10^3/\mu L$ (Reece and Swenson, 2004). Neutrophils are the most active cells in foreign cell phagocytosis, defending the body against infection (Reece and Swenson, 2004), but ruminants tend to have benign neutrophilia. Where there is an increase in neutrophils stimulated by adrenaline due to heavy physical exertion (Reece and Swenson, 2004; Tornquist and Rigas, 2010) or cortisol, when prolonged stress occurs (Bórnez et al., 2009; Catanese et al., 2013; Junqueira and Carneiro, 2013; Weiss and Walcheck, 2008), this cause the number of neutrophils to increase greatly and

rapidly. Perhaps lambs in the CTL group became more stressed during blood collection than lambs in the other groups, causing their cortisol to increase leading to elevated neutrophils.

Lambs from ewes in groups LE and STFP had lower red blood cells and hemoglobins, although the values for all treatments were within the reference value for sheep, 9 - 15 $x10^6/\mu L$ for red blood cells and 9 - 15 g/dL for hemoglobins (Reece and Swenson, 2004). Red blood cells are anucleated cells made up of 90% hemoglobin (Harvey, 2008), heme protein responsible for transporting oxygen and carbon dioxide within the body (Junqueira and Carneiro, 2013; Harvey, 2008; Reece and Swenson, 2004). After birth, a factor that impairs red cell formation in stem cells is iron deficiency (Birgel, 1999; Harvey, 2008). Nursing animals can quickly deplete their body iron stores as they grow since milk is very low in iron (Birgel, 1999; Harvey, 2008).

Gender had an influence on haemoglobin level and mean corpuscular haemoglobin (MCH), where females had higher values, but values for both genders were within the reference values for sheep, hemoglobin mentioned above and 8 - 12 pg for MCH (Reece and Swenson, 2004). This disagrees with Silva et al. (2014), who found the components of the red blood cells were higher in males, and postulated males respond more actively to a stress situation than females, stimulating the hematopoietic organs to release more cells into the bloodstream.

Females also had higher values for monocytes, but the values were within the average of the values found in the literature, which are between 0.8 – 4.0 $x10^2/\mu L$ (Ahmadi-hamedani et al., 2016; Badawi and AL-Hadithy, 2014; Dias et al., 2010; Lepherd et al., 2009).

The lambs that came from a twin pregnancy had lower values for red blood cells, hemoglobin, hematocrit, MCH and MCHC (Table 8), but all values found were within the reference values, with these for hemoglobin, red blood cell and MCH, are already outlined above, and the range for hematocrit is 27 - 45%, while the MCHC is 31 - 34% (Reece and Swenson, 2004). As stated above, iron deficiency can impair red blood cell formation. Iron is

part of the hemoglobin which is a heme protein, so when you have twins, competition for maternal milk, which no longer contains an adequate amount of iron, can further impair hemoglobin formation, consequently red blood cells, thus causing a smaller average weight and a smaller percentage of the volume occupied by hemoglobins present in the red blood cells. Fewer red blood cells also generate a lower percentage of hematocrit, as this is the percentage of the volume occupied by red blood cells in the total blood volume (Junqueira and Carneiro, 2013).

2.5. Conclusion

Ewes fed over the required metabolizable energy diet based on starch supplemented with chromium propionate, during gestation and lactation, resulted better performance of offsprings between birth and sixty days of age.

Single lambs showed greater blood proteins and creatinine concentration than the twin lambs regardless of maternal nutrition, pointing to improved overall protein metabolism and muscle mass index.

Conflict of interest statement

The authors declare no conflict of interest.

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Annex 1: Tables

Table 1. Composition of the experimental diets components.

Nutrient	Coast-cross Hay	Corn Silage	Ground Grain Corn	Soybean Meal	Fat Protected
Dry matter (%)	88.90	26.00	88.00	91.00	98.00
Crude protein (% DM)	7.30	7.00	9.00	40.00	
NDF (% DM)	72.30	70.00	9.00	15.00	
ADF (% DM)	41.00	44.00	3.00	10.00	
ME (Mcal)	1.90	1.98	3.20	3.00	5.41
TDN (% DM)	53.00	53.00	88.00	84.00	150.00
EE (% DM)	2.53	1.30	4.30	1.60	84.50
Mineral matter (% DM)	6.29	7.00	2.00	7.00	15.00
Calcium (% DM)	0.38	0.35	0.02	0.38	12.00
Phosphorus (% DM)	0.25	0.19	0.30	0.71	

DM = dry matter; NDF = neutral detergent fiber; FDA = acid detergent fiber; ME = metabolizable energy, calculated by the SRNS program; TDN = Total digestible nutrient; EE = Ether extract.

Table 2. Experimental diets ingredients and composition for ewes at first third of pregnancy.

	CTL	LE	ST	STCR	STFP
		Ingredients (% dry matter)			
Hay		99			
Corn silage	85		68	68	72
Fine ground corn	9		24	24	18
Soybean meal	5		6	6	7
Fat protected					2
Chromium				*0.01	
Mineral	1%	1%	1%	1%	1%
Calcite limestone			1%	1%	
		Composition (%)			
Dry matter (%)	29.10	89.00	33.50	33.50	32.44
Crude Protein (% DM)	8.80	7.23	9.55	9.55	9.46
TDN (%DM)	61.08	52.46	62.65	62.65	63.14
EE (%DM)	1.58	2.28	2.01	2.01	3.57
ME (Mcal)	2.06	1.88	2.26	2.26	2.27
ADF (%DM)	38.18	40.58	31.46	31.46	32.94
NDF (%DM)	61.08	71.57	50.99	50.99	53.12
Mineral matter (%DM)	7.29	7.84	7.04	7.10	6.89
Calcium (%DM)	0.53	0.65	0.67	0.67	0.71
Phosphorus (%DM)	0.27	0.31	0.29	0.28	0.29

DM = dry matter; NDF = neutral detergent fiber; FDA = acid detergent fiber; ME = metabolizable energy, calculated by the SRNS program; TDN = Total digestible nutrient; EE = Ether extract. LE = Low metabolizable

energy; CTL = Control; ST = High metabolizable energy with starch; STFP = High energy metabolizable with starch and protected fat; STCR = High energy metabolizable with starch and chrome.

Table 3. Experimental diets ingredients and composition for ewes at last third of the pregnancy period.

	CTL	LE	ST	STCR	STFP
		Ingredients (% dry matter)			
Hay		99			
Corn silage	70		50	50	58
Fine ground corn	19		37	37	27
Soybean meal	10		11	11	12
Fat protected					3
Chromium				*0.01	
Mineral	1%	1%	1%	1%	1%
Calcite limestone			1%	1%	
			Composition (%)		
Dry matter (%)	32.83	89.00	39.89	39.89	36.96
Crude Protein (% DM)	10.55	7.23	11.14	11.14	11.10
TDN (%DM)	62.25	52.46	68.32	68.32	68.46
EE (%DM)	1.89	2.28	2.42	2.42	4.61
ME (Mcal)	2.24	1.88	2.46	2.46	2.47
ADF (%DM)	32.63	40.58	24.53	24.53	27.58
NDF (%DM)	52.62	71.57	40.48	40.48	44.89
Mineral matter (%DM)	6.72	7.84	6.72	6.90	6.47
Calcium (%DM)	0.48	0.65	0.75	0.75	0.77
Phosphorus (%DM)	0.32	0.31	0.32	0.32	0.31

DM = dry matter; NDF = neutral detergent fiber; FDA = acid detergent fiber; ME = metabolizable energy, calculated by the SRNS program; TDN = Total digestible nutrient; EE = Ether extract. LE = Low metabolizable energy; CTL = Control; ST = High metabolizable energy with starch; STFP = High energy metabolizable with starch and protected fat; STCR = High energy metabolizable with starch and chrome.

Table 4. Experimental diets ingredients and composition for ewes at early lactation.

	CTL	LE	ST	STCR	STFP
		Ingredients (% dry matter)			
Hay		99			
Corn silage	60		41	41	47
Fine ground corn	26		44	44	35
Soybean meal	13		13	13	14
Fat protected					3
Chromium				*0.01	
Mineral	1%	1%	1%	1%	1%
Calcite limestone			1%	1%	
		Composition (%)			
Dry matter (%)	36.08	89.00	44.47	44.47	41.53
Crude Protein (% DM)	11.84	7.23	12.19	12.19	12.00
TDN (%DM)	65.76	52.46	71.91	71.91	72.17
EE (%DM)	2.10	2.28	2.65	2.65	4.65
ME (Mcal)	2.37	1.88	2.60	2.60	2.60
ADF (%DM)	28.74	40.58	20.83	20.83	23.23
NDF (%DM)	46.68	71.57	34.88	34.88	38.36
Mineral matter (%DM)	6.26	7.84	5.96	6.00	5.84
Calcium (%DM)	0.42	0.65	0.60	0.60	0.67
Phosphorus (%DM)	0.32	0.31	0.33	0.33	0.32

DM = dry matter; NDF = neutral detergent fiber; FDA = acid detergent fiber; ME = metabolizable energy, calculated by the SRNS program; TDN = Total digestible nutrient; EE = Ether extract. LE = Low metabolizable energy; CTL = Control; ST = High metabolizable energy with starch; STFP = High energy metabolizable with starch and protected fat; STCR = High energy metabolizable with starch and chrome.

Table 5. Birth weight, 60-day weight and average daily weight gain of lambs from different treatments, gender and type of birth.

	Treatments					Gender		Type of Birth	
Weight	CTL	LE	ST	STCR	STFP	Female	Male	Single	Twins
Birth weight (kg)	4.3±0.15	4.1±0.20	4.6±0.15	4.6±0.16	4.6±0.18	4.3±0.10	4.6±0.11	4.9±0.10^{a}	4.0±0.11^{b}
60 days weight (kg)	14.9±0.91^{c}	16.0±1.25^{bc}	18.2±0.96^{bc}	20.6±0.99^{a}	19.3±1.13^{ab}	17.7±0.63	17.9±0.70	20.5±0.64^{a}	15.1±0.69^{b}
Daily Weight gain (kg d^{-1})	0.18±0.01^{b}	0.20±0.02^{ab}	0.23±0.01^{ab}	0.27±0.02^{a}	0.24±0.02^{a}	0.22±0.01	0.22±0.01	0.26±0.01^{a}	0.18±0.01^{b}

LE = Low metabolizable energy; CTL = Control; ST = High metabolizable energy with starch; STFP = High energy metabolizable with starch and protected fat; STCR = High energy metabolizable with starch and chrome. ^{abc}Different letters in the row differ by Tukey´s test, $p < 0.05$.

Table 6. Blood biochemical parameters (mean ± s.e) for 60 day old lambs from different treatments, genders and type of lambing.

Blood Parameters	Treatments					Gender		Type of Birth	
	CTL	LE	ST	STCR	STFP	Female	Male	Singular	Twins
Glucose (mg dL^{-1})	81.0±2.78	83.6±3.72	83.6±2.94	81.1±3.01	89.8±3.43	83.3±1.92	84.4±2.11	83.4±1.92	84.4±2.11
Insulin (ng mL^{-1})	0.4±0.08	0.4±0.11	0.4±0.09	0.6±0.09	0.6±0.10	0.4±0.06^{a}	0.6±0.06^{b}	0.5±0.06	0.4±0.06
Urea (mg dL^{-1})	27.8±2.33^{b}	39.4±3.12^{a}	28.3±2.46^{ab}	32.0±2.53^{ab}	33.8±2.88^{ab}	33.7±1.61	30.9±1.77	30.0±1.61	34.5±1.77
Creatinine (mg dL^{-1})	0.8±0.02^{ab}	0.6±0.03^{c}	0.8±0.02^{a}	0.7±0.02^{bc}	0.7±0.03^{c}	0.7±0.02	0.7±0.02	0.8±0.02^{a}	0.7±0.02^{b}
CK (U L^{-1})	239.6±52.73	194.7±70.54	286.9±55.73	296.3±57.17	170.7±65.15	223.0±36.50	252.3±40.10	247.5±36.54	227.8±40.07
AST (U L^{-1})	81.3±3.16	90.7±4.22	76.6±3.34	85.4±3.42	83.66±3.90	82.9±2.18	84.1±2.40	78.4±2.19^{b}	88.6±2.40^{a}
GGT (U L^{-1})	71.6±3.46^{ab}	58.6±4.63^{b}	77.9±3.66^{a}	77.8±3.75^{a}	82.71±4.28^{a}	74.4±2.40	73.1±2.63	78.2±2.40^{a}	69.2±2.63^{b}
Total Protein (g dL^{-1})	5.2±0.09	4.9±0.12	5.3±0.09	5.1±0.09	5.10±0.12	5.1±0.06	5.1±0.07	5.3±0.06^{a}	4.9±0.07^{b}
Albumin (g dL^{-1})	2.8±0.07	2.7±0.09	3.0±0.07	3.0±0.07	2.8±0.08	2.9±0.05	2.8±0.05	3.0±0.05^{a}	2.7±0.05^{b}
Globulin (g dL^{-1})	2.4±0.08	2.1±0.11	2.33±0.09	2.1±0.09	2.34±0.10	2.2±0.06	2.3±0.06	2.3±0.06^{a}	2.2±0.06^{b}
Calcium (mg dL^{-1})	11.4±0.20	10.8±0.27	11.4±0.22	11.4±0.22	11.78±0.25	11.3±0.14	11.4±0.16	11.5±0.14	11.2±0.16
Phosphorus (mg dL^{-}	9.9±0.44	9.1±0.59	10.1±0.46	10.4±0.47	10.15±0.54	10.2±0.30	9.7±0.33	10.5±0.30^{a}	9.4±0.33^{b}

LE = Low metabolizable energy; CTL = Control; ST = High metabolizable energy with starch; STFP = High energy metabolizable with starch and protected fat; STCR = High energy metabolizable with starch and chrome. ^{abc}Different letters in the row differ by Tukey´s test, $p < 0.05$. CK = creatine kinase; AST = Aspartate aminotransferase; GGT = Gamma.

Table 7. Interaction between treatment and gender (mean ± s.e.) for Insulin (ng mL-1).

Treatments	Gender	
	Female	Male
CTL	0.53±0.11^{ab}	0.22±0.13^{b}
LE	0.37±0.12^{ab}	0.49±0.19^{ab}
ST	0.34±0.13^{ab}	0.49±0.12^{ab}
STCR	0.44±0.14^{ab}	0.87±0.12^{a}
STFP	0.29±0.14^{ab}	0.84±0.15^{ab}

LE = Low metabolizable energy; CTL = Control; ST = High metabolizable energy with starch; STFP = High energy metabolizable with starch and protected fat; STCR = High energy metabolizable with starch and chrome. ^{ab}Different letters differ by Tukey´s test, $p < 0.05$.

Table 8. Blood count (mean ± s.e) for 60 day old lambs from different treatments, genders and type of lambing.

Blood Count	Treatments					Gender		Type of Birth	
	CTL	LE	ST	STCR	STFP	Female	Male	Singular	Twins
Leukocyte (x10^{3} µL^{-1})	8.9±0.53	7.9±0.71	7.2±0.56	7.0±0.58	7.1±0.66	7.6±0.37	7.7±0.40	8.1±0.37	7.2±0.40
Neutrophil (x10^{3} µL^{-1})	4.0±0.33^{a}	2.9±0.45^{ab}	2.5±0.35^{b}	2.6±0.36^{b}	2.4±0.41^{b}	2.6±0.23	3.2±0.25	2.9±0.23	2.8±0.25
Lymphocyte (x10^{3} µL^{-1})	4.4±0.40	4.7±0.53	4.3±0.42	4.2±0.43	4.2±0.49	4.6±0.27	4.2±0.30	4.6±0.27	4.1±0.30
Monocyte (x10^{2} µL^{-1})	1.8±0.27	1.0±0.36	0.9±0.29	1.2±0.29	1.1±0.3	1.5±0.19^{a}	0.9±0.21^{b}	1.3±0.19	1.2±0.20
Eosinophil (x10^{2} µL^{-1})	1.7±0.46	1.7±0.62	2.6±0.49	1.5±0.50	2.4±0.58	2.0±0.32	1.9±0.35	2.7±0.32^{a}	1.3±0.35^{b}
Basophil (/µL)	68.9±17.96	53.1±24.02	64.0±18.98	55.6±19.47	60.8±22.18	44.0±12.43	77.0±13.65	66.4±12.44	54.6±13.64
Red blood cells (x10^{6} µL^{-1})	13.5±0.33^{a}	11.8±0.44^{b}	13.1±0.34^{ab}	13.0±0.35^{ab}	12.5±0.40^{ab}	12.9±0.23	12.6±0.25	13.3±0.23^{a}	12.2±0.25^{b}
Hemoglobin (g dL^{-1})	12.9±0.31^{a}	11.3±0.41^{b}	13.0±0.33^{a}	12.9±0.34^{a}	12.3±0.38^{ab}	12.9±0.21^{a}	12.0±0.24^{b}	13.3±0.21^{a}	11.7±0.23^{b}
Hematocrit (%)	37.2±0.91	33.4±1.22	37.5±0.96	36.9±0.99	35.5±1.13	37.0±0.63	35.2±0.69	38.0±0.63^{a}	34.2±0.69^{b}
MCV (fL)	27.7±0.38	28.4±0.50	28.6±0.40	28.5±0.41	28.3±0.46	28.7±0.26	28.0±0.29	28.6±0.26	28.0±0.29
MCH (pg)	9.5±0.12	9.6±0.17	9.8±0.13	9.9±0.13	9.7±0.15	9.9±0.09^{a}	9.5±0.09^{b}	9.9±0.09^{a}	9.5±0.09^{b}
MCHC (%)	34.5±0.30	33.9±0.40	34.6±0.31	34.8±0.32	34.5±0.37	34.7±0.21	34.2±0.23	34.8±0.21^{a}	34.1±0.23^{b}
Platelet (x10^{5} µL^{-1})	5.9±0.33	5.0±0.44	6.2±0.38	6.4±0.36	6.2±0.41	6.2±0.23	5.7±0.25	6.0±0.23	5.9±0.25

LE = Low metabolizable energy; CTL = Control; ST = High metabolizable energy with starch; STFP = High energy metabolizable with starch and protected fat; STCR = High energy metabolizable with starch and chrome. ^{ab}Different letters in the row differ by Tukey´s test, $p < 0.05$. MCV = Mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

3. INFLUENCE OF EWE ENERGY RESTRICTION OR SUPPLEMENTATION ON THE PHYSIOLOGICAL PERFORMANCE AND MEAT QUALITY OF THEIR LAMBS WHEN FED IN A FEEDLOT

Paper according guidelines of *Small Ruminant Research* – submission version

ABSTRACT

Inadequate nutrition of ewes during pregnancy and lactation are related to cause growth and metabolic problems that impact their offspring meat production, leading to economic losses. To study the impact on lamb performance and meat quality of the ewe under and overnutrition diets with different energy sources, at first and last third of pregnancy as well as during lactation, 72 ewes were randomly distributed into 5 different feeding groups: diet with 100% energy recommended by the NRC (2007), diet with 110% energy of the recommended level using three different sources, starch, starch with chromium propionate, starch with protected fat or a diet with 90% of the recommended energy. The male lambs were weaned at 90 ± 15 and placed in feedlot, where they remained for 60 days receiving the same diet. The lamb were weighed every 14 days, and blood samples were collected for biochemical parameters and blood count before slaughter. After slaughter, carcass parameters and meat quality were evaluated. Lambs from the starch diet and twin pregnancies showed greater concentration of glucose ($P < 0.05$), whereas those from the restricted maternal diet showed a higher concentration of urea ($P < 0.05$). The initial and final weight of the lambs, as well as the weight of the hot and cold carcass, were higher for lambs from ewes fed high energy maternal diets with either chromium or protected fat as were those from a single birth ($P < 0.05$). With the exception of thaw losses, which was lower for meat of lambs from a restricted maternal diet, and cooking losses, which was greater for lambs from a maternal diet with protected fat ($P < 0.05$), no meat quality parameters were affected. The results show the impact of nutrition during pregnancy and lactation, can cause problems in the production and animal health, especially glycemic control and that additives like chromium can mitigate the consequences of a high-energy diet.

Keywords: High energy;Starch; Fat protect; chrome

3.1. Introduction

The Brazilian sheep meat production is well distributed across the country, with emphasis on the Northeast region and the state of Rio Grande do Sul (IBGE, 2019). Despite the growth, the consumption of sheep meat is low compared to other types of meat (Firetti et al.,

2010). It has been reported factors such as low product standardization, lack of market supply and high prices as some of the possible reasons for the persistent low consumption (Andrade et al., 2016; Firetti et al., 2010). Sheep production in Brazil would benefit from adjustments to improve meat consistency and efficiency of its production.

A potential strategy that can be used to improve production, quality and consistency in obtaining the desired product is called "fetal programming". This is defined as a mammal's response to a specific challenge at the most critical stage of pregnancy, which will affect offspring development quantitatively and qualitatively resulting in permanent effects (Du et al., 2010). Inadequate maternal nutrition at the beginning, mid-pregnancy and late gestation can cause low birth weight and weight gain (Ford et al., 2007; Gardner et al., 2005), a reduction in the number of muscle fibers and a lower percentage and number of glycolytic fibers (Bee, 2004; Fahey et al., 2005), hyperinsulinemia (maternal overnutrition) (Jones and Ozanne 2009; McLean et al., 2018) and increased visceral fat, as well as increased connective tissue overall (Ford et al., 2007; Gardner et al., 2005; Karunaratne et al., 2005).

Therefore, adequate nutrition throughout the gestational period should be ensured so that a healthy lamb is born, capable of responding well post-natal nutrition, with adequate muscle mass growth so that the meat has adequate attributes valued by the consumer. In this context, it is known that the demand for energy from the ewe at the end of pregnancy and during lactation increases (NRC, 2007), a period in which, generally, the pasture is scarce and of low quality in Brazil. Therefore, strategies to improve energy supply in these stages are necessary. Starch is a very common energy source used to improve nutrition. It is a non-fibrous carbohydrate and favor the ruminal production of propionate, which consequently increases blood glucose (Silva et al., 2015). Chromium, on the other hand, acts to normalize metabolism and glucose uptake by tissues, that is, it increases tissue sensitivity to insulin so that animals can take better advantage of all available energy (Davis and Vicent, 1997; Sumner et al., 2007).

On the other hand, lipid supplementation in the ruminant diet is a way to attend to the energy demands, and when lipids are in protected form they increase their intestinal absorption potential, and in females this causes an increase in the synthesis of steroid hormones and growth factors (Gressler and Souza, 2009). However, a large energy intake also causes problems to the fetus, such as inflammatory responses in the fetus muscle (Tong et al., 2009)

Thus, the objective of this work was to observe if different energy levels of the maternal diet, as well as different energy sources could affect the performance of lambs subsequently fed in a feedlot, as well as the physiological state of these lambs pre-slaughter and carcass and meat quality parameters.

3.2. Material and methods

3.2.1. Experiment place and animals

The experiment was carried out at the Faculty of Animal Science and Food Engineering at the University of São Paulo (FZEA/USP), campus of Pirassununga. The project was approved by CEUA/FMVZ nº 8317201218.

Seventy-two crossbred (Dorper x Santa Inês) ewes were used, aged 2 to 4 years, randomly distributed in five paddocks, subjected to different experimental treatments, with each group having the same number of primiparous and multiparous ewes. All sheep were synchronized for oestrous and the mating success was recorded.

The 5 experimental diets were calculated and adjusted according to gestation phase, and ewes were fed a diet 100% of the NRC (2007) energy recommendations, or 110% of the recommended energy requirements, using three different sources, or 90% of the recommended energy requirements. The diets are described below. The experimental diets were offered at the beginning of pregnancy, right after mating (50 days), at the end of pregnancy (last 50 days) and during lactation (90 days). The animals were fed twice a day at 8 am and 4 pm, and received

water and mineral salt *ad libitum*. In the intermediate period of gestation (after 50 days) the sheep received the same diet, according to the NRC (2007) recommendation.

1) Low metabolizable energy (LE): below the requirement (10%) in energy according to the NRC (2007) (15 sheep);

2) Control (CTL): within the requirement (15 sheep);

3) High energy metabolizable using starch (ST): above the requirement (10%) with starch as a supplementary energy source (15 sheep);

4) High energy metabolizable using starch and protected fat (calcium salts of palm oil) (STPF): above the requirement (10%) using starch plus protected fat as a supplementary energy source (12 sheep);

5) High energy metabolizable using starch and chromium propionate additive (STCR): above the requirement (10%) with starch as a supplementary energy source and chromium propionate as additive (15 sheep).

After birth, the sheep were kept with their lambs, and the lambs received the same diet in the *creep* (18% Crude Protein and 2900 Mcal ME) up to 90 ± 15 days when the lambs were weaned. From weaning, the males (n=36) from the different treatments (8 CTL, 5 LE, 8 ST, 9 STCR, 6 STFP) were in fed in individual pens.

The diets were the same for all lambs, formulated for a weight gain of 300 grams per day, according to NRC (2007) recommendations, so that the effect of the maternal feeding on animal performance could be evaluated. The diet was offered twice a day at 8 a.m. and 3 p.m. Residual feed was weighed daily, allowing for the daily offer to be determined with a margin of 5%, to guarantee *ad libitum* consumption. The diet consisted of chopped coast-cross hay, ground corn, soybean meal and minerals. The total diet had 14% crude protein, 73% TDN, 29% NDF, 5% mineral matter, based on dry matter. The lambs were in fed for 60 days, where they reached ± 40kg; and animals were weighed every 14 days.

3.2.2. Metabolic profile

One day before the slaughter, blood samples were collected by venepuncture of the jugular vein in sterile tubes specific for each test from all lambs. Biochemical parameters were analysed in blood collected in vacuum collection tubes with 10 mL clot activator (except for glucose analysis, in which 4mL fluoride vacuum tubes were used), centrifuged for 20 minutes at 2500 rpm in a serological centrifuge (model:32/10 CS3.5, Inbras, Brazil) to obtain a serum blood aliquot, which was stored in 1.5 ml Eppendorf tubes frozen at −20 °C until analysis. The following assays were conducted: glucose, insulin, urea, creatinine, CK (creatine kinase) AST (aspartate aminotransferase), GGT (Gamma-Glutamyl Transferase), total protein, albumin, cholesterol, triglycerides, calcium and phosphorus. These serum blood samples were submitted to the automatic spectrophotometer (BS120, Mindray, China) at the Veterinary Clinical Laboratory at FZEA/USP, Campus Pirassununga, and specific Labtest kits were used for each analysis of the biochemical parameters.

For the blood count, a 4 mL EDTA vacuum tube was used to collect the sample which, was analysed on an automatic hematological counter (BC-2800Vet, Mindray, China). Differential leukocyte counts and morphological evaluation of the figurative elements of the blood were also performed by means of a Rosenfeld-stained blood smear.

3.2.3. Slaughter and after slaughter

The animals were fasted for 16h, with access to water. The animals were slaughtered at the FZEA / USP Slaughterhouse plant, following humanitarian standards (BRASIL, 2000) and good manufacturing practices.

Carcass, hot (shortly after slaughter) and cold (24 hours after slaughter, in a cold chamber at 2ºC), omental and mesenteric fat, and liver were weighed in a digital scale (model:

BK300, Balmak, Brazil) for carcass and a bench scale (model: 9094, Mettler – Toledo, USA) for fat and liver.

The pH was measured using a digital portable pH meter (model: DM-2, Digimed, Brazil, calibrated at two pH points, 7 and 4) and the temperature was measured with a digital spike thermometer (model: TP3001, NOVOTEST, Brazil). Two measurements were made, one soon after slaughter and the other 24 hours post slaughter.

Twenty-four hours after slaughter, the *Longissimus dorsi* (LD) loin eye area (LEA) was measured at the 13th rib. The contour of the muscle was traced on transparency plastic sheet and with a ruler two straight lines were drawn measuring the loin eye width (A). That is, the maximum distance from the muscle from the medial line to the lateral extremity of the *Longissumus thoracis* and *lomborum* and at depth (B) the maximum perpendicular distance width, located adjacent the lateral edge of the vertebrae. Then, the LEA was calculated from the equation $(A/2 \times B/2) \times \pi$, considering $\pi = 3.1416$. Subcutaneous fat thickness was also measured at the 13th rib with the aid of a caliper.

At boning, the LD muscle was collected and stored in a vacuum packaging in -80ºC freezer for further analysis, and 2 grams of this muscle was frozen quickly in liquid nitrogen for histology analysis.

3.2.4. Meat Quality Analyses

Thawing loss (TL). In this analysis, three steaks from the LD each 2.5 cm of thick were used. The samples were removed from the package, dried to remove exudate water, and weighed frozen and then they were held chilled at 2°C overnight. On the next day, the samples were dried and weighed again. The TL was determined by the difference between weights before and after thawing (Missio et al., 2010).

Cooking loss (CL). In this analysis, 3 steaks from the LD each 2.5 cm thick were used. The samples were weighed and cooked in a preheated electric oven. At 36ºC, the samples were turned over and kept in the oven until the internal temperature of 72ºC was reached (temperature was controlled by a digital thermometer). Afterward, the steaks were left to cool at room temperature and were weighed again. The CL was determined by the difference between the weight after and before cooking (Felício, 1999).

Shear force (SF). The procedure was performed according to the recommendations of the American Meat Science Association - AMSA (2010). The same samples used to assess CL were used shear force testing. Five to eight cylinders of 1.25cm of diameter, cut following the direction parallel to the muscle fibers, were removed from the steaks. The SF was measured using the Warner-Bratzler machine (G-R Manu-facturing Co. Manhattan, KS) equipped with a Warner-Bratzler'V' slotblade accessory (3.0 mm thickness and 60° triangular aperture) and aload cell of 500 N (basic force gauge) and speed of 20 cm/min. Results for each steak is the average of the cylinders and it is expressed in Newtons (N).

Myofibrillar fragmentation index (MFI). The MFI was determined in accordance to procedures described by Culler et al. (1978), with the following adaptations: 1g of muscle sample was homogenized in 10 MFI buffer volume. The protein concentration was determined by the biuret method described by Gornall et al. (1949). The protein concentration was adjusted to ensure the same protein concentration of 5 $mg.ml^{-1}$. The myofibrillar suspension was diluted and stirred, and absorbance was read immediately in the spectrophotometer (brand Unico, model 1205) at 540nm wavelength. The index was calculated according to Culler et al. (1978).

Sarcomere length (SL). Five sub-samples representing different anatomic positions (lateral, medial, intermediate, ventral, and dorsal) of LL muscle were taken from each steak. From each sub-sample, approximately 0.5 g of muscle tissue was homogenized with 5% glutaraldehyde in 0.1 MNaHPO4buffer with pH adjusted to 7.2. The homogenate was incubated

for 4 h, and then transferred to a fixation solution (0.2 M sucrose in buffer 0.1 M NaHPO4with pH 7.2), following the method described by Cross et al. (1981). The sarcomeres were measured with100× amplification on a Nikon Eclipse 80i light microscope (Nikon,Tokyo, Japan). For each sub-sample, five readings were taken. For each reading, between 10 and 20 sarcomeres were measured. Finally, the average of values obtained for each sub-sample represents the sarcomere length value of each animal and it is expressed in micrometers (μm)

Collagen determination. The analysis of total and soluble collagen will be performed according to Bergman and Loxley (1961) modified by Brown et al. (2001). The collagen content and its fractions were evaluated by quantifying the amino acid hydroxyproline after hydrolysis of the material. Results were calculated using a response curve. Expression of results refers to the hydroxyproline values that were obtained from the absorbance readings taken on a spectrophotometer. For the analysis of soluble collagen the samples were first cooked in a water bath at 80 ° C for 75 minutes and then centrifuged at 4000 rpm for 10 minutes at 20ºC. The conversion factor used for both analyses was 7.14 times the hydroxyproline concentration.

Muscle Fiber Area Measurement. The samples were wrapped in neutral talc and frozen in liquid nitrogen to be transported, also in nitrogen, to the Striated Muscle Biology Laboratory, Department of Morphology, UNESP - Botucatu Biosciences Institute. Slices (10 μm) were prepared using a cryostat. The samples were fixed with Baker's formalin calcium, stained with hematoxylin and eosin, alcohol and xylol. The HE (Hematoxylin-Eosin) technique were used to evaluate muscle morphology in general. The slides' photos were taken using a biological microscope (model: Biovideo, Bel Photonics, Italy) with 40× amplification and the fiber area was measured with the aid of the program ImageJ.

3.2.5. *Statistical analysis*

Statistical analyses were performed using PROC MIXED within SAS version 9.4 statistical package for Windows (SAS Institute Cary, NC, USA). A completely randomized experimental design was used in a 5 * 2 factorial arrangement (5 treatments and 2 types of birth). Treatment (sheep diets) and type of birth (single or twin) were considered fixed effects in the model and all possible interactions included in the model. For some data cofactors were used. Cold carcass weight was used as cofactor for fat thickness, LEA, dressing, omental and mesenteric fat weight and collagen. For pH values the temperatures were used as cofactor, while for cooking loss the pH at 24 hours was used as a cofactor and for liver weight analysis the final weight in confinement was used as cofactor. The original or transformed data were submitted to the 5% Tukey test.

3.3. Results

3.3.1. Blood parameters

The blood glucose concentration was influenced by both the diets applied to the ewe and the type of birth ($P > 0.05$, Table 1), where lambs from the ST treatment showed greater levels, as well as lambs that came from twin pregnancies. Insulin, on the other hand, had a greater concentration for STFP compared to CTL ($P < 0.05$, Table 1), and showed a tendency for ST to have a higher concentration than CTL.

The urea concentration of the lambs was influenced by the energy level or energy source of the ewe's diet ($P < 0.05$, Table 1), with lambs that came from the LE diet having a greater concentration than STCR. The concentration of creatinine in the lambs was not influenced by the diet of the ewe ($P> 0.05$, Table 1). None of the factors analysed were influenced by the type of birth.

For the evaluated enzymes, CK, AST and GGT, only CK showed an interaction between the evaluated factors, where single born lambs from the treatment of ewes fed with the STFP

diet showed a lower concentration than others single born lambs from ewes with a high energy diets (*ST vs STFP – P = 0.08*, Table 2), while twin lambs showed greater CK than single born from ewes fed STFP. AST, on the other hand, did not differ between treatments *(P > 0.05*, Table 1). The concentration of GGT in the lambs was influenced by the energy level from specific source of the ewe's diet and by the type of birth, where STFP showed a greater value than CTL and lambs single born showed greater values (*P < 0.05*, Table 1).

The concentration of total protein and albumin of the lambs were influenced by the diet of the ewe, where the ST treatment showed the highest value (*P < 0.05*, Table 1). However, the type of birth affected only albumin, with single born lambs having higher concentration.

The concentration of cholesterol and triglycerides in lambs was not influenced by any of the parameters evaluated (*P > 0.05*, Table 1). The concentration of phosphorus in the lambs was influenced by the energy level and diet source of the ewe, where the LE treatment had the highest value, and twins lambs also showed higher values than singles (*P < 0.05,* Table 1).

Red blood cells, haematocrit and haemoglobin, the concentration of red blood cells in the blood of the lambs showed a difference for the type of birth, where single born lambs had a greater value (*P < 0.05*, Table 1). The concentration of haemoglobin and haematocrit were affected by an interaction between the parameters evaluated, where for the concentration of haemoglobin the concentration was greater for lambs coming from the maternal diet STFP and single born (*P < 0.05*, Table 2). As for the concentration of haematocrit in lambs coming from the maternal diet STFP and twin born, the values were lower (*P < 0.05*, Table 2).

3.3.2. Feedlot performance and carcass parameters

The initial weight of the lambs in the feedlot was greater for those who came from ewes fed the STCR and STFP diets, and this pattern was maintained for the final weight, hot and cold carcass weight (*P < 0.05*, Table 3). Lambs single born also had greater values for the

aforementioned parameters (Table 3). Daily weight gain and fat thickness were not affected by any of the factors analysed (*P > 0.05*, Table 3). The dressing percentage was affected by the level and source of the ewes' energy, where the CTL treatment had a greater dressing percentage than the others treatments (*P < 0.05*, Table 3), but the type of birth did not affect this parameter (Table 3). The loin eye area was not affected by any of the analysed parameters (*P > 0.05*, Table 3). Omental and mesenteric fat weights and liver weight were also not affected by any of the factors studied (*P > 0.05*, Table 3).

The carcass temperature (0h and 24h) and pH (0h and 24h) were not affected by any of the analysed parameters (*P > 0.05*, Table 3).

3.3.3. Meat quality parameters

Thawing and cooking losses were influenced by the ewe diet, where the LE treatment obtained the least thawing loss, whereas for the cooking loss the lowest values were obtained for STFP treatment (*P < 0.05*, Table 4). The other parameters of meat quality analysed showed no difference for any of the factors studied (*P > 0.05*, Table 4).

3.4. Discussion

3.4.1. Blood parameters

Glucose showed a difference between the factors analysed, where lambs from mothers fed the ST diet had the highest concentration and those fed the STCR diet the lowest concentration, even though the values were within the ideal range stipulated for lambs, between 80-120 mg/dL (Reece and Swenson, 2008). Insulin showed statistical difference, where it was observed that the STFP treatment had a higher concentration than CTL. In work where ewes received three types of diets during the pregnancy; a control, a restriction (60% of the recommended nutrients) or obesogenic (140% of the recommended nutrients) it was shown lambs that came from ewes fed with the control diet had 0.33 ng/mL of insulin whereas those

fed the other two diets had 0.49 ng/mL of insulin (Hoffman et al., 2016). In the current study all treatments had much higher values than those reported by Hoffman et al. (2016). However, it was observed that ST and STFP, treatments with high energy, had both a greater glucose and insulin concentrations, which may be an indication that these treatments are prone to develop insulin resistance, once, the high concentration of glucose from a maternal diet with excessive energy during the gestation, stimulates beta cells of the not yet mature offspring pancreas by glucose that passes by placenta, may causing cell stress, impairing its sensitivity to glucose in adulthood (Jones and Ozanne 2009; Kongsted et al., 2014; McLean et al., 2018).

On the other hand, lambs from STCR treated ewes had a lower concentration of glucose (Table 1), and an insulin concentration that were not statistically different from CTL. This shows that chromium may have helped in the use of energy during pregnancy, not letting the high energy supplied to the ewe impair the development of the lamb in the fetal phase, since chromium acts in conjunction with insulin receptors in cells sensitive to this hormone, increasing glucose uptake, improving the use of the energy supplied (Tang et al., 2015; Vincent, 2000).

There was an influence of the type of pregnancy on glucose-insulin homeostasis, where lambs of twin pregnancy had a greater concentration of glucose ($P < 0.05$) without changes in insulinemia. Twin pregnancy can be considered a type of dietary restriction (Casellas and Caja, 2014; Cleal et al., 2007; Gootwine et al., 2007; Greenwood et al., 2000; McCoard et al., 2000; Rhind et al., 1980; Sales et al., 2018), and as stated above, restriction can impair pancreatic development and/or consequently insulin production and sensitivity.

Urea also showed a difference ($P < 0.05$, Table 1), where lambs coming from restricted maternal nutrition, LE, showed greater contraction than STCR diet, with the other showing concentration that did not differ from the divergent maternal diets. However, the values were above the stipulated range, where the reference value is 17.2 - 43.1 mg/dL (Kaneko et al, 2008).

Lambs in similar feedlot conditions as in the present study presented concentrations of urea in the same range found in this work (Costa et al., 2018; Gobindram et al., 2016). The creatinine concentration did not show any difference; however, the values were below the normal range, 1.2 - 1.9 mg/dL (Kaneko et al., 2008). The results indicate a hyperuremia in the lambs from LE fed ewes, even though no change were observed in the creatinine levels, which may indicate changes in the liver metabolism with changes in the kidney function. Changes in both organs in adult sheep offspring seems to be observed under a more drastic restritiction (50% of nutrients) that resulted in both hyperuremia and very low creatinine concentration (Khanal et al., 2016), which may be a result of reduced muscle mass an altered glomerular filtration rate. The type of birth did not influence the concentration of urea and creatinine (Table 1), and urea showed values within the concentration mentioned above.

Creatine kinase (CK) showed interaction between the maternal diets and the type of birth (Table 2), and with the exception of STFP coming from a simple pregnancy, all values were above the reference values 106 - 168 U/L (Kaneko et al., 2008), mainly lambs from a twin pregnancy of the maternal STFP diet, which presented a greater concentration of CK. There is no specific work in the literature that discusses the influence of the maternal diet on CK concentration, however it is known that an increase in serum CK is related to muscle fiber disturbances normally after exercise bout (Wilson et al., 1990), as a response to maintenance of energy balance during contraction. CK is the catalyst for the transfer of high-energy phosphate to ADP to become ATP (Hoffmann and Solter, 2008; Valberg, 2008). It may be that lambs born from ewes fed the STFP diet (Table 2), which as stated above, may have had insulin resistance and less control of glucose uptake, may have an increased AMP/ATP ratio, and possible activation of AMPK and phosphorylation of the CK enzyme, which would stimulate its translocation into the myofiber and possible externalization of the enzyme (Friedrichsen et al., 2013).

Values of serum aspartate aminotransferase (AST) for all maternal diets for both type of birth in the present study did not differ and they were within the range (Table 1) of reference values of 60 – 280 U/L (Kaneko et al., 2008) for sheep. This serum enzyme activity has been used as an indicator of neuromuscular disorders in domestic animals, and elevations have been reported in numerous muscle disorders (Valberg, 2008).

In the present study, we observed that the levels of gamma glutamyltransferase (GGT) were above the reference range of 20 - 52 U/L (Kaneko et al., 2008), similar to those reported for lambs in feedlot which presented a 61 - 71 U/L (Gallo et al., 2019), with the exception of STFP, which presented a value much higher than CTL diet. GGT is a liver enzyme used to indicate whether there are lesions in the liver (Hoffmann and Solter, 2008; Thompson and Pauli, 1981). Stimulations of exogenous origin, such as a high concentration of food and sudden changes in diets, can cause damage to liver cells and the dispersion of these enzymes in the blood, increasing their levels (Braun et al., 1983; Moreira et al., 2012). This can explain why GGT levels were high for all treatments and types of birth, since the feedlot diet was adjusted daily. This can also indicate liver may be the main target organ of the long-term fetal programming response, where metabolic adaptability in response to malnutrition in late pregnancy put the offspring at risk of irreversible damage to this organ and which can cause the early development of obesity (Khanal et al., 2016). However, there was no visible liver damage in the present study, such that the weight of the liver was unaffected by the treatments (Table 3). In addition, GGT can also be used as a marker for metabolic syndrome, which is characterized by insulin resistance, type II diabetes, hypertension and abdominal obesity (Araújo et al., 2005). Showing the tendency of lambs that came from a maternal STFP diet, which had a high concentration of GGT, to develop obesity in adulthood.

The total protein showed a difference between treatments, and all values were below the reference values, ranging from 6.0 to 7.9 g/dL (Kaneko et al., 2008) and the albumin

concentration also showed a difference between treatments, following the same pattern as the total protein concentration, but it was within the reference values, 2.4 - 3.0 g/dL (Kaneko et al., 2008), where lambs from the STFP fed ewes showed lower concentrations for both parameters (Table 1). There are no specific studies that link maternal nutrition during pregnancy and lactation with changes in blood protein concentrations in lambs on feedlot diet, however the synthesis of blood proteins can be impaired by two factors, severe liver damage or protein deficiency in the diet (González, 2018; Gonzáles and Silva 2006; Reece and Swenson, 2004). However, as shown above, lambs from the STFP fed ewes showed a disturbance in the concentration of GGT, indicating possible liver damage and metabolic disorders, which may have caused a low concentration of total protein and albumin in these lambs.

The present study corroborates with experiments that worked with sheep that received overnutrition and had restricted nutrition, in which the values for cholesterol and triglycerides were slightly outside the reference values, but also did not show any difference between the maternal nutrition levels analysed (Hoffman et al., 2014, 2016). Nevertheless, lambs from ewes who suffered restriction (50% of nutrients) during pregnancy and after birth showed a high concentration of circulating cholesterol at 6 months of age fed with a high-energy diet due to epigenetic changes in the liver cholesterol promoting gene 7α-hydroxylase (Khanal et al., 2016). The restriction in the present study may have been not enough to elicit the same response.

Phosphorus showed differences between treatments (Table 1), where ST and STCR had greater values. However, the concentrations found in the present study for all treatments are above the reference range, 5.0 – 7.3 mg/dL (Kaneko et al., 2008). There is no data in the literature on the influence of maternal nutrition during pregnancy, altering the amount of phosphorus in the offspring. But the high concentration of phosphorus in the blood may have been stimulated by a period of calcium stress, this stress stimulates the secretion of parathyroid hormone (PTH), and this increases the renal and salivary excretion of phosphorus, as well as

increases the concentration of phosphorus in the blood, to stimulate its bone resorption and release of calcium in the blood (Goff, 2000).

All values for red blood cells were within the reference range for sheep, 9 - 15x10^6/µ (Reece and Swenson, 2004). Haemoglobin level was impacted by diet and type of birth, where single born lambs from ewes fed the STFP diet had a higher level than their twin counterparts, despite the differences in concentrations, all treatments were within the reference range of 9 - 15 g/dL (Reece and Swenson, 2004).

There was also an interaction between treatments and type of birth for haematocrits, where lambs from a single pregnancy of the LE and ST treatments had higher values and lambs from a twin pregnancy from the STFP treatment the lowest value ($P < 0.05$, Table 2). The definition of haematocrit is the percentage of the volume occupied by red blood cells in the total blood volume (Junqueira and Carneiro, 2013), so the data in the present study are in agreement, where the lowest haemoglobin value is also the lowest percentage of haematocrit, these values may indicate a possible hemolysis in these animals, which may have been caused by an oxidative stress of haemoglobin in the liver (Vickers et al., 2010). Despite these differences, all values were within the reference range, which is between 27 - 45% (Reece and Swenson, 2004).

3.4.2. Feedlot performance and carcass parameters

The effect of fetal programming on the lambs in feeedlot was clear in the higher energy diets, where lambs that came from the STCR and STFP treatments were already heavier at feedlot entry and this pattern remained until slaughter (Table 2), since these lambs obtained no difference in daily weight gain ($P > 0.05$, Table 3). These data do not corroborate with the experiment carried out with Angus and Simental cows, where they were divided into 2 treatments, one control and the other with 25% more energy in the diet in the final third of gestation until weaning. The calves did not show any difference in weight at weaning, or at the

end of feedlot finishing, and did not differ in carcass weight (Wilson et al., 2016). On the other hand, both those authors and our study showed no interference in the average daily weight gain of the offspring from feeding the mother with high energy diet.

As shown in Table 3, the CTL and LE groups obtained the lowest weights, but the average daily weight gain did not differ. This corroborates with the literature, where authors observed that the general restriction of nutrients or the restriction of metabolizable energy, greater than the present experiment (between 30 – 50%), in different gestation phases did not affect offspring performance during the feedlot phase (Daniel et al., 2007; Geraseev et al., 2006; Ithurralde et al, 2019; Piaggio et al., 2018; Sibbald and Davidson, 1998). The factor that most influences the animal during confinement would be its initial weight, which is consequently influenced by the period of food restriction that the offspring may have suffered from birth to weaning (Bohnert et al., 2013; Café et al., 2009; Ithurralde et al, 2019; Mulliniks et al., 2016).

Lambs from a single gestation entered the feedlot heavier and maintained this pattern until slaughter (Table 3). It is known that for twin pregnancies the total mass of the placenta, as well as the ability to exchange nutrients is reduced, causing fetal growth restriction (Casellas and Caja, 2014; Cleal et al., 2007; Gootwine et al., 2007; Greenwood et al., 2000; McCoard et al., 2000; Rhind et al., 1980; Sales et al., 2018). This growth delay was not compensated until the time of slaughter, even though the performance of the lambs was not affected by the type of birth, since the daily weight gain did not differ (Table 3), confirming what was discussed above, that the weight of entry into the feedlot was determinant of the performance.

Despite the higher carcass weight of lambs from a high energy maternal diet, it was observed that dressing was higher in lambs from the CTL group, which may indicate that lambs from a mother with a high energy diet accumulate more fat overall and particularly visceral fat. The data from the present study do not reveal significant differences for omental and mesenteric fat and fat thickness, but there was a tendency for high energy treatments to have greater

measures and weight for the analyzed variables (Table 3). Results regarding fattening are extremely variable from experiment to experiment, since the effects of overnutrition during pregnancy are strongly linked not only to nutrition itself, but also to genotype, age of the lamb, body score of the sheep, and when and how much the sheep gained weight during pregnancy (Bell and Greenwood, 2016).

As stated above, there was no significant difference between the fat thickness, but it can be observed the tendency of the LE group to have a higher value than CTL, showing that growth can be impaired by the restriction, but the accumulation of subcutaneous fat is not affected (Daniel et al., 2007; Greenwood et al., 2004; Piaggio et al., 2018).

It can be seen in Table 3 that there was no difference in the loin eye area (LEA) for any of the parameters analyzed ($P > 0.05$) using cold carcass weight as covariable. Therefore, the carcasses from lambs coming from a high maternal energy diet during pregnancy/lactation, especially the STCR, presented bigger LEA. The data in literature are quite varied, and there are studies that show that both maternal over-nutrition and restriction can cause a reduction in the rib eye area, or have no effect on this characteristic (Hoffman et al., 2016; Huang et al., 2010; Ithurralde et al., 2019; Piaggio et al., 2018; Tong et al., 2009; Yan et al., 2013; Zhu et al., 2004, 2006).

There was no difference between carcass temperatures at slaughter, being within the expected range (Gardner et al., 2006), close to body temperature. The 24h temperature also showed no difference. There was also no significant difference between pH at 0h, and all of them were within the expected range close to neutral (Sañudo et al., 1992; Savell et al., 2005). The pH measured at 24h also showed no significant difference between the samples, and was within the expected range, between 5.3 - 5.8 (Sañudo et al., 1992; Savell et al., 2005), with values very close to those found for lambs in conditions similar to the present experiment (Jucá

et al., 2016; Souza et al., 2016). Lambs from different types of birth also showed no difference and are also within the values shown previously.

The weight for the liver showed no difference for any of the variables analyzed (Table 3). Long et al. (2009) observed that bovine fetuses that suffered restriction at the beginning of pregnancy did not show any difference. Another experiment showed that fetuses of sheep that suffered restriction at the beginning of pregnancy, regardless of whether they came from a single or double pregnancy, did not show any difference between the weight of the liver either (Cleal et al., 2007). In addition, organs such as the brain, heart and liver have a higher priority in the partition of nutrients during fetal development, when there is some disturbance in the availability of nutrients (Du et al., 2010; Zhu et al., 2006), they may not be as affected by changes in the maternal diet.

3.4.3. Meat quality parameters

The thawing loss was lower in the muscle of lambs from LE (1.8%) and STCR (3.7%) treatments (Table 4). However, as previously mentioned, there was no difference between the pH's and these were within the expected range. However, the pH drop was not followed in the present experiment, and the extent of the pH drop is important in the water retention capacity; if the pH drop is too fast while the carcass is still warm it will cause a denaturation of proteins that maintain the myosin bridges, and they will shrink and reduce the myofibrillar space, thus the water will be expelled increasing the drip loss (Oksbjerg et al., 2013; Young et al., 2009). This may have caused a great loss of water before the muscles' were collected. The type of pregnancy did not influence this factor ($P > 0.05$, Table 4).

There was also a significant difference in cooking losses, where the muscle coming from lambs under the STCR, ST and CTL treatment showed greater losses (35.8%, 33.7%, 33.1% respectively) and STFP showed less loss (28.9%). However, despite the difference, the

parameters are within what was seen in the literature, where lambs of the Santa Inês breed or lambs ½ Santa Inês X Dorper blood, reared in systems similar to the present study, presented cooking loss values between 27.7% and 40.1% (Costa et al., 2009; Souza et al., 2016). The type of birth also did not influence this factor (*P > 0.05*, Table 4), being within the parameters mentioned above.

There was no difference for any of the variables studied for shear force, MFI and sarcomere length (*P > 0.05*, Table). The literature shows that for sheep the restriction of ewes' food during pregnancy did not alter meat quality parameters (Krausgrill et al., 1999; Nordby et al., 1987; Piaggio et al., 2018; Tygesen et al., 2007). For cattle, these data vary a lot (Alvarenga et al., 2016; Blair et al., 2013; Maresca et al., 2019; Mohrhauser et al., 2015; Robinson et al., 2013; Underwood et al., 2010). However, there are no specific data for meat quality in overfed animals, however in the literature the data show that supplementation of animals or an obesogenic diet improves adipogenesis in the fetal period, increasing intramuscular adipocytes (Bee 2004; Duarte et al., 2014; Long et al., 2010; Tong et al., 2009; Yan et al., 2011; Zhu et al., 2008), consequently increasing the marbling of meat and its sensory characteristics, however, unfortunately the present study did not make this assessment.

There was also no difference in the concentration of collagen for any of the parameters analyzed *(P > 0.05,* Table 4), but it can be seen that the concentration of collagen tended to be higher for meat from lambs coming from the STFP treatment. In work with cattle that were overfed, there was an increase in the collagen gene expression in the Longissimus muscle (Duarte et al., 2014), whereas in work in which there was a restriction of feeding, the collagen concentration for the Longissimus muscle was not changed (Alvarenga et al., 2016; Maresca et al., 2019), whereas the concentration in semitendinosus muscle increased in animals with restricted feeding (Alvarenga et al., 2016). For pigs the restriction also caused an increase in collagen in the semitendinosus muscle of pigs (Karunaratne et al., 2005); it is known that

connective tissue varies in quantity and distribution among the different muscles, and that the semitendinosus muscle has a greater amount of connective tissue than Longissimus (Purslow, 2005; Rhee et al., 2004), perhaps this was also one of the factors that explains the absence of differences between the results, and that food restriction causes a delay in muscle fibrinogenesis (Du et al., 2010). In studies with 2.5 year old lambs (Huang et al., 2012) and fetal lambs (Huang et al., 2010), maternal overfeeding caused an increase in the amount of collagen in the Longissimus and Semitendinosus muscles, and this is manifest due to the inflammatory response that occurs in the offspring muscle from obese mothers which induces the expression of the transforming growth factor (TGF-β), and this stimulates fibrosis in the muscle, promoting an increase in connective tissue during muscle regeneration, and an increase the concentration of collagen in the muscle.

The average area of muscle fibers also did not show any difference for the analyzed parameters, however there was a tendency for lambs coming from STCR treatment and single gestation to have a larger area than the other treatments ($P > 0.05$, Table 4). The literature has shown that over-nutrition can negatively affect fiber size (Bayol, et al., 2005; Huang et al., 2010; Tong et al., 2009; Zhu et al., 2008). Nutrient deficiency during pregnancy can also cause a decrease in the size of muscle fibers (Du et al., 2010; Greenwood et al., 1999; Underwood et al., 2010), as well as in lambs from a twin pregnancy (McCoard et al., 2000). The fetal skeletal muscle is the largest insulin-sensitive tissue in the body and the primary site for the use of insulin-stimulated glucose (Beauchamp and Harper, 2016), and this may be an indicator for the tendency of the STCR treatment to have a larger area, since chromium can improve tissue energy utilization, as it increases tissue sensitivity to insulin (Davis and Vicent, 1997; Sumner et al., 2007; Vicent 2000), and this can also act as a growth factor (Brown, 2014). However, it should be noted that when you have a high insulin concentration, obesity can be an indicator of pre-disposition (Ford et al., 2007, 2009; Hoffman et al., 2016), that is, an increase in tissue

adipose rather than lean tissue in adulthood. However, as shown in Table 1, the insulin concentration was greater in treatments with higher energy, however lambs from the STCR treatment exhibited larger fiber areas, indicating that chromium may have improved the use of energy in muscle tissue.

3.5. Conclusion

It was concluded that malnutrition can affect the insulin-glucose axis, and that treatment with chromium can mitigate the consequences of overnutrition on insulin resistance. Moreover, chromium supplementation on maternal diet during gestation/lactation that would cause "metabolical syndrome" on offspring may contribute to regulated muscle and liver energy metabolism during lamb growth with no signs of physiological disturbances.

Meat quality was not affected by ewe's nutrition, however, further studies are needed on how maternal malnutrition, especially the mitigation by chromium supplementation, can affect myofibers size, connective tissue deposition within muscle with special attention to collagen and fat accumulation into the offspring muscles.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Annex 1: Tables

Table 1. Blood parameters (mean ± s.e.) for pre slaughter lambs from different treatments and type of birth.

Blood Parameters	Treatments					Type of Birth	
	CTL	LE	ST	STCR	STFP	Singular	Twins
Glucose (mg/dL)	88.3±2.01^{ab}	88.7±3.07^{ab}	92.8±2.01^{a}	83.3±1.84^{b}	90.1±2.51^{ab}	85.4±1.29^{b}	91.9±1.64^{a}
Insulin (ng/mL)	0.8±0.58^{b}	1.1±0.89^{ab}	2.7±0.58^{ab}	1.8±0.53^{ab}	3.1±0.69^{a}	1.8±0.36	2.1±0.47
Urea (mg/dL)	62.2±2.91^{ab}	69.0±4.45^{a}	56.4±2.91^{ab}	52.4±2.67^{b}	60.4±3.45^{ab}	58.4±1.80	61.8±2.38
Creatinine (mg/dL)	0.79±0.04	0.65±0.06	0.70±0.04	0.73±0.04	0.78±0.05	0.74±0.03	0.72±0.03
AST (U/L)	104.7±16.2	108.7±24.8	129.8±16.2	128.5±14.9	132.4±19.2	129.2±10.0	112.5±13.2
GGT (U/L)	61.3±4.48^{b}	63.7±6.85^{ab}	74.8±4.47^{ab}	74.3±4.11^{ab}	83.4±5.31^{a}	77.3±2.78^{a}	65.7±3.66^{b}
Total Protein (g/dL)	5.3±0.10^{b}	5.1±0.15^{b}	5.8±0.10^{a}	5.4±0.09^{b}	5.2±0.12^{b}	5.4±0.06	5.3±0.08
Albumin (g/dL)	3.08±0.07^{ab}	2.97±0.11^{ab}	3.31±0.07^{a}	3.08±0.07^{ab}	2.91±0.08^{b}	3.15±0.04^{a}	2.99±0.06^{b}
Cholesterol (mg/dL)	54.8±4.18	54.5±6.41	62.8±4.18	51.5±3.84	49.0±4.96	53.2±2.60	55.82±3.43
Triglycerides (mg/dL)	21.1±3.30	23.6±5.06	28.2±3.30	24.1±3.03	23.6±3.92	22.6±2.05	25.6±2.70
Phosphorus (mg/dL)	7.8±0.25^{b}	6.1±0.38^{c}	9.7±0.25^{a}	8.9±0.23^{a}	7.2±0.29^{bc}	7.6±0.15^{b}	8.3±0.20^{a}
Red blood cells (x10^{6}/µL)	11.9±0.44	11.4±0.68	12.7±0.44	11.8±0.41	11.8±0.52	12.4±0.27^{a}	11.4±0.36^{b}

LE = Low metabolizable energy; CTL = Control; ST = High metabolizable energy with starch; STFP = High energy metabolizable with starch and protected fat; STCR = High energy metabolizable with starch and chrome. ^{abc}Different letters in the row differ by Tukey´s test, $P < 0.05$. CK = creatine kinase; AST = Aspartate aminotransferase; GGT = Gamma glutamyltransferase.

Table 2. Interaction between treatment and type of birth (mean ± s.e.) for Creatine Kinase (U/L), Haemoglobin (g/dL) and Haematocrit (%).

Type of Birth	Treatments				
	CTL	LE	ST	STCR	STFP
	Creatine Kinase (U/L)				
Singular	180.4±23.01^{ab}	196.5±29.71^{ab}	217.3±29.71^{ab}	227.7±27.73^{a}	129.5±25.73^{b}
Twins	239.7±29.71^{a}	190.0±51.46^{ab}	236.8±23.01^{a}	164.0±23.01^{ab}	268.0±36.38^{a}
	Haemoglobin (g/dL)				
Singular	11.0±0.44^{ab}	12.4±0.49^{ab}	12.4±0.56^{ab}	11.2±0.49^{ab}	12.6±0.49^{a}
Twins	11.7±0.56^{ab}	9.9±0.98^{ab}	11.7±0.44^{ab}	10.9±0.44^{ab}	9.5±0.69^{b}
	Haematocrit (%)				
Singular	32.3±1.17^{ab}	36.4±1.31^{a}	36.1±1.51^{a}	32.1±1.31^{ab}	35.2±1.31^{ab}
Twins	34.3±1.51^{ab}	30.3±2.62^{ab}	33.2±1.17^{ab}	31.5±1.17^{ab}	27.6±1.85^{b}

LE = Low metabolizable energy; CTL = Control; ST = High metabolizable energy with starch; STFP = High energy metabolizable with starch and protected fat; STCR = High energy metabolizable with starch and chrome. ^{AB}Different letters in the column differ by Tukey´s test, $P < 0.05$. ^{ab}Different letters in the row differ by Tukey´s test, $P < 0.05$.

Table 3. Feedlot performance and carcass parameters (mean ± s.e.) for lambs from different treatments and type of birth.

	Treatments					Type of Birth	
	CTL	LE	ST	STCR	STFP	Singular	Twins
Initial weight (kg)	18.6±1.51[b]	20.4±2.32[ab]	22.7±1.39[ab]	27.3±1.39[a]	26.1±1.80[a]	26.0±0.94[a]	20.0±1.24[b]
Final weight (kg)	36.4±1.74[b]	40.5±2.67[ab]	42.6±1.74[ab]	46.8±1.60[a]	46.3±2.07[a]	45.3±1.08[a]	39.8±1.43[b]
Daily Weight gain (kg/d)	0.32±0.01	0.36±0.02	0.35±0.01	0.35±0.01	0.36±0.01	0.34±0.01	0.35±0.01
Hot carcass weight (kg)	17.6±1.05[b]	19.2±1.60[ab]	20.2±1.05[ab]	23.0±0.96[a]	22.1±1.24[ab]	22.1±0.65[a]	18.8±0.86[b]
Cold carcass weight (kg)	17.1±1.02[b]	18.6±1.56[ab]	19.6±1.02[ab]	22.3±0.94[a]	21.3±1.21[ab]	21.4±0.63[a]	18.2±0.84[b]
Fat thickness (mm)	1.2±0.21	1.6±0.24	1.9±0.18	1.7±0.19	1.9±0.22	1.8±0.12	1.5±0.15
Dressing (%)	48.2±0.52[a]	46.9±0.59[ab]	46.6±0.46[ab]	46.4±0.46[ab]	45.4±0.54[b]	46.7±0.31	46.6±0.37
Loin eye area (cm^2)	17.7±1.79	16.7±2.31	18.1±1.79	20.1±1.46	20.0±1.89	18.9±1.04	18.1±1.31
Temperature 0h (ºC)	32.6±0.74	32.5±1.14	33.2±0.74	35.1±0.68	34.4±0.88	33.8±0.46	33.31±0.61
Temperature 24h (ºC)	9.8±0.48	8.4±0.74	8.5±0.48	8.1±0.44	8.6±0.57	8.8±0.30	8.6±0.40
pH 0h	6.57±0.09	6.44±0.11	6.46±0.08	6.48±0.08	6.38±0.10	6.47±0.05	6.46±0.06
pH 24h	5.72±0.04	5.60±0.05	5.65±0.04	5.55±0.04	5.64±0.04	5.63±0.02	5.63±0.03
Omental and mesenteric fat (kg)	1.2±0.20	1.2±0.23	1.3±0.18	1.7±0.18	1.4±0.21	1.4±0.11	1.3±0.14
Liver weight (kg)	0.8±0.05	0.9±0.05	0.9±0.04	0.8±0.04	0.8±0.05	0.8±0.03	0.9±0.03

LE = Low metabolizable energy; CTL = Control; ST = High metabolizable energy with starch; STFP = High energy metabolizable with starch and protected fat; STCR = High energy metabolizable with starch and chrome. [ab]Different letters in the row differ by Tukey´s test, $P < 0.05$.

Table 4. Meat quality parameters (mean ± s.e.) for lambs from different treatments and type of birth.

Meat Quality Parameters	Treatments					Type of Birth	
	CTL	LE	ST	STCR	STFP	Singular	Twins
Thaw loss (%)	7.6±0.75[a]	1.8±0.91[c]	6.5±0.70[ab]	3.7±0.70[bc]	6.5±0.81[ab]	5.3±0.44	5.2±0.52
Cooking loss (%)	33.3±1.03[a]	32.5±1.25[ab]	33.7±0.96[a]	35.8±0.96[a]	28.9±1.11[b]	33.0±061	32.7±0.72
Shear force (N)	59.8±4.9	62.8±7.5	54.9±4.9	65.7±4.5	66.7±5.8	62.8±3.0	61.8±4.0
MFI	72.5±6.48	75.7±9.92	67.2±6.48	70.0±5.95	69.7±7.68	69.0±4.01	73.0±5.30
Sarcomere length (µm)	1.7±0.03	1.7±0.05	1.7±0.03	1.7±0.03	1.7±0.04	1.7±0.02	1.7±0.02
Collagen total (mg/g)	13.7±2.03	13.2±2.31	11.1±1.77	12.4±1.80	16.9±2.10	12.4±1.18	14.5±1.43
Collagen soluble (mg/g)	6.2±0.90	6.1±1.02	5.4±0.78	5.5±0.80	8.1±0.93	5.7±0.52	6.8±0.63
Collagen insoluble (mg/g)	7.5±1.78	7.1±2.02	5.6±1.55	6.9±1.58	8.8±1.85	6.6±1.03	7.8±1.25
Fiber área (μm^2)	1030.7±193.06	1673.9±256.99	1624.8±184.76	1733.1±167.69	1405.6±193.06	1598.4±100.38	1388.8±170.02

LE = Low metabolizable energy; CTL = Control; ST = High metabolizable energy with starch; STFP = High energy metabolizable with starch and protected fat; STCR = High energy metabolizable with starch and chrome. [ab]Different letters in the row differ by Tukey´s test, $P < 0.05$. MFI = myofibrillar fragmentation index.