



Effect of colostrum treated by heat on immunological parameters in newborn lambs

A. Loste, J.J. Ramos, A. Fernández*, L.M. Ferrer, D. Lacasta, M.T. Verde, M.C. Marca, A. Ortín

Department of Animal Pathology, Veterinary Faculty, Zaragoza University, Miguel Servet 177, 50013-Zaragoza, Spain

Received 4 July 2007; received in revised form 21 November 2007; accepted 5 December 2007

Abstract

Seventeen newborn lambs were fed with untreated colostrum (group A) and 18 lambs were fed with colostrum treated by heat (56 °C, 30 min) (group B). Blood samples were obtained at seven time points from day 0 to 38 of life. Clinical status and body weight were recorded and serum protein fractions and IgG levels were determined in blood. Phagocytosis of neutrophils from the lambs and opsonic capacity of their serum were examined using a flow cytometry method. As an indicator of *in vivo* cellular immunity, reactions to intradermal injection of phytohemagglutinin (PHA) were examined at 8 and 16 days of age. There were no clinical signs of disease and no significant differences in body weight between groups. Lambs from group B had lower total protein initially and lower γ -globulin and IgG levels until day 32 compared to lambs from group A ($P < 0.01$). Heat treatment of colostrum had no significant effect on serum opsonic capacity or phagocytosis by neutrophils and these functions increased with age. However, responses to PHA in lambs from group B were lower ($P < 0.01$) than group A at day 8 of life, but not at day 16, suggesting that cellular immunity was affected by the treatment of colostrum. These results showed that colostrum treated by heat impaired cellular and IgG levels in lambs, but not phagocytic function. However, these changes did not affect the health or performance of the lambs.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Lambs; Heat treatment of colostrum; Immunity response

1. Introduction

In ruminants, syndesmochorial placentation prevents the transmission of immunoglobulins *in utero*, and animals are born with a very low serum concentration of immunoglobulin G (IgG). Thus, in neonatal lambs the acquisition of passive immunity by the early ingestion and absorption of maternal immunoglobulins from colostrum

is critical for survival (Waelchli et al., 1994; Nowak and Poindron, 2006). Colostrum also contains nutrients, minerals, trace elements, hormones, growth factors, and other non-specific immunologic substances (Blum, 2006; Nowak and Poindron, 2006), necessary for normal development. Colostral immunoglobulins ingested by the newborns are transferred intact from the intestinal lumen and reach the circulation by a non-selective transport mechanism (Sheldrake and Husband, 1985). Ingestion of colostrum during the first 24–36 h after birth is essential for the acquisition of passive immunity, because intestinal absorption of the immunoglobulins decreases rapidly and ceases by this time (Weaver et al., 2000).

* Corresponding author. Tel.: +34 976 76 10 00x1574; fax: +34 976 76 16 12.

E-mail address: afmedica@unizar.es (A. Fernández).

The serum concentration of Ig in neonates is ultimately determined by the quantity in the colostrum, the volume of colostrum ingested and the time of ingestion. Failure of passive transfer of immunity (FPT) is a well-documented cause of infection-related illness and death in large animal neonates (Aldridge et al., 1992; Besser and Gay, 1994; Weaver et al., 2000). The most common causes for FPT include inadequate quality or production of colostrum, delays in colostrum administration, weak neonates or death of the dam (Aldridge et al., 1992; Besser and Gay, 1994). Neonatal animals can be fed with colostrum preparations or substitutes, but effectiveness may vary. Castro et al. (2005) found high serum IgG levels in goat kids using lyophilized colostrums, whereas Foster et al. (2006) reported that a commercial product failed to provide adequate serum IgG concentrations in cattle.

Some infectious diseases can be transmitted via colostrum to the lambs, most importantly Maedi–Visna virus (MVV). Most infected animals with MVV remain asymptomatic for several years, with lambing a time of high lentivirus expression, which facilitates the spread of infection. Virus present in lung secretions, colostrum and milk are the sources of infection to offspring and other sheep in the flock. Since animals cannot be treated or vaccinated successfully, disease control is based on artificial rearing of replacement lambs with MVV-free colostrum and milk (Pépin et al., 1998; Preziuso et al., 2004; Alvarez et al., 2005). However, farmers do not know which animals are uninfected to overcome this lambs can be raised using colostrum supplements or colostrum replacements free of MVV.

Colostrum pasteurization can play an important role in preventing MVV transmission (Pépin et al., 1998). However, Ig and other constituents of the colostrum are thermolabile and the immunological status of the neonate can be compromised. Inadequate levels of serum IgG, lactoferrin and neutrophil function have been observed in calves fed with pasteurized bovine colostrum (Meylan et al., 1996; Lakritz et al., 2000; Godden et al., 2003) and in goat kids (Fernández et al., 2006).

Most studies of FPT have only considered immunoglobulin levels, because it plays a crucial role in disease protection. However, other components of the immune system should be considered, such as phagocytosis by neutrophils and serum opsonic activity, which are the first line of defense against bacterial infection (Tizard, 2002) or delayed-type hypersensitivity (DTH) which is an *in vivo* manifestation of cell-mediated immune response (CMIR) (Sandbulte and Roth, 2004; Hernández et al., 2005).

In order to contribute to knowledge of the passive transfer of immunity in neonatal lambs, this study describes the effect of colostrum treated by heat on some

immunological parameters and growth of lambs during the first month of life.

2. Materials and methods

2.1. Animal management

The Ethics Committee of Zaragoza University approved this experimental protocol. Rasa Aragonesa lambs from forty, pluriparous, ewes on a commercial farm in Zaragoza (Spain) were used. Parturitions were observed and only 35 female lambs resulting from uncomplicated births were included in the study. The lambs were separated from the ewes immediately after birth, avoiding any colostrum intake, their umbilical cords disinfected, and they were injected with a vitamin AD₃E dose. Their weights were recorded at day 0 and weekly until the end of the study.

To assess clinical status, animals were observed twice daily during the experimental period for any symptoms of disease. Rectal temperature was also taken every day during the first 14 days of the experiment.

2.2. Feeding protocol

The lambs were randomly allocated into two experimental groups. Lambs from group A ($n=17$) received pooled fresh ovine colostrum obtained from ewes of the same farm a few weeks before. Lambs included in group B ($n=18$) were fed with the same colostrum which had been treated by heat at 56 °C for 30 min in a bath. No coagulation in the heat-treated colostrum was observed. Aliquots from both colostrums were stored at –20 °C for subsequent IgG analysis. Untreated colostrum and colostrum treated by heat were distributed into containers and frozen at –20 °C until the time to feed the neonates.

In both groups, the total amount of colostrum (180 ml/kg body weight) was divided into three equal parts and given at 3, 10 and 17 h after birth using a nipple bottle. Lambs that did not suckle were stomach tubed. After completion of colostrum administration, lambs were fed with artificial powdered milk, reconstituted in hot water (40 °C), three times a day using a nipple bottle. Twelve days after birth a commercial started feed and barley straw were introduced.

2.3. Blood samples

Blood samples (4 ml) without anticoagulant and with heparin were taken from the jugular vein of lambs at birth just before suckling and on 2, 4, 8, 16, 32 and 38 days of age. Serum and plasma were harvested after centrifugation and stored at –20 °C for further analysis.

2.4. Protein serum electrophoresis

Serum total protein concentration was measured by use of an automated biochemical analyzer (Technicon RA-500, Dublin, Ireland) using the manufacturer's reagent and recommended

procedures. Serum protein fractions were segregated by electrophoresis with cellogel acetate strips (2.5 × 17 cm, ATOM cat. Number 19000) and were read in a photodensitometer (Shimadzu CS-9000, Kyoto, Japan).

2.5. IgG levels in colostrum and serum and efficiency of IgG absorption

Colostrum and serum IgG concentration was determined by use of a commercially available radial immunodiffusion assay (Bethyl Laboratories cat. Number R50–100, Montgomery, Texas, USA). Samples were evaluated in duplicate according to the specifications of the manufacturer.

To calculate the efficiency of IgG absorption for untreated and treated colostrum, it was assumed that 7.5% of body weight was equivalent to the blood plasma volume in lambs. The following equation was used:

$$\text{IgG absorption} = \left[\frac{\text{Birth weight} \times 0.075 \times \text{serum IgG concentration}}{\text{total IgG fed}} \right] \times 100.$$

2.6. Opsonization assay

Fifteen microliter of labelled propidium iodine (PI) *E. coli* serovar (O157:H7) (1.5×10^7) was added to 15 μl of serum from the lambs and incubated at 39 °C for 30 min to opsonized the bacteria. Control tubes contained bacteria in sterile phosphate buffer solution (PBS). Donor leukocytes were prepared by lysing 10 ml of heparinized blood from an adult healthy sheep with ammonium chloride and Tris (9+1). The donor leukocytes were suspended in sterile PBS at a concentration of 5×10^6 cells/ml. These cells were labelled with 5 μM dihydrorodamine (DHR) for 15 min at 39 °C. Then, 100 μl of leukocytes were added to the opsonized *E. coli* to achieve a bacteria-to-leukocyte ratio of 30:1. The samples were incubated with continuous mixing for 30 min at 39 °C, then placed on ice to stop phagocytosis, protected from light, and surface fluorescence quenched by the addition of 10 μl of 0.4% trypan blue. Flow cytometric (Epics Elyte Coulter, Hialeah, FL, USA) analysis was performed within 2 h of preparation. The

excitation source employed was the 488 nm line of an ILT argon laser at a power of 20 mW. The neutrophil population was selected from the cytogram generated with forward and the scatter. The DHR fluorescence was recorded with a 525 ± 20 nm BP filter. Results from 10,000 events per sample were acquired, and reported as a percentage of blood neutrophils that had ingested one or more bacteria.

2.7. Phagocytic assay

In vitro phagocytic activity of the lamb's neutrophils in heparinized blood was quantified by flow cytometry using the Phagotest® kit (cat. number 341060, Orpegen Pharma, Heidelberg, Germany), and following the same technique described previously (Fernández et al., 2006).

2.8. Intradermal PHA test

Wool was clipped from a 13 × 8 cm rectangle on both sides of the costal area of the lambs. Each lamb was injected intradermally on day 8 and 16 with 0.1 ml of 2.5 mg/ml phytohemagglutinin (cat. Number L8754, PHA, Sigma Chemical, Co.) in sterile isotonic saline solution in the clipped area. Approximately 10 cm from this site, 0.1 ml of saline solution was injected, serving as a control. Measurements of the wheal thickness that developed in response to each injection were obtained immediately and 3, 6, 12, 24 and 48 h after injection using a constant tension calliper. Results were expressed as a ratio of the skin thickness at PHA-injected compared to saline injected sites.

2.9. Statistical analysis

Data are reported as mean ± SD. A non-parametric Mann–Whitney *U* rank sum test was used for comparisons between groups. A *P* value of <0.05 was considered significant. Simple linear regression analysis was performed to evaluate the correlation between serum IgG concentration and serum γ -globulin. Correlation coefficients are shown as rank correlation coefficients (r_s), and *P* values were calculated using Spearman's test.

Table 1

Serum protein fractions (g/l) from lambs 2 to 32 days of age fed with untreated (group A) or heat-treated (group B) colostrum

	Day 2		Day 4		Day 16		Day 32	
	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B
Total proteins	59.3 ± 10	47.4 ± 1.2***	54.3 ± 9.3	47.9 ± 4.7*	47.1 ± 5.7	43.1 ± 8	48.1 ± 7	45.6 ± 5.6
Albumin	28.5 ± 3.3	26.8 ± 2.6	27.0 ± 3.1	25.0 ± 7.7	27.0 ± 3.9	26.3 ± 5.2	28.7 ± 4.9	28.4 ± 1.9
α -globulins	3.7 ± 0.8	3.7 ± 0.5	3.3 ± 0.6	3.4 ± 0.3	2.8 ± 0.5	2.6 ± 0.5	3.3 ± 0.8	3.2 ± 0.6
β -globulins	7.7 ± 0.6	7.6 ± 1.2	8 ± 1.1	8.1 ± 1.1	7.6 ± 1.1	7.4 ± 1.3	7.7 ± 1.2	7.5 ± 0.8
γ -globulins	19.6 ± 7.6	9.2 ± 1.6***	15.5 ± 6.4	9.5 ± 1.7***	10.2 ± 3.4	6.5 ± 1.5***	8.6 ± 2.1	6.4 ± 1.2***
Globulins	31.0 ± 7.7	20.5 ± 1.6***	26.8 ± 6.9	21.0 ± 2**	20.73 ± 3.9	16.5 ± 3.1***	19.6 ± 3.3	17.1 ± 2.2**
A/G ratio	0.96 ± 0.23	1.31 ± 0.15***	1.03 ± 0.22	1.19 ± 0.36	1.34 ± 0.31	1.6 ± 0.19**	1.48 ± 0.26	1.67 ± 0.27*

Values are mean ± SD, **P* < 0.05; ***P* < 0.01; ****P* < 0.001 comparing groups A and B at each time point.

A/G ratio = albumin/globulin ratio.

3. Results

3.1. Clinical status and body weight

No signs of disease or death were recorded in the lambs during the time of the experiment. The mean birth weight did not differ significantly between the two groups of lambs (group A: 3.57 ± 0.51 kg; group B: 3.83 ± 0.36 kg). Significant differences in body weight between groups were not observed at any time during the experiment. At the end of the study, the mean body weight in group A was 7.14 ± 1.35 kg and in group B was 7.28 ± 0.93 kg respectively. Rectal temperatures of the lambs fluctuated between 38.5 °C and 39.6 °C, and no significant differences between groups were recorded ($P > 0.05$).

3.2. Serum protein electrophoresis

Table 1 show results obtained from the analysis of serum protein fractions. At day 2, levels of total proteins, γ -globulin fractions and globulins were lower and albumin/globulin ratio (A/G ratio) was higher in lambs given colostrum treated by heat ($P < 0.001$). The significant differences persisted to day 32 for γ -globulin fractions, globulins and A/G ratio in group B, but TP was not significantly different after day 4. A significant correlation between serum IgG concentration and γ -globulin in both groups of lambs was observed at all sampling times (range $r^2 = 0.431$ – 0.531 ; $P < 0.05$ – 0.01).

3.3. IgG levels in colostrum and serum and efficiency of IgG absorption

IgG concentration in fresh colostrum was 64.18 mg/ml, whereas in colostrum treated by heat the concentration was 42.06 mg/ml, 34.48% lower than fresh colostrum.

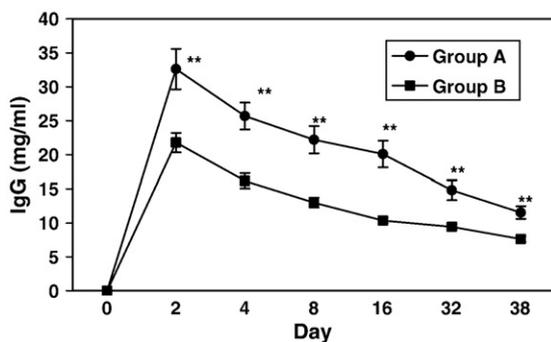


Fig. 1. Serum IgG concentration (mg/ml) (mean \pm SEM) in lambs fed untreated colostrum (group A) or heat-treated colostrum (group B). ** $P < 0.01$.

Table 2

Opsonic capacity of serum and phagocytic activity of neutrophils in lambs from birth to 32 days of age fed with untreated (group A) or heat-treated (group B) colostrum

Age (days)	Opsonic capacity (%)*		Percent phagocytosis (%)*	
	Group A	Group B	Group A	Group B
0	22.7 ± 6.5	25.5 ± 8.1	39.7 ± 7.3	43.3 ± 7.2
4	31.8 ± 5.5	30.1 ± 5.6	50.9 ± 19.4	50.6 ± 8.7
16	48.5 ± 11.4	48.1 ± 10.5	61.9 ± 13.3	58.1 ± 13.7
32	64.7 ± 12.1	64.1 ± 11.3	77.3 ± 21.3	65.6 ± 13.8

Values are means \pm SD.

*Results are percentage of neutrophils phagocytosing 1 or more *E. coli* organisms.

None of the lambs had measurable serum IgG concentrations prior to feeding (Fig. 1). Mean serum IgG concentration of lambs fed with fresh colostrum (group A) was significantly higher than that of lambs fed with heat-treated colostrum (group B) at all sampling times ($P < 0.01$). Mean serum IgG concentration peaked at 48 h in both groups.

Heat treatment of colostrum had no significant effect on lamb IgG absorption efficiency (group A 22.2% , group B 23.9%).

3.4. Opsonic capacity of the serum and phagocytosis by neutrophils

The phagocytic activity of the neutrophils and opsonic capacity of the serum from the lambs are shown on Table 2. In both groups, the phagocytosis by neutrophils and the opsonic capacity was lowest at birth, and increased with age. However, no effect of heat treatment of colostrum was observed.

Table 3

Intradermal PHA test in lambs fed with untreated (group A) and heat-treated (group B) colostrum

Time from injection	Day 8		Day 16	
	Group A	Group B	Group A	Group B
0	1.48 ± 0.33	1.28 ± 0.21	0.96 ± 0.86	0.98 ± 0.61
3	1.98 ± 0.68	$1.49 \pm 0.30^{**}$	1.45 ± 0.23	1.35 ± 0.23
6	1.85 ± 0.55	$1.53 \pm 0.27^*$	1.31 ± 0.16	1.35 ± 0.15
12	1.76 ± 0.50	$1.46 \pm 0.27^*$	1.24 ± 0.14	1.25 ± 0.10
24	1.60 ± 0.40	1.42 ± 0.23	1.17 ± 0.08	1.16 ± 0.08
48	1.61 ± 0.49	1.33 ± 0.26	1.11 ± 0.10	1.15 ± 0.17

Results (mean \pm SD) were expressed as the ratio of skin thickness at sites injected with PHA compared to those injected with saline.

* $P < 0.05$; ** $P < 0.01$.

3.5. Intradermal PHA test

Table 3 shows results of DTH test. Animals fed heat-treated colostrum (group B) showed significantly lower responses to PHA injection at 8 days, but not 16 days of age. These differences on day 8 occurred at 3 ($P < 0.01$), 6 and 12 h after injection ($P < 0.05$). The skin response to PHA was maximal at 3 h following intradermal injection in lambs from group A on days 8 and 16. In group B, the largest responses to PHA was at 6 h postinoculation on day 8 and were similar at 3 and 6 h on day 16.

4. Discussion

In our experiment, no mortality or clinical signs of disease in lambs of either group were recorded. Moreover, no significant differences in body weight between groups were reported during the study. These results show that, in our experimental conditions, heat treatment of colostrum had no influence on the lambs' health or growth. Our results agree with observations in kids (Sherman et al., 1990; Fernández et al., 2006) and calves (Godden et al., 2003) that received pasteurized colostrum. Another study has shown a high morbidity and mortality in kids fed with colostrum treated by heat, and this was consistent with low serum immunoglobulin concentration in these kids (O'Brien and Sherman, 1993). Many factors such as environmental and management conditions, stress, concurrent disease and virulence of pathogens may also contribute to the development of disease. Pasteurization of colostrum has been regarded as an effective way to control microorganisms in colostrum and limit the transmission of infectious diseases from the dam to the newborn (McGuirk and Collins, 2004). It has been demonstrated that pasteurization decreased the viability of pathogens from colostrum at a laboratory scale (Godden et al., 2006; Trujillo et al., 2007), and would be adequate to control transmission of infectious diseases through colostrum, as recommended to prevent MVV infection (Pépin et al., 1998). Because pasteurization is associated with low immunoglobulin concentration and viscosity problems, it is not routinely recommended now to control colostrum microorganisms (Godden et al., 2003; McGuirk and Collins, 2004). However, more recent investigations have demonstrated that bovine colostrum heated at 60 °C for up to 120 min did not change viscosity or Ig concentration (McMartin et al., 2006). In our case, no coagulation or viscosity problems were detected following heating at 56 °C for 30 min. Because these difficulties occur when large volumes of colostrum were pasteurized (Godden et al., 2003), more research is needed to improve large scale commercial pasteurization.

Serum protein fractions concentrations were within normal ranges although TP and globulin levels were slightly low (Ramos et al., 1994) in both groups of lambs. The highest initial concentrations of TP, γ -globulin and total globulins, was due to colostrum intake. Colostrum is rich in IgG, IgA and IgM which are absorbed by gut in the first 24–36 h after birth (Besser and Gay, 1994; Waelchli et al., 1994). The differences observed between groups in serum protein fractions was a reflection of the amount of IgG absorbed by the lambs from the colostrum. This also has been observed in other experiments when pasteurized colostrum was given to goat kids (Fernández et al., 2006) and to calves (Godden et al., 2003). There was a positive significant correlation between serum IgG levels and γ -globulin concentrations in lambs from both groups, which indicates γ -globulin composition is mainly IgG (Fernández et al., 2006; Massimini et al., 2006). Measurement of serum γ -globulin concentrations by electrophoresis is the most accurate tool for estimation of FPT in lambs because approximately 95% of the variation in serum IgG levels in 1-day-old lambs associated with serum γ -globulin concentration (Massimini et al., 2006). Moreover, the level of serum TP has been used as a practical and economic test to evaluate passive immunity in ruminants younger than 1 week old (McGuirk and Collins, 2004; Massimini et al., 2006).

The destruction of immunoglobulins with heat treatment of colostrum depends on the temperature, time and size of the batch, and different types of colostrum (Godden et al., 2003, 2006; McMartin et al., 2006; Trujillo et al., 2007). In our study, heating colostrum at 56 °C for 30 min caused a 34.48% reduction in IgG levels, but no increase in viscosity was observed. The IgG concentration found in fresh colostrum in this study (64.18 mg/ml) was similar to that reported by Waelchli et al. (1994) (65.5 mg/ml), and Maden et al. (2003) (60.52 mg/ml), and slightly lower than that found by Al-Sabbagh et al. (1995) (79 mg/ml). Previous studies have demonstrated a significant increase in colostrum IgG destruction when higher temperatures of pasteurization were used (Lakritz et al., 2000; Godden et al., 2003) while lower temperature (<63 °C) may not harm the colostrum antibody or activity (Godden et al., 2006; Trujillo et al., 2007).

The lambs had no detectable serum IgG prior to colostrum intake. The IgG absorption peak was observed at 48 h in both groups. Serum IgG concentration in the first 48 h of life depends on the total colostrum IgG mass received, body weight and efficiency of absorption (Weaver et al., 2000). Given that heat treatment caused a 34.48% reduction in colostrum IgG levels, it is not unexpected that serum IgG concentrations were significantly lower in

lambs that were fed with heat-treated colostrum. Similar percentage of reduction of IgG concentration has been reported following pasteurization of goat colostrum (Argüello et al., 2003; Fernández et al., 2006). Other studies in ruminants also have found a significant decrease in serum IgG levels, following heating of colostrum (Lakritz et al., 2000; Tyler et al., 2000; Godden et al., 2003). However, the efficiency of IgG absorption appeared slightly higher in lambs fed with heat-treated colostrum and possibly the method of processing may influence IgG absorption or half-life.

The dividing line between hypogammaglobulinemia and normal serum IgG concentration in neonatal lambs has not yet been universally accepted. Previous studies have shown that lambs with serum concentrations of IgG < 500 mg/dl (Vihan, 1988), and < 150 mg/dl (Hunter et al., 1977) and serum concentrations of IgG₁ < 60 mg/dl (McGuire et al., 1983) and < 100 mg/dl (Gilbert et al., 1988) at 24 to 36 h of age are less likely to survive. In our study, serum IgG concentrations in 48-hour-old lambs in group A (326 mg/dl) and in group B (217.8 mg/dl) were between the levels above and were in accordance with those reported for normal lambs (Vihan, 1988; Waelchli et al., 1994; Massimini et al., 2006). Because no mortality or clinical signs of diseases were reported, the levels of serum IgG at 48 h of life found in this experiment in both groups could be considered adequate to prevent FPT in lambs reared under excellent management conditions.

While the effects of heat treatment of colostrum on serum IgG levels in neonates have been reported, no studies have been performed on serum opsonic capacity or neutrophilic phagocytic activity of the newborn. Serum opsonic capacity of the lambs increased following ingestion of colostrum, whether heat-treated or not. This activity increased with age, and, at 32 days of life was similar to that recorded for other adult animals (Bernadina et al., 1991; Gröndahl et al., 1999). The main plasmatic opsonins are IgG and complement (Tizard, 2002), but in our experiment this capacity was independent of IgG concentration as has been observed in kittens (Hanel et al., 2003). In foals, Gröndahl et al. (1999, 2001) have found a negative correlation between serum opsonic capacity and serum IgG concentration. Our results indicate that lambs are initially deficient in serum opsonic capacity which is independent of the serum IgG concentration and heat treatment of the colostrum.

Our results show that colostrum feeding is followed by an increase of phagocytic activity by neutrophils from lambs in both groups. These results are in accordance with previous reports in small ruminants (Bernadina et al., 1991; Fernández et al., 2006), calves (Lombardo et al., 1979; Menge et al., 1998) and foals (Gröndahl et al., 1999,

2001). It is likely colostrum ingestion enhanced phagocytosis of *E. coli* due to increased serum opsonic capacity as discussed above. Our study showed that colostrum treated by heat had little influence on phagocytic activity of neutrophils. These indicated that sheep colostrum contains some factors that were not substantially altered by heat under our experimental conditions. Levels of phagocytosis at 32 days of life were similar to that neutrophils from adults (data from our laboratory), in accordance with results obtained by other researchers (Bernadina et al., 1991; Gröndahl et al., 1999).

PHA stimulates growth and cell division of T lymphocytes and responses following intradermal injection have been regarded as an indication of CMIR mediated by T lymphocytes (Ekkel et al., 1995; Tizard, 2002). However, PHA did not induce a typical delayed reaction in cattle (Hernández et al., 2005). Results of this study show a maximal response to PHA injection between 3 to 6 h following intradermal injection which was similar to those observed in pigs (Ekkel et al., 1995) and cows (Hernández et al., 2005). Those lambs fed with heated colostrum developed a smaller response to PHA injection at 8 days of age as reported in kids (Fernández et al., 2006). These results may indicate that heat treatment of colostrum destroyed some components promoting cellular immunity in lambs. Mammary secretions contain a large variety of components that are immunologically active such as cells, cytokines, growth factors and hormones. Up to 67% of cells in sheep milk are T lymphocytes (Le Jan, 1996; Blum, 2006). These lymphocytes are transported from intestine to the mesenteric lymph nodes and are another mechanism by which the ewe may transfer immunity to the lamb (Sheldrake and Husband, 1985). Further, it has been demonstrated that following colostrum cell ingestion there is a higher response of lymphocytes to non-specific mitogens (Riedel-Caspari, 1993; Le Jan, 1996). Additionally, the transfer of colostrum cytokines to the neonate may promote immunological function (Le Jan, 1996; Blum, 2006). Heat treatment may damage both colostrum cells and cytokines and thereby affect the immunological capacity of the neonatal lamb.

In conclusion, the results presented indicate that feeding heat-treated sheep colostrum (56 °C, 30 min) alters some immunological parameters in lambs, mainly IgG concentration and the response to PHA. However, no adverse effects were observed for phagocytic function or performance of the lambs.

Acknowledgements

This research was funded by a grant from DGA (P 218/242, group A30). The authors wish to express

their appreciation to María Angeles Lostao and Dr. Desireé Pereboom from Servicio de Separación Celular, Zaragoza University, for their technical assistance.

References

- Aldridge, B., Garry, F., Adams, R., 1992. Role of colostrum transfer in neonatal calf management: failure of acquisition of passive immunity. *The Compendium* 14, 265–270.
- Al-Sabbagh, T.A., Swanson, L.V., Thompson, J.M., 1995. The effect of ewe body condition at lambing on colostrum immunoglobulin G concentration and lamb performance. *Journal of Animal Science* 73, 2860–2864.
- Alvarez, V., Arranz, J., Daltabuit-Test, M., Leginaikoa, I., Juste, R.A., Amorena, B., de Andrés, D., Luján, L., Badiola, J.J., Berriatúa, E., 2005. Relative contribution of colostrum from Maedi–Visna virus (MVV) infected ewes to MVV-seroprevalence in lambs. *Research in Veterinary Science* 78, 237–243.
- Argüello, A., Castro, N., Capote, J., Ginés, R., Acosta, F., López, J.L., 2003. Effects of refrigeration, freezing–thawing and pasteurization on IgG goat colostrum preservation. *Small Ruminant Research* 48, 135–139.
- Bernadina, W.E., van Leeuwen, M.A.W., Hendriks, W.M.L., Rutenberg, E.J., 1991. Serum opsonic activity and neutrophil phagocytic capacity of newborn lambs before and 24–36 h after colostrum uptake. *Veterinary Immunology and Immunopathology* 29, 127–138.
- Besser, T.E., Gay, C.C., 1994. The importance of colostrum to the health of the neonatal calf. *Veterinary Clinics of North America. Food Animal Practice* 10, 107–117.
- Blum, J.W., 2006. Nutritional physiology of neonatal calves. *Journal of Animal Physiology and Animal Nutrition* 90, 1–11.
- Castro, N., Capote, J., Alvarez, S., Argüello, A., 2005. Effect of lyophilized colostrum and different colostrum feeding regimen on passive transfer of immunoglobulin G in Majorera goat kids. *Journal of Dairy Science* 88, 3650–3654.
- Ekkel, E.D., Huypers, A.H., Counotte, G.H.M., Tielen, M.J.M., 1995. The phytohaemagglutinin (PHA) skin test as an indicator of stress-induced changes in immune reactivity in pigs. *Veterinary Quarterly* 17, 143–146.
- Fernández, A., Ramos, J.J., Loste, A., Ferrer, L.M., Figueras, L., Verde, M.T., Marca, M.C., 2006. Influence of colostrum treated by heat on immunity function in goat kids. *Comparative Immunology, Microbiology and Infectious Diseases* 29, 353–364.
- Foster, D.N., Smith, G.W., Sanner, T.R., Busso, G.V., 2006. Serum IgG and total protein concentrations in dairy calves fed two colostrum replacements products. *Journal of the American Veterinary Association* 229, 1282–1285.
- Gilbert, R.P., Gaskins, C.T., Hillers, J.K., Parker, C.F., McGuire, T.C., 1988. Genetic and environmental factors affecting immunoglobulin G₁ concentrations in ewe colostrum and lamb serum. *Journal of Animal Science* 66, 855–863.
- Godden, S.M., Smith, S., Feirtag, J.M., Green, L.R., Wells, S.J., Fetrow, J.P., 2003. Effect of on-farm commercial batch pasteurization of colostrum on colostrum and serum immunoglobulin concentrations in dairy calves. *Journal of Dairy Science* 86, 1503–1512.
- Godden, S., McMartin, S., Feirtag, J., Stabel, J., Bey, R., Goyal, S., Metzger, L., Fetrow, J., Wells, S., Chester-Jones, H., 2006. Heat-treatment of bovine colostrum. II: effects of heating duration on pathogen viability and immunoglobulin G. *Journal of Dairy Science* 89, 3476–3483.
- Gröndahl, G., Johannisson, A., Dimmers, S., Waern, M.J., 1999. Influence of age and plasma treatment on neutrophil phagocytosis and CD18 expression in foals. *Veterinary Microbiology* 65, 241–254.
- Gröndahl, G., Sternberg, S., Jensen-Waern, M., Johannisson, A., 2001. Opsonic capacity of foal serum for the two neonatal pathogen *Escherichia coli* and *Actibobacillus equuli*. *Equine Veterinary Journal* 33, 670–675.
- Hanel, R.M., Crawford, C., Hernández, J., Benson, N.A., Levy, J.K., 2003. Neutrophil function and plasma opsonic capacity in colostrum-fed and colostrum-deprived neonatal kittens. *American Journal of Veterinary Research* 64, 538–542.
- Hernández, A., Yager, J.A., Wikie, B.N., Leslie, K.E., Mallard, B.A., 2005. Evaluation of bovine cutaneous delayed-type hypersensitivity (DTH) to various test antigens and a mitogen using several adjuvants. *Veterinary Immunology and Immunopathology* 105, 45–58.
- Hunter, A.G., Reneau, J.K., Williams, J.B., 1977. Factors affecting IgG concentration in day-old lambs. *Journal of Animal Science* 45, 1147–1151.
- Lakritz, J., Tyler, J.W., Hoestler, D.E., Marsh, A.E., Weaver, D.M., Holle, J.M., Steevens, B.J., Densbigh, J.L., 2000. Effects of pasteurization of colostrum a subsequent serum lactoferrin concentration and neutrophils superoxide production in calves. *American Journal of Veterinary Research* 61, 1021–1025.
- Le Jan, C., 1996. Cellular components of mammary secretions and neonatal immunity: a review. *Veterinary Research* 27, 403–417.
- Lombardo, P.S., Todhunter, D.A., Scholz, R.W., Eberhart, R.J., 1979. Effect of colostrum ingestion on indices of neutrophil phagocytosis and metabolism in newborn calves. *American Journal of Veterinary Research* 40, 362–368.
- Maden, M., Altunok, V., Birdane, F.M., Asla, V., Nizamlioglu, M., 2003. Blood and colostrum/milk serum γ -glutamyltransferase activity as a predictor of passive transfer status in lambs. *Journal of Veterinary Medicine. Series B* 50, 128–131.
- Massimini, G., Peli, A., Boari, A., Britti, D., 2006. Evaluation of assay procedures for prediction of passive transfer status in lambs. *American Journal of Veterinary Research* 67, 593–598.
- McGuire, T.C., Regnier, J., Kellon, T., Gates, N.L., 1983. Failure in passive transfer of immunoglobulin G₁ to lambs: measurement of immunoglobulin G₁ in ewe colostrums. *American Journal of Veterinary Research* 44, 1064–1067.
- McGuirk, S.M., Collins, M., 2004. Managing the production, storage, and delivery of colostrum. Managing the transition cow to optimize health and productivity. *Veterinary Clinics of North America. Food Animal Practice* 20, 593–603.
- McMartin, S., Godden, S., Metzger, L., Feirtag, J., Bey, R., Stabel, J., Goyal, S., Fetrow, J., Wells, S., Chester-Lones, H., 2006. Heat treatment of bovine colostrum. I: effects of temperature on viscosity and immunoglobulin G level. *Journal of Dairy Science* 89, 2110–2118.
- Menge, Ch., Neufeld, B., Hirt, W., Schmeer, N., Bauerfeind, R., Baljer, G., Wieler, L.H., 1998. Compensation of preliminary blood phagocyte immaturity in the newborn calf. *Veterinary Immunology and Immunopathology* 62, 309–321.
- Meylan, M., Rings, D., Shulaw, W.P., Kowalski, J.J., Bech-Nielsen, S., Hoffsis, G.F., 1996. Survival of *Mycobacterium paratuberculosis* and preservation of immunoglobulin G in bovine colostrum under experimental conditions simulating pasteurization. *American Journal of Veterinary Research* 57, 1580–1585.
- Nowak, R., Poindron, P., 2006. From birth to colostrum: early steps leading to lamb survival. *Reproduction Nutrition Development* 46, 431–446.

- O'Brien, J.P., Sherman, D.M., 1993. Serum immunoglobulin concentrations of newborn goat kids and subsequent kid survival through weaning. *Small Ruminant Research* 11, 71–77.
- Pépin, M., Vitu, C., Russo, P., Mornex, J.F., Eterhans, E., 1998. Maedi–Visna infection in sheep: a review. *Veterinary Research* 29, 341–367.
- Prezioso, S., Renzoni, G., Allen, T.E., Taccini, E., Rossi, G., DeMartini, J.C., Braca, G., 2004. Colostral transmisión of Maedi–Visna virus: sites of viral entry in lambs born from experimentally infected ewes. *Veterinary Microbiology* 104, 157–164.
- Ramos, J.J., Verde, M.T., Marca, M.C., Fernández, A., 1994. Clinical chemical values and variations in Rasa Aragonesa ewes and lambs. *Small Ruminant Research* 13, 133–139.
- Riedel-Caspari, G., 1993. The influence of colostral leukocyte on the course of an experimental *Escherichia coli* infection and serum antibodies in neonatal calves. *Veterinary Immunology and Immunopathology* 35, 275–288.
- Sandbulte, M.R., Roth, J.A., 2004. Methods for cell-mediated immunity in domestic animal species. *Journal of the American Veterinary Medical Association* 225, 522–530.
- Sheldrake, R.F., Husband, A.J., 1985. Intestinal uptake of intact lymphocytes by neonatal rats and lambs. *Research in Veterinary Science* 39, 10–15.
- Sherman, D.M., Arendt, T.D., Gay, J.M., Maefsky, V.A., 1990. Comparing the effects of four colostrum preparations on serum Ig levels of newborn kids. *Veterinary Medicine* 8, 908–913.
- Tizard, I.R., 2002. *Inmunología Veterinaria*, Sexta edición. MacGraw-Hill Interamericana, México.
- Trujillo, A.J., Castro, N., Quevedo, J.M., Argüello, A., Capote, J., Guamis, B., 2007. Effect of heat and high-pressure treatments on microbiological quality and immunoglobulin G stability of caprine colostrum. *Journal of Dairy Science* 90, 833–839.
- Tyler, J.W., Lakritz, J., Hostetler, D.E., Douglas, V., Weaver, D.M., Steevens, B.J., Holle, J., Denbidh, J., 2000. Effect of pasteurization at 76 and 63 °C on the absorption of colostral IgG in calves. *Journal of Dairy Research* 67, 619–623.
- Vihan, V.S., 1988. Immunoglobulin levels and their effect on neonatal survival in sheep and goats. *Small Ruminant Research* 1, 135–144.
- Waelchli, R.O., Müller, Ch., Hässig, M., Rüschi, P., 1994. Immunoglobulin concentrations in colostrum and serum of lambs of dairy sheep breeds. *The Veterinary Record* 135, 16–17.
- Weaver, D.M., Tyler, J.W., VanMetre, D.C., Hostetler, D.E., Barrington, G.M., 2000. Passive transfer of colostral immunoglobulins in calves. *Journal of Veterinary Internal Medicine* 14, 569–577.