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PASSIVE TRANSFER OF IMMUNOGLOBULINS FROM EWE TO LAMB

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Abstract

The neonatal period in small ruminants in the Northern Hemisphere usually starts in December. It is around this critical period that 10-12% of lambs and kids die in the first 72 hours of life because of poor colostrum quality and quantity. Concerning this fact, it is of importance that all pregnant ewes have adequate access to forages for a good colostrum quality, indispensable for the lasting growth of newborns lambs. To demonstrate the passive transfer of immunoglobulins from the colostrum, 10 samples of colostrum were collected from 10 individual ewes, followed by 10 samples of serum from their resulting lambs in the first day after lambing and at 8 days of age. The colostrum and protein serum levels were tested with: refractometry, using zinc sulfate turbidity test, qualitative sodium sulfite turbidity test. From all the colostrum samples collected, just 2 of them presented an acceptable quality of immunoglobulins. By negative correlation, the lambs' levels of serum protein in day 1 and day 8 were high in all newborns. Although the current preliminary results are somewhat inconclusive, they outline the importance and practical significance of colostrum quality monitorization in ewes.

Key words: *colostrum, ewes, immunoglobulins, lambs, protein levels.*

INTRODUCTION

For decades it is known that the ruminants' synepitheliochorial placenta is very similar to the equines and swine epitheliochorial one. Thus, the six tissue layers of these types of placenta prevent the passage of maternal antibodies to the fetus (Vejlsted, 2010; Borghesi et al., 2014). Even new world camelids, like llama and alpacas that are not considered true ruminants, present failure of passive transfer due to their particular type of placenta (Weaver et al., 2000). Consequently, the immune system of newborns ruminants for defending against microorganisms is received throughout colostrum ingestion, passively (Maden et al., 2003).

The term "colostrum" represent the first lactic secretion enriched with elements of blood serum, including antimicrobial proteins such as lactoferrin, lactoperoxidase, lysozyme, proline-rich polypeptides and immunoglobulins (Igs) (Tyler et al., 1999; Loste et al., 2008; Vejlsted, 2010; Lopreiato et al., 2017). Colostrum acts as an important nutritive source, due to the high content of vitamins and minerals, having a laxative effect (Boucher, 2014).

In order to reduce the failure of passive transfer, and at the same time morbidity and mortality in foals, piglets, calves, lambs, kids and alpaca crias, colostrum ingestion and absorption immediately after birth is requires (Nowak & Poindron, 2006), due to the drastic decrease over time of Igs content (Weaver et al., 2000). Therefore, for a maximum efficacy, the offspring must ingest the colostrum during the first two hours of life, as Santiago et al. (2020) reported. These antibodies are absorbed via pinocytosis by epithelial cells in the jejunum and ilium, and then they are transferred by the vascular system into the thoracic duct (Riddle, 2003).

There are five classes of immunoglobulins known, however a high importance for passive immunity belongs to IgG, IgM and IgA. Out of these, IgG_1 levels decrease significantly within the first 10-12 hours post lambing and after 24 hours postpartum, the IgG levels become unsatisfactory (Santiago et al., 2020).

The yellowish color and viscosity of the colostrum can be used as indicator of the colostrum IgG concentrations (Meo-Scotoni & Machado Neto, 1992), nevertheless, a more precise method is to evaluate the total protein content of colostrum (Borghesi et al., 2014).
To evaluate the total protein content of the colostrum and the lambs blood serum, Quigley et al. (2013) proposed the use of Brix refractometer as a tool. Few years later, Alves et al. (2015) evaluated the passive transfer of immunity from ewes to lambs using indirect enzyme-linked immunosorbent assay (ELISA) test and the enzymatic colorimetric kits.
Currently, in practice there are used different methods of passive transfer evaluation like: radial immunodiffusion of IgG_1 concentration, sodium sulfite turbidity assay, zinc sulfate turbidity assay, ELISA, refractometry, glutaraldehyde coagulation test etc (Tyler et al., 1996; Tyler et al., 1999; Boucher et al., 2014; Alves et al., 2015).
According to Vatankhah (2013), the immune-globulin levels that are present in the ewes colostrum are directly correlated with the immunoglobulin levels found in lambs blood serum. Up-to-date, few studies have been conducted in order to estimate the optimal passive immunity transfer in lambs, to the best of our knowledge, being the first study on this subject from Romania.
The main goal of this paper, was to compare the results of passive transfer of immunity in newborn lambs, evaluated throughout techniques of refractometry, qualitative zinc sulfate turbidity test, and qualitative sodium sulfite turbidity test, to conclude if there is a correlation between them, and which of these could be reliable in practice at farm level.

MATERIALS AND METHODS

The authors of this study respected all rights of animals' welfare in correlation to European Union legislation (Directive 2010/63/EU), and none of them suffered of any painful procedures.

Animals
For this experiment, 10 crossbred ewes were used (F_1 Texel x Țurcană) together with their resulting 10 lambs, belonging to a 200 heads herd from Poiana Marului, Brasov county. All ewes were primiparous, at a proper body condition score, ranging between 2.5-3 BCS.
The ewes were naturally breeded during late August, and they were confirmed pregnant two months later after transabdominal ultrasonographically examination (Tringa Linear VET®, Esaote, The Netherlands), as Jones et al. described in 2016. All ewes taken into the study were carefully monitored to ensure that none was affected by any pathological condition. At the same time, each animal received dried hay (7 kg) and corn grains (500-700 g), twice per day, and the water intake was *ad libidum*.

Experimental design
Based on aspects such as udder shape and consistency, used as prodromal signs of lambing, the ewes were separated around ten days before parturition of other flockmates. Between January and February 2020, all ewes had monotocous eutocic deliveries, without the need of assistance. In maximum 4 hours after lambing, from each ewe were collected 20 ml of colostrum into 30 ml vials (Urocultor, EasyCare®, Romania), and from each lamb blood samples were taken by jugulare vain puncture into 4 ml cloat activator vials (Vacutest, Kima®, Italy).
Using the same protocol, eight days later, another 10 blood samples were collected from neonatal lambs.

Colostrum assessment
Each colostrum sample was evaluated in the first hour after milking, looking for the color, consistance and IgG level. To determine IgG level of each colostrum sample, it was used a Brix refractometer that was calibrated before. According to Bielmann et al. (2010), the Brix refractometer is not sensitive to the temperature of the colostrum at the time of analysis.
After a gentle mixture of each colostrum sample, it was pipetted one drop from each sample, and after it was covered with the refractometer prism, the value was read using natural light. For precise results, this procedure was repeated 30 minutes later, considering that during this time, the colostrum composition will not be affected.

The obtained values were expressed in Brix percentages that later were converted into mg/ml according to statement of Hameed et al. (2019) witch said that a Brix value of 22% corresponds to 50 mg/ml of IgG.

Blood samples evaluation
After sampling, blood was kept around one day at room temperature for clot sedimentation, then centrifugated for 10 minutes at 500 rpm, then the serum was separated into Eppendorf vials and freezed untill all samples were obtained.

1. Refractometry
To assess total serum protein level, an ordinary refractometer was utilized in the same manner as the Brix refractometer. Serum IgG_1 (being the most abundant in serum) concentration could be estimated based on serum total protein concentration using the following formula:

$$\textbf{Serum IgG1}\ \left(\frac{mg}{dl}\right) = -3615 + \left[901\ x\ total\ serum\ protein\ \left(\frac{g}{dl}\right)\right]$$

2. Zinc sulfate turbidity test
A 0.1 ml aliquot of each serum sample was added to 6 ml of zinc sulfate solution in 10 ml sterile tubes. The solution was mixed carefully, then incubated for 1 hour at 23°C, and placed in front of a text. Positive results were considered if text was not legible through the sample tube, turbidity confirming the presence of IgG into the serum by precipitation of gamma globulin.

3. Sodium sulfite turbidity test
Using distilled water, sodium sulfite solutions were prepared at14%, 16% and 18%. A quantity of 0.1 ml of serum aliquot was mixed with 1.9 ml of each concentration of sodium sulfate. Tubes were mixed, incubated for 15 minutes at 23°C, and evaluated in the same manner as the previous test was performed. The test results were recorded on a 0 to 3 scale, where 0 represented no turbidity in all 3 tubes, 1 for turbidity in 18% solution tube, 2 for turbidity in 18% and 16% solution tube, and 3 for turbidity in all tubes. All assays were investigated by the same reader.
The results obtained from the turbidity tests were compared and correlated with the refractometry results using Office Excel 2016.

RESULTS AND DISCUSSIONS

None of the sheep was excluded from this study due to pathological conditions or other nonmedical causes. At the same time, all ewes registered a BCS according to their condition, ranging between 2.5 and 3.

Colostrum assessment
After the evaluation of colostrum quality by Brix refractometer, using the rule of three, it was calculated the level of IgG (mg/ml) (Figure 1) for each of the ten samples. Values between 29.55 and 53.41 mg/ml were obtained, results that coincide with a good quality colostrum in ewes, similar to the report of Alves et al. (2015) that used in their study the ELISA method.
Berge et al. (2018) showed that from frozen-thawed Awassi ewes, the colostrum values range between 14.40 and 17.10 Brix %, compared to the present study, where data ranged between 13 and 23.5 Brix %. By comparison, Boucher et al. obtained in 2014, higher values in Marino breed ewes (from 92.32 to 131.90 mg/ml), and in Dorper breed (from 75.69 to 81.20 mg/ml) supplemented with wheat or canola.

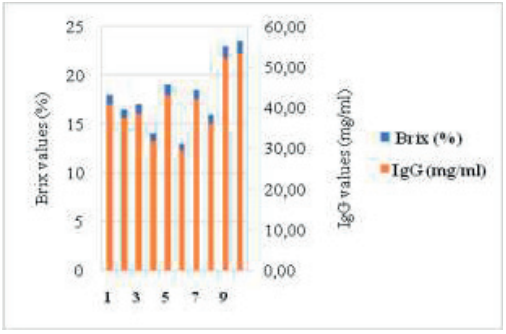

Figure 1. Graphic representation of colostrum quality expressed in Brix % and the values of IgG (mg/ml)

Recently, Kessler et al. (2019) observed in Santa Ines ewes, IgG colostrum values ranging between 1.2 and 60.7 mg/ml. Massimini et al., (2006) showed that serum proteins concentrations range from 4.0 to 8.2 mg/dl, signicatvely smaller values compared to our results.

Serum refractometry
Even if by testing the serum refractometry, only IgG_1 results were obtained, it can be

concluded that this is the main component of IgG, from all four subtypes (IgG_2, IgG_3, and IgG_4), thus the data is not quite pertinent to extrapolate the whole IgG levels of maternal colostrum.

Although we evaluated just the colostrum of first lactating ewes, the current results suggest that this is method is proper for the use in assessing the passive transfer of IgG in newborn lambs. The highest value registered was in ewe number 10, and the minimum value, observed in ewe number 6, this values were not correlated with abnormalities during their pregnancy or lambing, showing that animals from the same flock can present a large difference in the values of IgG, and at the same time, to be physiological sound.

Colostrum ingestion took place before blood sampling in 6 out of the10 lambs, according to belly palpation and refractometry results (Figure 2). The thawing of serum samples took place at room temperature for one hour, then samples were evaluated using refractometry.

Values of serum total protein (Table 1) were converted using the above formula.

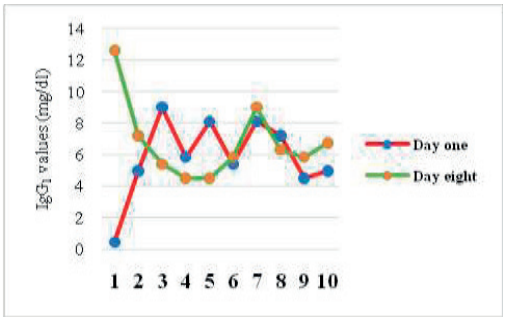

Figure 2. IgG_1 values obtained from blood samples collected from lambs on day 1 and 8 of life

Table 1. Serum proteins values obtained from lambs at 1 day and 8 days of life

Serum proteins (g/dl)	
1st day of life	8th days of life
4.5	18
9.5	12
14	10
10.5	9
13	9
10	10.5
13	14
12	11
9	10.5
9.5	11.5

Table 2. Comparative data of IgG concentration colostrum and serum IgG_1

Serum IgG_1 values (mg/dl) in lambs		Colostrum IgG values (mg/dl)
1st day	8th days	
0.44	12.60	4,091
4.94	7.20	2,727
9.00	5.40	2,273
5.85	4.49	2,045
8.10	4.49	2,045
5.40	5.85	2,386
8.10	9.00	3,182
7.20	6.30	2,500
4.49	5.85	2,386
4.94	6.75	2,614

Thus, evaluating Table 2, it can be observed that in most lambs, a very small amount of IgG_1 was absorbed from the total volume of IgG from the maternal colostrum. Boucher et al., metioned in 2014, concentrations ranging between 700 and 1100 mg/dl of the serum IgG on 2 days old lambs, and Alves et al. (2015) found in Santa Ines lambs values of serum IgG of 182 mg/dl in males and 293 md/dl in females, using refractometry. For the same breed, Kessler et al. (2019) showed values ranging between 120 and 6070 mg/dl.

According to Massimini et al. (2007), an increased risk of illness and death is associated with the concentration of IgG. In their work, the concentration of IgG serum 24 hours after birth ranged from 0 to 524 mg/dl. Similar results were obtained by Hashemi et al., in Karakul breed, with an average of260 mg/dl.

Recently, Gokce & Atakisi (2019) registered serum IgG concentration, 24 hours after birth at a mean of 2198 mg/dl.

Moreover, Daniels et al. (2000), working on supplementing vitamin E in ewes, registered 1.96 mg/dl for serum IgG concentration in lambs from ewes that received vitamin E supplementation one month prior to lambing. Stewart et al. (2013), after supplementing ewes before lambing with selenium, estimated that IgG concentration of lamb serum was at a mean of 2670 mg/dl.

Zinc sulfate and sodium sulfite turbidity tests

Regarding the zinc sulfate test, the turbidity was present for all samples both in the first day and in the eight day after lambing, suggesting that IgG was present in the serum of the lambs. The score registered by the sodium sulfite test

for al serum samples is in contradiction with the previous test results, because on the first day of the lambs life, it was registered the maximum score of 3+ just in one serum sample, for other two was 0.

Even after eight days, the results of both tests were still in contradiction, this time the serum samples presenting in the first day the score 0 for the sodium sulfite test, presented during the eight days interval the score 3+, respectively 1+, however, the turbidity of the zinc sulfate test was still observed at this moment for all samples (Table 3).

Brujeni et al. (2010), same as Demis et al. (2020) used in lambs a quantitative zinc sulfate turbidity test to estimate the total immunoglobulin levels, however, not for IgG concentrations. Comparing to the recent literature, it seems that this is the only study on lambs, that used a qualitative zinc sulfate test, could be reliable for use at farm level.

Tabel 3. Qualitative score of sodium sulfite and zinc sulfate test used for first and eight day serum samples in newborn lambs

First day of life		Eight day of life	
Sodium sulfite turbidity test	Zinc sulfate turbidity test	Sodium sulfite turbidity test	Zinc sulfate turbidity test
0	Turbid	3+	Turbid
0	Turbid	1+	Turbid
1+	Turbid	0	Turbid
2+	Turbid	0	Turbid
2+	Turbid	0	Turbid
2+	Turbid	0	Turbid
3+	Turbid	0	Turbid
2+	Turbid	0	Turbid
2+	Turbid	0	Turbid
0	Turbid	0	Turbid

Based on the good quality of the colostrum in this study, it can be estimated that there is a strong correlation with a good IgG absorption by the lambs small intestines established by the positive score of zinc sulfate test.

CONSLUSIONS

All of the described and used assay methods from this study need rigorously handling, however, require minimal instruments.

The IgG colostrum quantity and levels of absorbtion can be easily estimated throughout the use of Brix refractometer and by zinc sulfate turbidity test, respectivelly. The clear relation between these two methods, recommends them to be performed in a routine manner for the perinatal management of lambs at farm-level.

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