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Preliminary Study of Microbiological and Immunological Quality of Sheep Colostrum: Influence on Early Postnatal Weight Change

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Abstract

Colostrum is crucial for the survival, development, and the future productivity of newborns. In this study, we evaluated the immunological and microbiological quality of colostrum in 28 Rasa Aragonesa ewes and its relationship with offspring growth during the first 48 h postpartum. Colostrum samples were collected by hand milking immediately after parturition. Immunoglobulin concentration was assessed using Brix refractometry and the samples were categorised according to their immunoglobulin content: high (>24 Brix value), medium (19–23 Brix value), and low (<19 Brix value). Bacterial counts of aerobes and coliforms were determined with the 3M Petrifilm™ system and the weight of the lambs was recorded using a digital suspension scale. The mean aerobic count (AC) was 3.63 ± 0.69 $\log_{10}$ CFU/mL after 24 h of incubation and the mean coliform count (CC) was 1.59 ± 0.82 $\log_{10}$ CFU/mL after 24 h of incubation. Colostrum with a high immunoglobulin concentration had lower aerobic count after 48 h of incubation than that with poor immunological quality. In relation to coliform counts, similar values were found in all groups. No significant differences were observed in terms of lamb weight gain according to colostrum quality. In conclusion, the immunological quality of colostrum affected the AC determined, but it did not affect CC or early postnatal lamb weight. These findings offer preliminary insights into the usefulness of the Petrifilm™ system in microbiological quality determination of colostrum and its relationship with immunological quality determined *in vitro*.

Keywords: ewe colostrum; microbiological quality; Petrifilm™; weight gain

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1. Introduction

Colostrum is the first secretion produced by the mammary gland after birth. It plays a crucial role in establishing passive immunity in newborns, especially in ruminants [1]. This fluid is rich in immunoglobulins, proteins, vitamins, minerals, and growth factors that are involved in a multitude of processes that determine lamb survival [2]. Colostrum not only performs an immunological function but also provides essential nutrients and contains microbiota that can affect lamb health [3,4]. Therefore, its quality must be understood from a multidimensional perspective that includes physicochemical, immunological, and microbiological aspects.

The immunological quality of colostrum is fundamentally defined by its immunoglobulin content, especially IgG, which represents more than 80% of the total [5]. The gold-standard techniques for quantifying immunoglobulins include radial immunodiffusion and

ELISA, which have been used in various studies to analyse immune transfer in sheep [6]. However, both methodologies have practical limitations: they require specific equipment and qualified technical personnel and are not feasible on farms [7]. As a result, several authors have proposed the use of more accessible tools such as the Brix refractometer [8], which is a practical tool for indirectly estimating the immunological content of colostrum, especially in field conditions. The Brix refractometer measures the refractive index of light, which is deflected when passing through media with different optical densities, and therefore the amount of light depends on the concentration of dissolved solids in the solution; thus, a higher concentration of solutes such as immunoglobulins, fats, lactose, and other proteins translates into higher values on the Brix scale [8]. Colostrum with Brix values $\geq$ 22 and 50 g/L of IgG is considered to have high immunological quality in bovines [9]. However, there is considerable variability in the Brix values of colostrum among different species and between breeds [10]. In addition, determining which IgG concentration threshold to use for ewe colostrum quality is not simple, as there is limited data to support either 20 g/L [10] or 50 g/L [11]. Therefore, the use of a colostrum bovine threshold for immunological quality evaluation in ovine colostrum is an exploratory classification approach and has some limitations such as different total protein content or colostrum intake [10].

On the other hand, recent studies have shown that in terms of physiological conditions, both milk and colostrum contain diverse bacteria (microbiota) [12,13]. These intramammary microbiota appear to play a role in local immune homeostasis, as well as in protecting against pathogenic microorganisms [12]. Ref. [14] characterised the microbiota using 16S rRNA massive sequencing, with their results identifying a common bacterial core (core microbiota) present in most samples, composed mainly of Staphylococcus, Lactobacillus, Corynebacterium, Streptococcus, Escherichia, and Shigella. These findings are relevant to the microbiological quality of colostrum, as its composition is directly influenced by the mammary gland health status at the time of calving [15]. Although the presence of certain bacteria does not necessarily imply a risk to the newborn, high concentrations or the predominance of pathogenic species can compromise colostrum safety, increase digestive infection risk, and reduce immunoglobulin absorption [16,17]. There is limited information on the threshold at which contamination can become problematic in sheep, although data used in cattle are often extrapolated [18,19]. The current standards for bacterial contamination in bovine colostrum are <100,000 CFU/mL aerobic count and <10,000 CFU/mL coliform count, with huge variations between dairy systems and colostrum management. These thresholds have not yet been validated in sheep colostrum. The bacteria count in colostrum were previously determined by different microbiological culture media; however, these laboratory techniques were costly and time consuming [19]. In recent years, a new simple method, the Petrifilm™ system (3M, Madrid, Spain), has been tested to measure colostrum contamination in cattle, with promising results [20,21].

The objective of this study was to describe the microbiological quality of colostrum produced by Rasa Aragonesa sheep using the Petrifilm™ system. In addition, we analysed the influence of the immunological quality of colostrum on the microbiological quality and early postnatal weight change in lambs.

2. Materials and Methods

2.1. Animals and Colostrum Sample Collection

The study was carried out in accordance with ARRIVE guidelines and Spanish Policy for Animal Protection RD 53/2013, which meets European Union Directive 2010/63/UE on animal protection. All experimental protocols were approved by the Ethical Committee of Animal Experimentation of the University of Zaragoza (nº PD24/24). This observational

study was conducted in a single farm with a total of 2000 Rasa Aragonesa breed sheep, which is located in La Muela (Zaragoza, Spain), during 3 weeks in February 2025. Reproductive management on the farm was based on natural mating with three parturitions every two years. Animals were in a semi-intensive system; they were moved to the pastures during the first 2/3 of the gestation period and were housed indoors in groups during the 1 month before parturition. Sheep were fed with a complete ration with roughage, silage, and concentrates and had free-access water. Lambing was monitored daily, and after birth, the ewes were moved to an individual space to enable mother and lamb care.

A total of 28 sheep (4.80 ± 0.73 years and 55 kg of weight) with twin births were involved in this study. This was a convenience sample from a single farm and no sample size calculation was performed. Colostrum were obtained by hand milking mammary glands within 2 h after parturition before suckling of lambs. The teat was not disinfected, but the first 2–3 streams of milk were discarded. Approximately 10 mL of colostrum were taken from each teat in 15 mL sterile tubes, avoiding contact with the udder skin. Duplicate samples were obtained. The analysis of colostrum quality was carried out within two hours of sample collection.

2.2. Colostrum Refractometry Test

The immunological quality of colostrum was assessed with the optical Brix refractometer (BRX-242, Hanstronik, Hong Han GmbH, Shenzhen, China; scale range: 0 to 32 Brix value). The refractometer was calibrated with distilled water before each analysis. Drops of colostrum were placed on the refractometer's glass prism, and the Brix values were measured. After analysing each sample, the refractometer was washed and dried. Colostrum was considered to be high in IgG content when the Brix values were greater than or equal to 24, to have middle levels of IgG when Brix values were between 19 and 23, and to have poor IgG content when the Brix values were lower than 19 [9]. This was an exploratory classification of colostrum quality according to the threshold of bovine colostrum used for internal comparison. All samples were tested in duplicate.

2.3. Colostrum Microbial Analysis

Once in the laboratory, colostrum samples were vortexed, diluted, and examined for aerobic count (AC) and coliform count (CC) using the corresponding Petrifilm™ system (3M, Madrid, Spain) according to the protocol described by [22]. The Petrifilm™ CCs were incubated for 24 h at 35 °C +/− 2 °C and ACs were incubated at 32 °C +/− 2 °C for 48 h according to the Petrifilm™ system protocol. After incubation, the growing bacterial colonies were counted and the number of colony-forming units per millilitre (CFU/mL) of colostrum was determined. The recommended quantification limits of the Petrifilm™ system range from 0 to 150–300 colonies (e.g., Aerobic (<100) or Coliforms (<150).

2.4. Lamb Weight Determination

Fifty-six lambs (twins from the same ewe) were weighed using a digital suspension scale (Venostal, Schoffel, De Goorn, The Netherlands). The values obtained were recorded manually on individual cards associated with each lamb and its mother by ear tag. The weight of the lambs was recorded twice during the first 6 h of life and 48 h postpartum.

2.5. Statistical Analysis

The statistical analysis was performed using SPSS version 22.0 for Windows (Chicago, IL, USA). The Shapiro–Wilk test was used to verify the normality of the studied parameters' values. The CFU counts did not follow a normal distribution; therefore, the values were transformed by natural logarithm in order to achieve normality and the homoscedasticity assumption for linear regression model use. Data from colostrum quality parameters and

lamb weights were analysed as individual observations using the ANOVA test. When analysis of variance showed a significant effect, values were compared using Tukey's HSD test. The data were expressed as mean value ± standard deviation (SD) and differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Immunological and Microbiological Characteristics of Sheep Colostrum

The immunological quality descriptive data expressed in Brix values and the microbiological quality (AC and CC) of sheep colostrum samples are described in Table 1. The average Brix value recorded in the colostrum samples was 22.67 ± 3.95, ranging from 16 to 30 Brix values. In relation to bacterial growth using 3M™ Petrifilm™ plates, the mean AC of colostrum was 3.63 ± 0.69 $\log_{10}$ CFU/mL, ranging from 2.00 to 4.72 $\log_{10}$ CFU/mL. The mean CC was 1.59 ± 0.82 $\log_{10}$ CFU/mL, ranging from 0 to 2.55 $\log_{10}$ CFU/mL.

Table 1. Descriptive statistics for Brix value and microbiological quality (aerobes count and coliform count after 24 h of incubation) in colostrum ewe samples (n = 28).

Item	Mean	Standard Deviation	Minimum	Maximum	Range
Brix Value (° Brix)	22.67	3.95	16	30	14
Aerobic Count ($\log_{10}$ CFU/mL)	3.63	0.69	2.00	4.72	2.72
Coliform Count ($\log_{10}$ CFU/mL)	1.59	0.82	0	2.55	2.55

The data distributions for AC and CC are shown in Figures 1 and 2. In relation to ACs, the values were mainly concentrated between 3 and 4 $\log_{10}$ CFU/mL, showing moderate dispersion and no extreme values. The distribution of CCs was skewed towards low values, with most samples below 2 $\log_{10}$ CFU/mL and not exceeding the critical threshold.

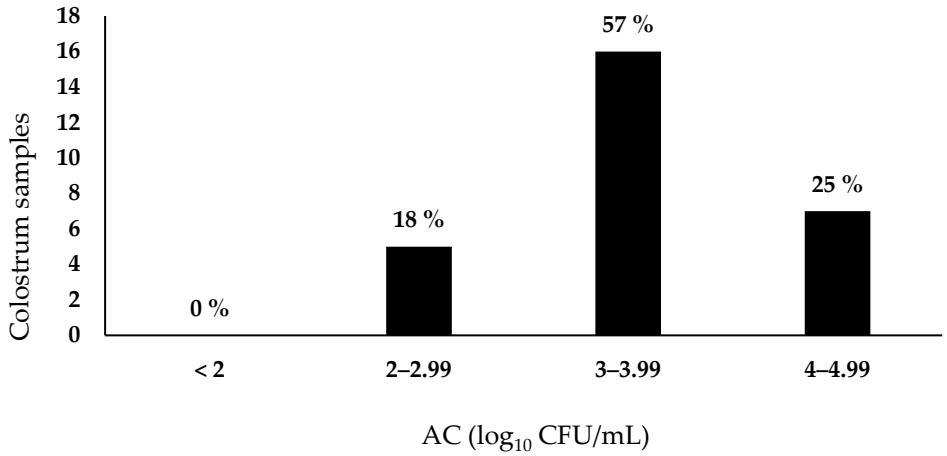

Figure 1. Distribution of aerobic count (AC) in ewe colostrum samples after 24 h of incubation (n = 28).

3.2. Relationship Between Immunological Quality and Bacterial Load of Colostrum

Figure 3 describes the evolution of AC growth at 24 h and 48 h of incubation at 37 °C, categorising the samples according to immunological quality (high, medium, and low) based on Brix refractometry values. No differences were determined between the different categories of colostrum analysed in AC values after 24 h of incubation. However, high-immunological-quality colostrum had significantly lower AC growth than medium- and low-quality colostrum at 48 h ($p < 0.05$). In relation to CC, no differences were found between colostrum with different immunological quality levels (Figure 4).

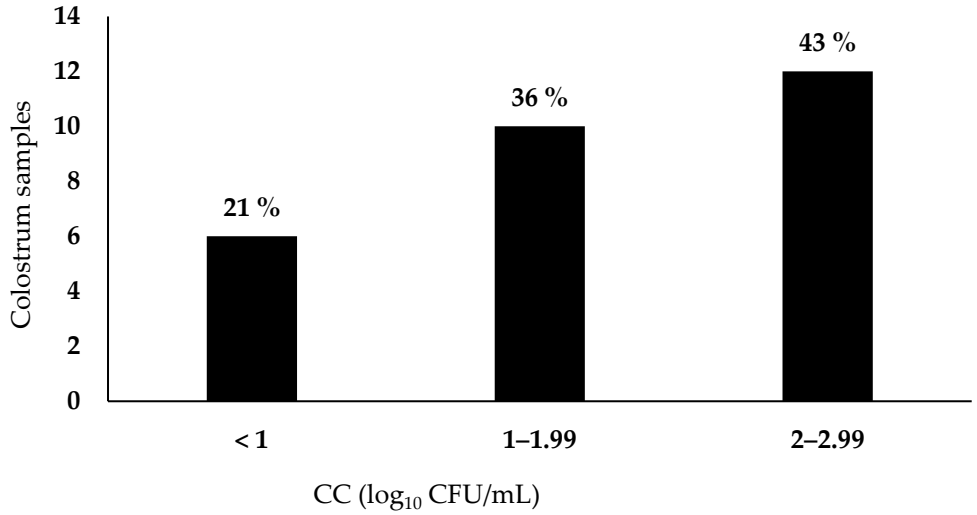

Figure 2. Distribution of coliform count (CC) in ewe colostrum samples after 24 h of incubation (n = 28).

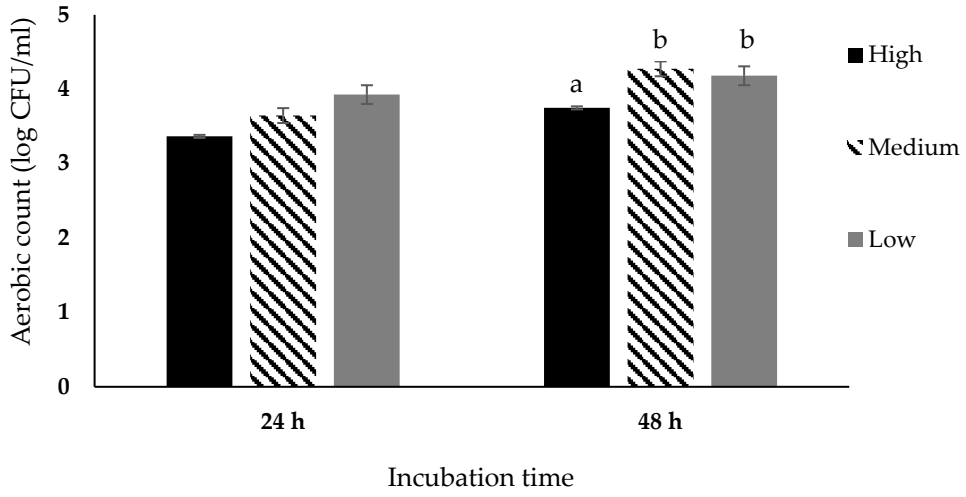

Figure 3. Effect of immunological quality in Brix value (high, medium, and low) on aerobic count of ewe colostrum after 24 h and 48 h of incubation (n = 28). Results are expressed as mean ± S.D. Different letters (a and b) indicate significant differences ($p < 0.05$).

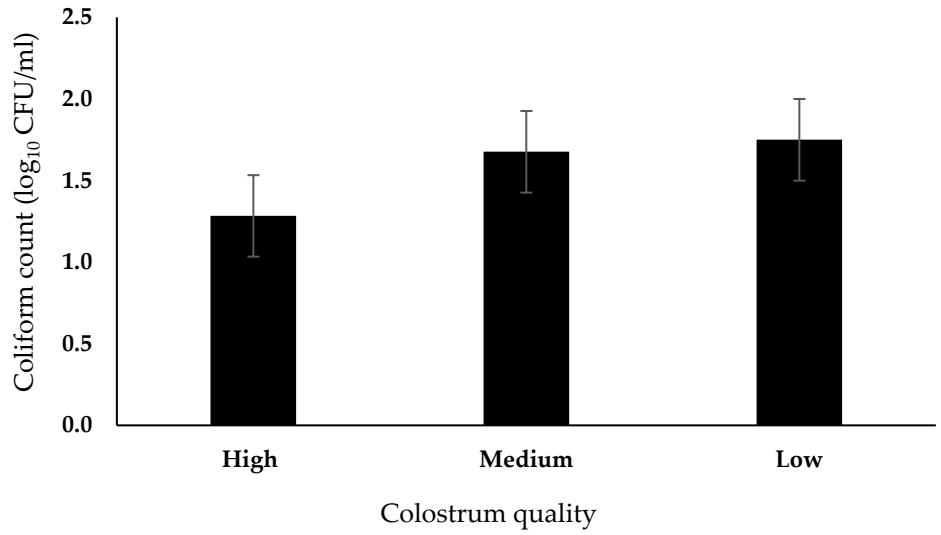

Figure 4. Effect of immunological quality in Brix value (high, medium, and low) on coliform counts of ewe colostrum after 24 h of incubation (n = 28). Results are expressed as mean ± S.D.

3.3. Relationship Between Immunological Colostrum Quality and Lamb Body Weight

The mean body weight value of lambs at birth was 3.92 ± 0.09 kg, increasing to 4.30 ± 0.1 kg at 48 h postpartum. In Figure 5, the average body weights of lambs after birth and at 48 h postpartum are represented according to the quality of colostrum received. No differences were observed between colostrum quality groups at any time analysed.

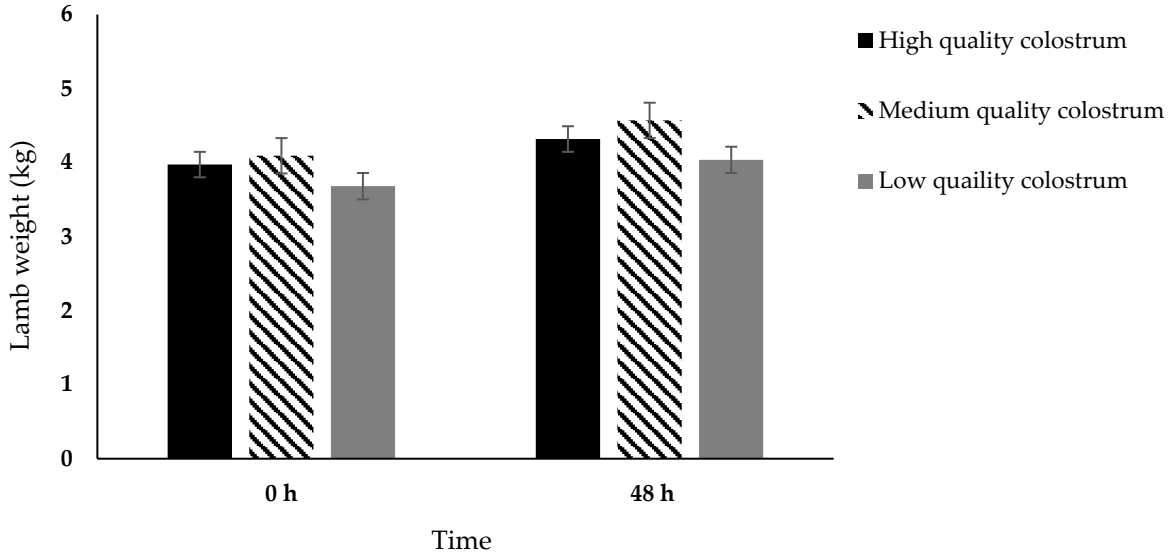

Figure 5. Effect of immunological quality in Brix value (high, medium, and low) on lamb weight gain after birth and 48 h (n = 56; high quality: 24 lambs; medium quality: 16 lambs; low quality: 16 lambs). Results are expressed as mean ± S.D.

4. Discussion

Colostrum quality is a determining factor for the survival and optimal growth of lambs; however, different bacteria and pathogens can be transmitted in colostrum and affect the transfer of passive immunity and animal health [17]. In this study, we determined a range of values for AC (from 3 to 4 $\log_{10}$ CFU/mL) and CC (<2 log10 CFU/mL) in ewe colostrum samples using the Petrifilm™ bacteriology culture system. These values are below the proposed risk thresholds for cattle (<5 $\log_{10}$ CFU/mL for AC and <4 $\log_{10}$ CFU/mL for CC) because sheep-specific limits are not well established, indicating that the colostrum analysed had a low microbial load [17,20,21]. The low proportion of colostrum containing coliform bacteria is consistent with low faecal contamination and, consequently, a low probability of colostrum transmitting pathogens that could cause serious illness in lambs. The observed data are consistent with recent studies that have used Petrifilm™ for bacterial quantification in bovine colostrum [20,21]. Ref. [20] observed that in dairy herds in Quebec, most samples classified as hygienically acceptable had counts clustered in similar ranges (3–4 $\log_{10}$ CFU/mL for aerobe counts), with a small proportion of samples showing significant coliform contamination. Similarly, ref. [21] described that, on Scottish cattle farms, the values obtained using Petrifilm™ usually showed an asymmetrical distribution: most colostrum samples showed low aerobe loads and only a small number of samples had high contamination values. This trend is similar in our study, where the concentration of samples in the lower range confirmed that the colostrum obtained showed good overall hygienic quality despite the absence of teat disinfection, which could be a potential source of environmental contamination. It should be noted that these results reinforce the fact that, beyond the mean value, the shape of the distribution of bacterial counts is useful for detecting deficiencies in hygiene and management patterns. In our study, the absence of isolated peaks of contamination suggests generally good hygienic conditions.

Generally, colostrum's microbiological quality is assessed by plate cultures on agar media following standardised methods according to bacterial isolation [23]. These procedures generally involve performing serial dilutions of the sample, sowing on specific agar, and incubating at different temperatures and times. However, in recent years, other methods such as the Petrifilm™ system have begun to be used to determine microbiological growth in numerous areas of the food industry, such as in dairy products [20]. The use of Petrifilm™ could be an advantage over traditional agar plates because it reduces preparation times, may reduce handling-related variability, and facilitates reading results through colour changes and bubbles generated by microbial activity. Furthermore, recent studies support the diagnostic validity of Petrifilm™ in bovine colostrum evaluation but not in ovine colostrum. Ref. [21] confirmed a high diagnostic concordance between Petrifilm™ and standard culture methods in cow colostrum, highlighting its usefulness in identifying samples with excessive contamination. Ref. [20] also demonstrated that this method is relatively reliable for detecting colostrum with bacterial counts above critical thresholds in cattle herds, allowing for rapid decision-making regarding colostrum administration or disposal. The main problem described by these authors is that this method may slightly underestimate contamination compared to laboratory tests and could be a limitation in this study. In addition, the samples were obtained from a limited number of ewe, from one farm, and therefore the results should be interpreted with caution.

On the other hand, we analysed the effect of the immunological quality on microbiological counts of ewe colostrum. The samples with high immunological quality (>24 Brix value) showed lower AC than those with the worst immunological quality. This pattern indicates a possible inverse association between immunoglobulin and total solids content and bacterial proliferation in colostrum, which is of interest for interpreting the microbiological quality of this fluid. The reason for this may be that colostrum that is richer in IgG and other bioactive components has a greater capacity to limit microbial proliferation, either through direct or indirect mechanisms. Lactoferrin has demonstrated antibacterial, anti-inflammatory, and protective properties against gastrointestinal infections in newborns [24]. In vitro experiments have shown that it can inhibit the growth of *Listeria monocytogenes* and *Escherichia coli* [25,26].

Another relevant bioactive protein is lysozyme, which is an enzyme with bactericidal activity that acts by hydrolysing the peptidoglycan in the cell wall, causing the lysis of both Gram-positive and Gram-negative bacteria [27]. Finally, lactoperoxidase is an important component of colostrum's natural antimicrobial system. This enzyme catalyses the formation of reactive compounds from hydrogen peroxide (H_2O_2) and thiocyanate (SCN^-), which can inhibit the growth of bacteria, fungi, and protozoa [26]. The functional combination of these proteins—lactoferrin, lysozyme, and lactoperoxidase—together with immunoglobulins, is considered a passive defence that we hypothesised that could explain, at least in part, the greater microbiological stability observed in high-quality colostrum. However, unfortunately these mechanisms were not directly assessed in the present study.

Colostrum is essential for lamb health, growth, and productivity during the first days of life [3,4]. We determined that immunological colostrum quality did not affect early postnatal weight change in lambs during the first 48 h after birth. This result can be interpreted in several ways. First, previous studies have indicated that the total amount of colostrum ingested has a more decisive effect on neonatal weight gain than the concentration of immunoglobulins due to a minimum level needing to be reached to ensure passive transfer [2,28]. In this study, we did not determine the effect of the total volume of colostrum in lambs on hydration or on early postnatal weight change. Furthermore, the absence of significant differences may be partly explained by the farm management conditions, characterised by strict postnatal care, draught-free housing, routine disinfection, and good

immunisation of mothers prior to lambing. This environment, combined with the low microbial load detected in the colostrum, would have minimised the impact of individual variations in immunological quality on lamb weight change at 48 h, allowing all animals to develop uniformly. Likewise, there is the possibility that these variations could be observed during the later stages of animal growth, as well as in carcass yield [29,30], although these characteristics were not evaluated in this study. It could therefore be suggested that under optimal health conditions and with sufficient colostrum intake, the immunological quality of colostrum may not be immediately reflected in weight gain, although its role remains essential for protection against subclinical infections and medium-term survival.

5. Conclusions

Low microbial contamination was found in ewe-harvested colostrum using the Petrifilm™ system. Colostrum with high immunological quality showed low aerobic count after 48 h of incubation but no variation in coliform count. Early postnatal weight changes were similar in all types of colostrum regardless of Brix value colostrum. The preliminary results showed that the Petrifilm™ system was a practical tool for assessing the microbiological quality of sheep colostrum, with potential for field application. However, further studies are needed due to the limitations related to the single-farm design, small sample size, lack of gold standard comparison, and very short observation period.

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Institutional Review Board Statement: The animal study protocol was approved by Ethics Committee of the University of Zaragoza (PD24/24), with an approval date of 21 December 2024.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. (The data are not publicly available due to privacy of the owner's sheep farm).

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Conflicts of Interest: The authors declare no conflicts of interest.

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