





Biochemical parameters and assessment of passive immunity transfer in elite Santa Inês lambs born from single and twin births

Parâmetros bioquímicos e avaliação da transferência de imunidade passiva em cordeiros Santa Inês de elite nascidos de partos simples e gemelar

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Abstract: Colostrum intake within the first hours of life is essential for neonatal survival. Lambs born from twin pregnancies may experience failure in passive immunity transfer (PIT), which can affect the serum concentrations of several metabolites. This study aimed to evaluate serum biochemical constituents and PIT in Santa Inês lambs born from single (Sg; n=19) and twin (Tw; n=28) births. Blood samples were collected at 0, 24, 48, 72, and 96 hours after birth, and analyzed for total protein, albumin, globulin, urea, creatinine, cholesterol, triglycerides, calcium (Ca), phosphorus (P), magnesium (Mg), and the enzymatic activities of aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT). Lambs in the GS group showed higher birth weight (3.98 kg vs. 3.03 kg; $p<0.05$), total protein (6.12 g/dL vs. 5.32 g/dL), globulin (4.35 g/dL vs. 3.64 g/dL), and GGT (322.93 U/L vs. 543.90 U/L) at 24 hours post-partum. Considering the association between globulin and immunoglobulin levels, the data suggest partial PIT failure in twin-born lambs. Additionally, significant differences ($p<0.05$) were observed in the mean values of albumin (1.87 vs. 1.80 g/dL), P (7.92 vs. 7.24 mg/dL), and Mg (2.51 vs. 2.37 mg/dL), with higher levels in the GS group. The remaining parameters remained within the reference ranges for the species. It is concluded that lambs from twin pregnancies are at greater risk of PIT failure and present lower serum levels of constituents related to neonatal immunity and metabolism, highlighting the importance of assisted colostrum management in such cases.

Key-words: colostrum; liver enzymes; minerals; neonates; total protein.

Resumo: A ingestão de colostro nas primeiras horas de vida é fundamental para a sobrevivência do neonato. Os cordeiros oriundos de partos gêmeares podem apresentar falha na transferência de imunidade passiva (TIP) com reflexo nas concentrações séricas de diversos metabólitos. Este estudo objetivou avaliar



os constituintes séricos e a TIP em cordeiros Santa Inês nascidos de partos simples (GS; n=19) e gemelares (GG; n=28). Amostras de sangue foram coletadas nas 0, 24, 48, 72 e 96 horas pós-nascimento, e analisadas quanto à proteína total, albumina, globulina, ureia, creatinina, colesterol, triglicerídeos, cálcio (Ca), fósforo (P), magnésio (Mg) e atividades das enzimas aspartato aminotransferase (AST) e gama-glutamilttransferase (GGT). Cordeiros do grupo GS apresentaram maior peso ao nascer (3,98 kg vs. 3,03 kg; $p<0,05$), proteína total (6,12 g/dL vs. 5,32 g/dL), globulina (4,35 g/dL vs. 3,64 g/dL) e GGT (322,93 U/L vs. 543,90 U/L) 24 horas após o parto. Considerando a associação entre globulina e imunoglobulinas, os dados indicam falha parcial na TIP em cordeiros gemelares. Além disso, observou-se diferença significativa ($p<0,05$) nos níveis médios de albumina (1,87 vs. 1,80 g/dL), P (7,92 vs. 7,24 mg/dL) e Mg (2,51 vs. 2,37 mg/dL), favorecendo o grupo GS. Os demais parâmetros mantiveram-se dentro da faixa de referência para a espécie. Conclui-se que cordeiros oriundos de partos gemelares apresentam maior risco de falha na TIP e menores valores de constituintes séricos relacionados à imunidade e ao metabolismo neonatal, sugerindo a importância de estratégias de colostragem assistida nesses casos.

Palavras-chave: colostro; enzimas hepáticas; minerais; neonatos; proteína total.

1. Introduction

The first few hours after birth are critical for lamb survival, and adequate quality and volume of colostrum intake are essential for this outcome⁽¹⁾. Colostrum is a vital source of nutrients, immunoglobulins, leukocytes, and cytokines, and contains a complex mixture of proteins, lipids, vitamins, lactose, minerals, hormones, enzymes, and other peptides⁽²⁾. Lambs should ingest at least 30 g of immunoglobulin G (IgG) within the first 24 hours of life to ensure adequate passive immunity transfer (PIT)⁽³⁾.

Colostrum volume generally increases with ewe age; thus, primiparous ewes carrying twins may fail to provide adequate colostrum⁽⁴⁾. Lambs from multiple pregnancies face further challenges, because they are usually smaller and weaker, which may limit their ability to ingest sufficient colostrum⁽⁵⁾. Conversely, multiparous ewes carrying triplets may produce lower-quality colostrum, as indicated by reduced Brix values, reflecting lower IgG intake by lambs⁽⁶⁾. Some sheep breeds such as Santa Inês—originating from the northeastern region of Brazil and characterized by rusticity, strong maternal ability, and high reproductive rates—require special attention regarding colostrum feeding in twin lambs⁽⁷⁾.

Although milk yield tends to be higher in ewes with multiple pregnancies, chemical composition does not change significantly⁽⁷⁾. Studies have shown that twin-born lambs experience significant fluctuations in their biochemical indices during the first days of life^(6,8–10). The evaluation of biochemical constituents in newborn lambs helps establish and interpret reference values for this age group, as these values differ from those of adult animals^(13–16).

In this context, the present study aimed to determine the biochemical parameters and evaluate PIT in Santa Inês lambs born from single and twin pregnancies.

2. Material and methods

2.1 Study area and animals

The experimental protocol was approved by the Animal Ethics Committee (protocol no. 5/2017). The study was conducted on a purebred Santa Inês sheep farm (purebred of origin, PO) located in the municipality of Estância, in the eastern Sergipe mesoregion (11°16'07" S; 37°26'32" W), in a transitional

zone between the Agreste and coastal Zona da Mata regions of southern Sergipe State, Brazil. The area is characterized by soil and climate conditions typical of a tropical subhumid climate, with an average annual temperature of 24.9 °C and mean annual rainfall of 1,400 mm. The flock comprised 970 ewes, including donors, breeding females, and recipients of high zootechnical value. Five breeding rams were managed under intensive and semi-intensive production systems, with emphasis on the selection and sale of breeding males and females.

A total of 69 ewes, aged between 1.5 and 7 years (4.05 ± 1.49), with a mean body weight of 67.23 ± 10.97 kg and a body condition score (BCS) of 3 to 3.5 (3.35 ± 0.49), were enrolled in the study. Mucous membrane coloration ranged from score 4 to 5 according to the Famacha® system, and fecal egg counts (FEC) were below 500 eggs per gram (EPG) of feces ⁽¹⁶⁾. The animals were housed in collective pens with sand-bedded floors and received standardized feeding management. The roughage diet consisted of elephant grass (*Pennisetum purpureum*), forage maize (*Zea mays*), gliricidia (*Gliricidia sepium*), and banana pseudostems (*Musa paradisiaca*). They received a concentrate composed of wheat bran, soybean meal, ground maize, and a mineral premix as a supplement. Chopped roughage mixed with concentrate was provided *ad libitum* twice daily in the feed troughs.

2.1.1 Reproductive management

For estrus synchronization and natural mating, the ewes were subjected to a hormonal protocol for ovulation synchronization, consisting of the insertion of intravaginal sponges containing medroxyprogesterone acetate (Progespon®, Zoetis, Brazil) on day 0, which were removed on day 14, followed by the intramuscular administration of 1.25 mg sodium cloprostenol (Sincrocio®, Ouro Fino, Brazil). After sponge removal, teaser rams fitted with marking harnesses were used to identify ewes in estrus, which were then naturally mated 12 h later with one of the four rams previously approved through andrological examination.

Pregnancy was confirmed in 34 ewes by transrectal ultrasonography performed 40 days after natural mating. Two weeks before lambing, ewes were moved to a maternity barn equipped with individual pens. No animals exhibited clinical signs of toxemia or other systemic diseases. The mammary glands were examined and palpated to detect either unilateral or bilateral mastitis.

All lambings occurred without the need for cesarean sections. To standardize the experimental groups, one ewe that gave birth to quintuplets was excluded. Two groups were established: 19 lambs from single births (Sg) and 28 lambs from twin births (Tw). The lambs remained in continuous contact with their respective dams, and colostrum intake occurred naturally without intervention. Following the first suckling, the umbilical cords were disinfected with 10% iodine solution. Individual lamb records included the date and time of birth, sex, litter size, and birth weight.

2.2 Sample collection and biochemical analysis

A total of 4 mL of blood was collected from each lamb into vacuum tubes via jugular vein puncture using a 25 × 0.8 mm hypodermic needle at five time points: immediately after birth (0 h) and after colostrum ingestion at 24, 48, 72, and 96 h. Samples were centrifuged at 3,000 g for 10 min to obtain serum, which was subsequently aliquoted into 2 mL microtubes and stored at –20 °C until analysis.

Serum aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) activities, along with serum concentrations of total protein, albumin, urea, creatinine, cholesterol, triglycerides, calcium (Ca), phosphorus (P), and magnesium (Mg), were measured. Globulin concentrations were calculated by subtracting albumin from the total protein values. GGT, total protein, albumin, and globulin levels were used as indicators of PIT. Analyses were performed using the kinetic method with commercial reagents (Labtest Diagnóstica S/A, Minas Gerais, Brazil) according to the manufacturer's instructions, on a LABMAX 240® automated biochemical analyzer (Labtest). All assays were conducted at the Laboratory of Deficiency and Metabolic Diseases of Domestic Animals, Department of Veterinary Medicine, Federal Rural University of Pernambuco, Recife, Brazil.

2.3 Statistical Analysis

Data were analyzed using the Statistical Analysis System software (version 9.4; SAS Institute Inc., Cary, NC, USA, 2015) and tested for normality and homoscedasticity. Because the parameters did not meet the assumption of residual normality, even after attempted transformations, the data were subjected to the nonparametric Kruskal–Wallis test to assess differences between groups and time points. The results are expressed as mean \pm standard error, with a 95% confidence interval. The level of significance for all analyses was set at $p < 0.05$.

3. Results and discussion

The neonatal period is a critical stage that requires specific care for the newborn, as numerous morphological and functional changes occur that enable adaptation to extrauterine life. In sheep, the syndesmochorial placenta limits the transplacental transfer of immunoglobulins from the dam to the fetus, resulting in lambs being born with hypogammaglobulinemia⁽¹⁷⁾. Colostrum ingestion within the first hours of life is essential for survival because it meets nutritional and metabolic demands while providing immune protection⁽¹⁸⁾. However, lambs from twin pregnancies face greater challenges, and the success of passive immunity transfer (PIT) may be compromised in low-birthweight or lethargic neonates. In the present study, single-born lambs had a higher mean birth weight compared with twins—3.98 kg versus 3.03 kg ($p < 0.05$)—consistent with previous reports^(6, 10, 11, 19). The risk of PIT failure increases in lambs weighing less than 3 kg and in those from multiple births⁽¹⁰⁾.

Total serum protein level can serve as an indirect estimate of immunoglobulin concentration, due to the strong association between these two variables⁽²⁰⁾. Thus, the total protein assessment can serve as an indirect predictor of PIT failure. In both experimental groups, total serum protein concentrations increased significantly at 24 h post-colostrum ingestion ($p < 0.05$), reaching mean values of 6.12 g/dL in the single-birth group (GSg) and 5.32 g/dL in the twin-birth group (GTw), then stabilized thereafter (Table 1).

Table 1. Mean values and standard error of the mean of serum constituents of total protein, albumin, globulin (g/dL), urea, and creatinine (mg/dL) of Santa Inês lambs born from single and twin births at 0, 24, 48, 72, and 96 hours of life.

Parameter	Birth type	Valuation time points (hours)					SEM	p
		0	24	48	72	96		
Total protein (g/dL)	Sg	4.34 ^b	6.12 ^a	5.76 ^a	5.52 ^a	5.30 ^a	0.22	0.0032
	Tw	4.44 ^b	5.32 ^a	5.11 ^a	5.02 ^a	5.21 ^a	0.18	<.0001
	T	4.40 ^b	5.72 ^a	5.44 ^a	5.27 ^a	5.26 ^a	0.14	<.0001
Albumin (g/dL)	Sg	2.01 ^a	1.77 ^b	1.90 ^{ab}	1.84 ^{ab}	1.84 ^{ab}	0.05	0.0562
	Tw	1.94 ^a	1.68 ^b	1.74 ^b	1.75 ^b	1.82 ^b	0.41	0.0002
	T	1.97 ^a	1.72 ^b	1.81 ^b	1.79 ^b	1.83 ^b	0.33	<.0001
Globulin (g/dL)	Sg	2.33 ^b	4.35 ^a	3.87 ^a	3.67 ^a	3.46 ^a	0.22	0.0003
	Tw	2.50 ^b	3.64 ^a	3.36 ^a	3.26 ^a	3.39 ^a	0.18	<.0001
	T	2.42 ^b	3.99 ^a	3.62 ^a	3.47 ^a	3.42 ^a	0.14	<.0001
Urea (mg/dL)	Sg	34.98 ^b	69.79 ^a	98.73 ^a	87.71 ^a	71.76 ^a	7.71	<.0001
	Tw	36.07 ^d	79.51 ^b	107.02 ^a	85.14 ^b	66.12 ^c	6.35	<.0001
	T	35.53 ^d	74.65 ^{bc}	102.88 ^a	86.43 ^b	68.94 ^c	4.99	<.0001
Creatinine (mg/dL)	Sg	1.73 ^a	0.99 ^b	0.58 ^{bc}	0.54 ^c	0.50 ^c	0.12	<.0001
	Tw	1.91 ^a	0.67 ^b	0.54 ^c	0.50 ^c	0.46 ^c	0.09	<.0001
	T	1.81 ^a	0.83 ^b	0.56 ^c	0.51 ^{cd}	0.48 ^d	0.08	<.0001

Sg = single birth, Tw = twin birth, T = all births (single and twin), and SEM = standard error of the mean. *Values followed by different lowercase letters in the same row differ significantly from each other ($p < 0.05$).

This post-colostrum increase was mainly attributable to the intestinal absorption of immunoglobulins, consistent with other studies reporting higher immunoglobulin concentrations in single-born lambs (9,11,17,21). Based on Turquino *et al.* (10), who defined total protein concentrations between 5.1 and 6.0 g/dL as indicative of partial PIT failure, the GTw lambs in this study likely did not ingest an adequate colostrum volume. Chagas *et al.* (6) also reported partial PIT failure in triplet lambs. Greater competition for nursing among the less vigorous twin lambs may contribute to reduced colostrum intake.

Although the present study did not include a physicochemical analysis of colostrum, its composition directly influenced neonatal biochemical parameters and PIT efficiency. Factors such as density, immunoglobulin content, lipid content, and total protein content, vary according to age, body condition score, parity, and litter size (3,4). In twin births, increased colostrum demand, combined with potentially lower IgG and lipid concentrations, may impair both immune and energy transfer, resulting in reduced serum total protein, albumin, and globulin levels during the first days of life. Therefore, it is plausible that differences in colostrum composition and volume between single and twin births contributed to the observed variations, underscoring the importance of management strategies for supplementary feeding or assisted colostrum administration.

Albumin concentrations decreased significantly in both GSg and GTw lambs at 24 h post-colostrum ingestion ($p < 0.05$), with minor variations thereafter. Similar findings have been reported by Silva *et al.* (21) in Dorper \times Santa Inês crossbred lambs. This decline during the first few hours after birth was likely due to rapid plasma volume expansion following colostrum ingestion (22). Conversely, globulin concentrations increased sharply after colostrum intake, reaching 4.35 g/dL in GSg lambs and 3.64 g/dL in GTw lambs. In Santa Inês ewes, immunoglobulins account for approximately half of the total protein concentration in the colostrum (23), explaining this early life increase.

Urea is a useful indicator of dietary protein intake and is produced in the liver via the urea cycle to detoxify ammonia, a byproduct of protein catabolism ⁽²⁴⁾. In the present study, urea concentrations increased significantly ($p < 0.05$) at 24 h, peaking at 48 h postpartum in GSg (98.73 mg/dL) and GTw (79.51 mg/dL). This increase reflects the metabolism of colostral proteins ⁽²⁵⁾. Creatinine, formed by phosphocreatine breakdown to supply energy to skeletal muscle ⁽²⁶⁾, was highest in both groups at birth compared to post-colostrum values. This may be related to an expansion in plasma volume after the first feeding and the onset of glomerular filtration ⁽¹²⁾. Elevated creatinine levels at birth likely reflect increased creatine metabolism due to higher energy demands for muscular activity immediately postpartum ⁽²⁷⁾.

As shown in Table 2, serum AST activity increased significantly ($p < 0.05$) in GSg (138.46 U/L) and GTw (145.96 U/L) groups at 24 h, followed by a decline by day two. This elevation may result from the presence of AST in colostrum and its subsequent intestinal absorption ⁽²⁸⁾. However, AST should not be used as a stand-alone PIT predictor because its concentration may not correlate with serum IgG levels; increases may instead be attributable to endogenous production ⁽²⁹⁾.

Table 2. Mean values and standard error of the mean of serum constituents of AST, GGT (U/L), cholesterol, and triglycerides (mg/dL) of Santa Inês lambs born from single and twin births at 0, 24, 48, 72, and 96 hours of life.

Parameter	Birth type	Valuation time points (hours)					SEM	p
		0	24	48	72	96		
AST (U/L)	Sg	36.47 ^c	138.46 ^a	64.39 ^b	60.88 ^b	63.63 ^b	14.54	<.0001
	Tw	42.70 ^c	145.96 ^a	71.86 ^b	90.65 ^b	85.17 ^b	11.97	<.0001
	T	39.59 ^c	142.21 ^a	68.12 ^b	75.76 ^b	74.40 ^b	9.42	<.0001
GGT (U/L)	Sg	97.57 ^b	322.93 ^a	699.20 ^a	482.32 ^a	346.95 ^a	83.56	<.0001
	Tw	66.72 ^c	543.90 ^{ab}	663.37 ^a	475.79 ^{ab}	396.58 ^b	68.83	<.0001
	T	82.12 ^c	433.41 ^{ab}	681.29 ^a	479.05 ^{ab}	371.77 ^b	54.13	<.0001
Cholesterol (mg/dL)	Sg	19.56 ^{bc}	14.61 ^c	43.42 ^a	29.48 ^b	27.83 ^b	3.55	<.0001
	Tw	22.44±2 ^{bc}	14.79 ^c	36.23 ^a	22.60 ^{bc}	27.37 ^b	2.93	0.0002
	T	21.00 ^b	14.70 ^c	39.83 ^a	26.04 ^b	29.00 ^b	2.44	<.0001
Triglycerides (mg/dL)	Sg	31.87 ^b	60.36 ^{ab}	74.32 ^a	79.85 ^a	58.80 ^{ab}	11.88	0,0070
	Tw	43.19 ^a	65.52 ^a	57.97 ^a	67.26 ^a	64.60 ^a	9.78	0,2430
	T	37.53 ^b	62.94 ^{ab}	66.14 ^a	73.55 ^a	61.70 ^a	7.69	0,0015

Sg = single birth, Tw = twin birth, T = all births (single and twin), and SEM = standard error of the mean. *Values followed by different lowercase letters in the same row differ significantly from each other ($p < 0.05$).

In the present study, GGT activity increased significantly ($p < 0.05$) from pre-colostrum to 24 h post-ingestion in both groups. Several authors have regarded GGT activity as a reliable PIT indicator ^(28–31). Gokce *et al.* ⁽³¹⁾ proposed that serum GGT values > 500 U/L at 24 h indicate adequate PIT in lambs. Despite the higher GGT activity in GG lambs, they were classified as having partial PIT failure, as previously discussed. This weak correlation between GGT activity and IgG concentration after colostrum ingestion was previously noted by Silva *et al.* ⁽³²⁾, who observed a low correlation in goat kids.

The primary energy source of colostrum is fat, which leads to elevated plasma lipid levels ⁽³³⁾ and consequent increases in triglyceride and cholesterol concentrations in lambs after ingestion ^(25,34). In this study, triglyceride concentrations peaked over the first three days in both groups, consistent with the findings of Oztabaki and Ozpinar ⁽³⁵⁾. This dynamic aligns with the colostrum fat profile of Santa Inês ewes, in which a linear decline occurs within the first 36 h postpartum ⁽³⁾.

In the present study, electrolyte concentrations (Ca, P, and Mg) were within the normal reference ranges for neonatal lambs ⁽¹³⁾, with minimal variation between the groups and time points (Table 3). In ewes, these mineral concentrations remain unchanged up to 24 h postpartum, with no compensatory increase in those bearing twins or triplets ⁽³⁶⁾. For P and Mg, the reference intervals differ markedly between neonates (0–6 months) and adults, whereas Ca values are similar across age groups ⁽¹³⁾.

Table 3. Mean values and standard error of the mean of serum constituents of calcium, phosphorus, and magnesium (mg/dL) of Santa Inês lambs born from single and twin births at 0, 24, 48, 72, and 96 hours of life.

Parameter	Birth type	Valuation time points (hours)					SEM	p
		0	24	48	72	96		
Calcium (mg/dL)	Sg	9.64 ^{ab}	9.49 ^{ab}	9.93 ^{ab}	10.22 ^a	9.25 ^b	0.22	0.0387
	Tw	10.08 ^a	9.29 ^a	9.61 ^a	10.01 ^a	9.69 ^a	0.18	0.0500
	T	9.86 ^{ab}	9.39 ^b	9.77 ^{ab}	10.12 ^a	9.47 ^b	0.14	0.003
Phosphorus (mg/dL)	Sg	6.16 ^b	7.70 ^a	8.46 ^a	8.65 ^a	8.61 ^a	0.32	<.0001
	Tw	6.09 ^c	6.67 ^b	7.64 ^a	7.52 ^a	8.24 ^a	0.26	<.0001
	T	6.13 ^c	7.19 ^b	8.05 ^a	8.09 ^a	8.43 ^a	0.21	<.0001
Magnesium(mg/dL)	Sg	2.14 ^b	2.49 ^b	2.30 ^b	2.75 ^a	2.84 ^a	0.97	<.0001
	Tw	2.07 ^d	2.25 ^c	2.16 ^{dc}	2.60 ^b	2.76 ^a	0.08	<.0001
	T	2.11 ^d	2.37 ^c	2.23 ^{cd}	2.67 ^b	2.80 ^a	0.06	<.0001

Sg = single birth, Tw = twin birth, T = all births (single and twin), and SEM = standard error of the mean. *Values followed by different lowercase letters in the same row differ significantly from each other ($p < 0.05$).

As shown in Table 4, GSg lambs had higher mean concentrations of albumin (1.87 g/dL), P (7.92 mg/dL), and Mg (2.51 mg/dL) than GTw lambs ($p < 0.05$). Although no significant differences were found between the groups for total protein, globulin, creatinine, cholesterol, triglycerides, P, and Mg, the GSg values tended to be higher, possibly because of the absence of nursing competition. Future research should include colostrum physicochemical analysis and a third group of triplet-bearing ewes to assess the biochemical dynamics in neonatal lambs.

Table 4. Mean values and standard error of the mean of serum constituents of Santa Inês lambs born from single and twin births.

Parameter	Birth type		SEM	p	Reference ⁽¹³⁾
	Single	Twin			
Total protein (g/dL)	5.41	5.02	0.17	0.214	5.5 – 5.7
Albumin (g/dL)	1.87	1.8	0.043	0.034	3.3 – 3.4
Globulin (g/dL)	3.54	3.23	0.09	0.514	2.1 – 2.2
Urea (g/dL)	72.6	74.54	3.15	0.633	43.7 – 47.6
Creatinine (g/dL)	0.87	0.81	0.04	0.139	0.65 – 0.70
Cholesterol (g/dL)	27.62	25.5	1.53	0.6	64.71 – 73.09
Triglycerides (g/dL)	61.35	59.71	9.71	0.67	24.87 – 28.04
AST (U/L)	72.76	87.27	5.92	<0001	76.4 – 82
GGT (U/L)	389.8	429.27	34.07	0.18	61.7 – 66.6
Calcium (mg/dL)	9.7	9.74	0.09	0.859	6.62 – 13.48
Phosphorus (mg/dL)	7.92	7.24	0.263	0.009	4.97 – 13.1
Magnesium (mg/dL)	2.51	2.37	0.069	0.041	1.76 – 2.96

Legend: SEM = standard error of the mean.

4. Conclusion

The dynamics of biochemical parameters differed between lambs with single and twin pregnancies throughout the evaluation period. Twin-born lambs exhibited a partial failure of (PIT) and significantly lower concentrations of certain serum constituents, likely due to greater competition among littermates, lower birth weight, and reduced vigor for voluntary colostrum intake. For twin-born lambs, it is recommended to provide assisted suckling directly from the dam, hand milking, bottle feeding, and/or establish a farm-level colostrum bank to ensure adequate immune and nutritional support.

Conflicts of interest statement

The authors declare that there is no conflict of interest.

Data availability statement

The full dataset supporting the results of this study is available upon request from the corresponding author.

Author contributions

Conceptualization: H. Rizzo and J.S. Carvalho. Funding acquisition: H. Rizzo. Methodology: H. Rizzo, J.S. Carvalho, and C.M.V. Ulian. Resources: H. Rizzo, J.S. Carvalho, and C.M.V. Ulian. Supervision: H. Rizzo and C.M.V. Ulian. Writing – original draft: H. Rizzo and J.S. Carvalho. Writing – review & editing: H. Rizzo, J.S. Carvalho, C.M.V. Ulian, and V. Ribeiro Jr. Formal analysis: V. Ribeiro Jr. Data curation: G.O.A. Delfino, J.M.N. Oliveira, H.C.O. Menezes, and J.S. Carvalho. Investigation: G.O.A. Delfino, J.M.N. Oliveira, H.C.O. Menezes, C.M.V. Ulian, and J.S. Carvalho. Project administration: H. Rizzo and J.S. Carvalho.

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