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Influence of colostrum treated by heat on immunity function in goat kids

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Abstract

The aim was to evaluate the influence of goat colostrum treated by heat on immune function in kids. Thirteen newborn kids were fed untreated colostrum (group A) and 13 kids were fed colostrum treated by heat (56 °C, 30 min) (group B). Blood samples were obtained at eight time points between the ages of 0 h to 28 days. Serum protein fractions, IgG levels and phagocytic activity of neutrophils were determined. A delayed type hypersensitivity (DTH) test was used, clinical status and body weight was recorded. There were no clinical signs of disease and no differences ($P>0.05$) on body weight between groups were noted. Kids from group B had less total protein levels and γ-globulins than kids from group A ($P<0.05$). A decrease in serum IgG levels ($P<0.05$) was observed in kids from group B during all experiment. DTH response in kids from group B was lower ($P<0.01$) than group A, suggesting alteration on cellular immune system. No effects on phagocytic activity of the neutrophils were observed when both groups were compared ($P>0.05$). These results showed

Abbreviations: DTH, delayed type hypersensitivity; IgG, immunoglobulin G; CMIR, cell-mediated immune response; FPT, failure of passive transfer; CAE, caprine arthritis-encephalitis; PBMC, peripheral blood mononuclear cells

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that colostrum treated by heat impaired some immunological parameters in kids, but these changes did not affect on clinical status or performance.

Keywords: Kids; Colostrum; DTH; Phagocytosis; Immunoglobulins

Résumé

Le but de ce travail a été l'évaluation des effets du chauffage sur l'ingestion par les chevreaux du colostrum des chèvres. Treize chevreaux nouveau-nés ont été nourris avec le colostrum non chauffé (groupe A) tandis que 13 autres (groupe B) ont reçu le colostrum chauffé (56ºC pendant 30 minutes). Huit prélèvements de sang ont été effectués chez tous les animaux entre la naissance (heure 0) et le jour 28. On a déterminé dans le sang des animaux, les différentes fractions sériques, le niveau des IgG et l'activité phagocytaire des neutrophiles. Un test d'intradermoréaction (IDR) a été réalisé en même temps qu'un examen clinique et le suivi du poids. Ni le poids des animaux, ni leur santé n'ont été modifiés significativement ($P > 0,05$) dans les groupes A et B. En revanche, le taux sérique des IgG a diminué significativement dans le groupe B de même que le taux de protéines et des ß-globulines. La réponse à l'intradermoréaction (IDR) a été également inférieure dans le groupe B. Ces résultats suggèrent que la réponse immunitaire de type cellulaire a été altérée. Cependant, l'activité phagocytaire des neutrophiles s'est montrée normale. Ces résultats montrent que le traitement du colostrum par chauffage est susceptible de modifier quelques réponses immunologiques chez les chevreaux, mais ces changements n'ont pas affecté l'état clinique de ces animaux.

Mots Clés: Colostrum; Chevreaux; Inmunoglobulines, Intradermoréaction; Phagocytose

1. Introduction

In ruminants, the synepitheliochorial placenta forms a syncytium between the maternal endometrium and the fetal trophectoderm, separating the maternal and fetal blood supplies and preventing the transmission of immunoglobulins (Ig) in utero. Consequently, the neonates are born agammaglobulinemic, depending of colostral Ig intake to reach an adequate passive immunity [1]. Colostrum is the first mammary secretion produced after birth in the mammalian species, and early ingestion of colostrum by the newborn is critical for its survival. Through colostrum, Ig and other important factors in disease protection, as well as hormones, growth factors and essential nutrients are transferred. Failure of passive transfer (FPT) of colostral Ig is associated with increased morbidity and mortality from neonatal diseases and is well documented for the neonates of farm animals [1,2]. The management of livestock can influence the levels of Ig and is better in kids suckling the colostrum from their dams. Sometimes, this is not possible because the newborn is orphaned or the amount of colostrum is insufficient. In this case, goat kids can be successfully fed with other colostral preparations from caprine livestock [3,4] or with

commercial sheep colostrum, but without to obtain the necessary immunity to protect them during the first month of life [5].

Some diseases can be transferred to newborn by colostral or milk secretions such as caprine arthritis-encephalitis (CAE) and various approaches have been tried to control it [3,6,7]. Colostrum treated by heat is a possibility because the virus of CAE is killed by heat [6,7]. However, Ig and other constituents of the colostrum are thermolabile and the immunological status of the animals can be compromised because a decrease in serum IgG, lactoferrin concentration and neutrophil function have been observed in calves fed with pasteurized bovine colostrum [8–10].

Only the level of Ig has been considered in most studies of FPT because this plays a crucial role in disease protection. However, other components of the innate immune system should be considered, such as phagocytosis by neutrophils, which are the first line of defense against bacterial infection [11] or delayed-type hypersensitivity (DTH) which is an in vivo manifestation of cell-mediated immune response (CMIR) [12].

The purpose of this study was to determine the influence of colostrum treated by heat on some immunological parameters in goat kids during the first month of life.

2. Materials and methods

2.1. Animals

The Ethics Committee of the Zaragoza University approved this experimental protocol. Thirteen, pluriparous, pregnant, Murciano-Granadina goats breed from a commercial milk producer farm in Zaragoza (Spain) were used. All births were double and kids were eligible for the study only if they had no trouble at birth and they were colostrum-deprived. The kids were immediately separated from their dams, their umbilical cords disinfected, injected with a vitamin AD_3E dose, and their weights were recorded at day 0 and weekly until the end of the study.

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

2.2. Feeding protocol

Two kids from each goat were used: one was randomly chosen and assigned to receive colostrum without treatment pooled from a local goat farm (group A, $n = 13$), and the other one was fed with the same colostrum which had been treated by heat at 56 °C for 30 min at Food Technology Department in Veterinary Faculty (group B, $n = 13$). 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.

A volume of colostrum to be fed was calculated (120 ml/kg body weight) to the first 24 h of life. In both groups, the total amount of colostrum was dividing into

three equal parts and given at 3, 10 and 17 h after birth using a nipple bottle. All kids included in this study drunk all colostrum calculated by nipple bottle. When the kids of both groups were 24 h old, they were fed with artificial powdered milk, reconstituted in hot (40 °C) water, three times a day using nipple bottles. At 12 days old, a starter feed and barley straw was introduced.

2.3. Blood samples

Blood samples (4 ml) were collected from the jugular vein and were distributed into two tubes: one of them without anticoagulant to obtain serum and the second tube with heparin for phagocytic assays. Samples were obtained at birth, just before suckling (day 0) and on 2, 4, 8, 12, 16, 20 and 28 days old.

2.4. Protein serum electrophoresis

Serum protein fractions were segregated by electrophoresis with cellogel acetate strips (2.5×17 cm, ATOM cat. number 19000) and were read with a photodensitometer (Shimadzu CS-9000, Kyoto, Japan). Serum total protein was performed with an autoanalyser (Technicon RA-500, Dublin, Ireland) using the manufacture's reagent and recommended procedures.

2.5. IgG levels in colostrum and serum

IgG levels in the colostrum was analyzed by a non-competitive sandwich ELISA with specific antibodies (Calokit-Cabra, Zeu-Immunotec S.L., Zaragoza, Spain) following the manufacturer's instructions.

The serum IgG was determined using a radial immunodiffusion test. Plates from Bethyl Laboratories (cat. number R50-100, Montgomery, Texas, USA) were used. The plates were incubated at room temperature for 72 h, after which the ring of precipitation was measured. Samples were evaluated in duplicate and three standards of known concentrations were included in each analysis.

2.6. Phagocytic assay

In vitro phagocytic activity was quantified by flow cytometry and determined using the Phagotest® kit (cat. number 341060, Orpegen Pharma, Heidelberg, Germany). The kit has FITC-labelled *Escherichia coli* to promote phagocytosis by neutrophils and the test was performed according to the manufacturer instructions with minor modifications. Briefly, 20 μl of bacterial suspension (1×10^9 *E. coli*/ml) was added to 100 μl of whole heparinized blood in a tube. After vortexing all tubes, they were incubated at 39 °C in a shaking water bath for 10 min. A control tube remained on ice, inhibiting phagocytosis. Following incubation, phagocytosis was stopped by placing the tubes in icewater. To eliminate non-phagocytosed bacteria, 100 μl of "quenching solution" for lysis of erythrocytes and fixation of the leukocytes were added. The phagocytic activity was quantified by flow cytometry (Epics Elyte

Coulter, Hialeah, FL, USA). 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 the forward scatter and the side scatter. The FITC 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. Delayed type hypersensitivity test

DTH test was performed to assess in vivo cellular immunity. Each kid was injected intradermically on day 7 and 28 with 0.1 ml of 2.5 mg/ml phytohemagglutinin (cat. number L8754, PHA, Sigma Chemical Co.) in isotonic saline solution in the centre of the costal area. Approximately 10 cm from this site, 0.1 ml of saline solution was injected, serving as a control. Double skinfold thickness was measured using a constant tension calliper, immediately and 3, 6, 12, 24 and 48 h after injection. Data were expressed as a ratio of skin thickness of PHA-injected to that of saline solution.

2.8. Statistical analysis

Data are reported as mean $\pm$ SD. A non-parametric Mann–Whitney U rank sum test was used for comparisons between groups. A P value of $P < 0.05$ 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). P values were calculated using Spearmann's test.

3. Results

3.1. Clinical status and body weight

Kids in both groups appeared to be clinically normal and no deaths were recorded. No variations in rectal temperature were noted and were always normal (range 38.5–39.3 °C, $P > 0.05$) and no statistically significant differences between groups were recorded, neither on body weight between groups during all experiment ($P > 0.05$). At day 0, body weight was 2.66 ± 0.55 kg in group A (range 1.6–3.4) and 2.43 ± 0.29 kg in group B (range 2.1–3.1), and at the end of the study, the body weight in group A was 5.91 ± 0.64 kg (range 4.5–6.8) and in group B was 5.6 ± 0.6 kg (range 4.85–6.4) ($P > 0.05$).

3.2. Protein serum electrophoresis

Table 1 shows data obtained from analysis of serum protein fractions. At day 0, no statistically significant differences ($P > 0.05$) were found between the groups, and no γ-globulin fractions were detected. In the following days, levels of total proteins

Table 1
Serum protein fractions (g/l) from kids fed with untreated colostrum (group A) and colostrum treated by heat (group B)

	Day 0		Day 2		Day 4		Day 12		Day 28	
	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B
Total proteins	36.5±2.7	37±2.3	68.7±6.2	62.8±13	65.1±8.2	58±8[a]	63.8±5.7	55.6±8[b]	62±3.1	58.3±6[b]
Albumin	25±1.7	27±2.3	26±2.1	27±3.1	30±2.5	29.5±2.3	31.7±3.4	29±4.2	31.7±3.3	30±3.1
α-globulins	4.6±1.6	5±2	4.9±1.2	5.2±2.2	4.5±0.6	4.3±1	3.9±0.8	3.9±1	5.7±1.3	5.8±1.7
β-globulins	5.3±0.5	4.3±0.9	7.9±1.5	8.3±2	7.3±1.5	6.7±0.9	7.4±0.8	6.8±0.8	10.9±1.4	9.9±1.6
γ-globulins	n.d.	n.d.	30.1±7	22.4±8[a]	23±5.8	17.1±7[a]	20.7±3.7	16±6.3[b]	13.7±0.2	12.5±0.4[a]
Globulins	11±1.3	10±3.1	42.9±5.5	35.9±10[a]	34.9±6.6	28±7.6[a]	32.1±4.9	27±5.7[a]	30.4±3	28.2±5.7[a]
A/G ratio	2.3±0.23	2.8±0.7	0.91±0.3	1.3±0.4[a]	0.89±0.1	1.1±0.3[a]	1±0.15	1.13±0.3	1.05±0.17	1.1±0.22

Values are mean±SD ($n = 13$); n.d. = not detected; [a]$P<0.05$; [b]$P<0.01$; comparing groups A and B at each time point.
A/G ratio = albumin/globulin ratio.

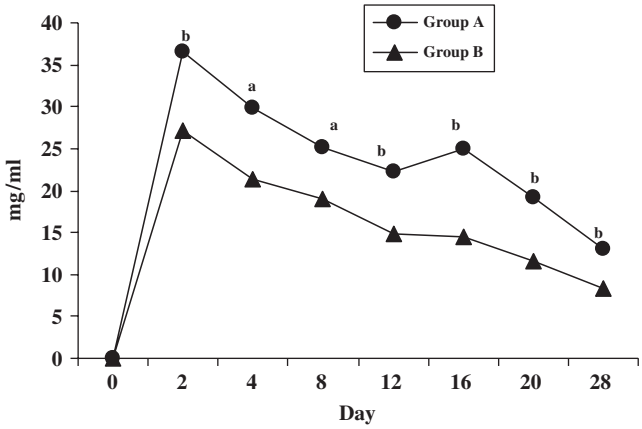

Fig. 1. IgG concentration (mg/ml) in serum from kids fed untreated colostrum (group A) and colostrum treated by heat (group B). ($n = 13$). [a]$P<0.05$; [b]$P<0.01$, comparing groups A and B at each time point.

(TP), γ-globulin fractions and globulins were lower and albumin/globulin ratio (A/G ratio) in group B was higher ($P<0.05$) than in group A. At the end of the experiment, TP levels in group B were lower than group A ($P<0.01$), and there was still a lower concentration of γ-globulin and globulin levels in group B ($P<0.05$). A significant correlation between serum IgG concentration and γ-globulin concentration in both groups of kids was detected (range $P<0.05$–0.001; range $r^2 = 0.394$–0.857).

3.3. IgG levels in colostrum and serum

The IgG concentration in untreated colostrum was 19.97 mg/ml, whereas in the colostrum treated by heat the concentration was 14.13 mg/ml, 29.2% lower than untreated colostrum.

None of the kids had detectable serum IgG at birth (Fig. 1). The mean peak serum IgG concentration on day 2 was significantly higher ($P<0.01$) in kids fed colostrum without treatment than kids fed colostrum treated by heat. IgG serum levels were decreasing, but were always significantly lower ($P<0.01$) in group B until the end of the experimental period.

3.4. Phagocytosis by neutrophils

The percentage of phagocytic activity of the neutrophils is shown in Table 2. The lowest phagocytic activity was observed at birth in both groups without significant differences ($P>0.05$). This activity increased, but no effect of colostrum treated by heat was noted ($P>0.05$).

Table 2
Percentage of phagocytic neutrophils from kids fed untreated colostrum (group A) and colostrum treated by heat (group B)

Day	Group A	Group B
0	61.3±9.8	55.6±10.7
4	73.1±9.4	75.1±4.5
12	69.6 ±13.9	62.9±11.7
20	70.7±8.8	74.1±7.6
28	68.5±8.8	68.3±6.2

Data are mean±SD ($n = 13$).

Table 3
Delayed type hypersensitivity test of kids fed untreated colostrum (group A) or colostrum treated by heat (group B)

	Day 7		Day 28	
Hour	Group A	Group B	Group A	Group B
0	1.04±0.14	1.01±0.21	1.0±0.1	0.97±0.07
3	2.47±0.49	2.04±0.28[a]	2.35±1.11	1.66±0.44[a]
6	1.96±0.28	1.58±0.24[b]	2.0±0.78	1.42±0.29[a]
12	1.83±0.39	1.42±0.35[a]	2.05±0.5	1.81±0.66
24	1.69±0.24	1.39±0.2[b]	1.81±0.29	1.46±0.26[b]
48	1.49±0.21	1.35±0.13	1.61±0.29	1.41±0.24

Data is expressed as ratio between thickness of the skin with PHA and isotonic saline solution.
Data are mean±SD ($n = 13$). [a]$P<0.05$; [b]$P<0.01$, comparing groups A and B at each time point.

3.5. Delayed type hypersensitivity test

Table 3 shows results of DTH test. The group B had less response to PHA injection at both 7 and 28 days. These differences were higher ($P<0.01$) on day 7 at 6 and 24 h after injection. The greatest difference between the groups was observed 24 h after injection ($P<0.01$).

4. Discussion

In this experiment, no mortality or clinical signs of disease in any kids of both groups were recorded. Furthermore, there were no differences in the body weight between groups. These results show that, in our experimental conditions, the source of colostrum (untreated or treated by heat) had no effects on these parameters. Other studies have shown a high mortality and sick in kids that received colostrum treated

by heat being in correlation with their low serum immunoglobulin levels [13]. However, in other experiments no death were recorded after pasteurizated colostrum were given to newborn [3,10]. Many factors such as environmental and management conditions, stress, concurrent disease and virulence of the pathogens many also contribute to the development of disease. Management and experimental conditions of this study were excellent and significantly different from observed in the majority of caprine farms, so could explain the good outcome recorded.

Results obtained from this study are important because the aim of heating colostrum is to destroy the CAE virus as an effective strategy for preventing of CAE virus infection on kids [3,6,7,14] while guaranteeing survival. Pasteurization of colostrum and milk has been demonstrated to be effective to prevent transmission of infectious microorganisms in cattle [8,15]. An appreciable advantage of this method is that colostrum can be from the same farm as the kids. Also, heat treated colostrum can be frozen for later use, such as management practices recommended for the control of CAE virus infection [7], and no negative effect of the frozen pasteurized colostrum has been observed with bovine colostrum [16].

The segregation of serum proteins showed normal values for all fractions and slightly lower for TP levels [17]. Also, showed that after the kids were fed colostrum, TP and globulin levels quickly increased (day 2). This observation is because colostrum is rich in IgG and IgA and they are quickly absorbed by the gut in the first 12–24 h of life [2]. Most of the IgG is included in the γ-globulin fraction, thus the analysis of this fraction of globulins provides an approximation to serum IgG concentration. The less concentration of γ-globulins and IgG found in group B compared with group A at day 2 together with the positive and significant correlation found between γ-globulin and IgG concentrations, suggest that γ-globulin levels are directly derived from colostrum. Thus, TP levels, total globulins, γ-globulins and the A/G ratio may be considered to evaluate if a kid has received sufficient colostrum, or a colostrum of good quality, being a simple method to perform in a veterinary clinical laboratory. Electrophoretic separation of serum proteins have been used to compare total serum globulins with IgG concentration in dairy cattle [18], being a reliable indicator of IgG levels. Also, we can obtain information about other globulin and albumin fractions.

After heat treatment of the colostrum, our results showed that the reduction in colostral IgG (29.2%) was similar to that reported for cow's pasteurized colostrum [8,10] and lower to that reported by Argüello et al. [19] in goat colostrum. Serum IgG levels in our study were higher than others previously reported for kids [3,13]. In this study a low serum IgG content in the kids fed heat treated colostrum was observed. There are several studies that have investigated the effect of colostrum pasteurization on IgG levels in newborn calves [9,10,16]. All authors have found lower values of serum IgG in the samples treated by heat. The decrease in IgG levels when cow's colostrum is pasteurized depended on the temperature and volume of the batch. In this way, Tyler et al. [16] found a very important reduction in calf serum IgG when colostrum was heated at 76 °C. Godden et al. [10] recorded a percentage reduction in serum IgG levels of 58.5% when the volume of the batch of pasteurization of the colostrum was greater (95 l) in comparison which a smaller batch (57 l). Under our

experimental conditions, the temperature was low (56 °C) and the volume of the batch was only 24 l, so the pasteurization conditions used in this experiment had little effect on colostral IgG.

We have not found any references about the evaluation of phagocytic activity of the neutrophils by flow cytometry in goats. From the scientific literature there are reports in large animal neonates that have shown that phagocytic activity was correlated with serum IgG levels [20,21], and also by other plasma components such as opsonins or complement which may be of great importance in phagocytosis [22–24]. In contrast, there are other reports that found phagocytic activity of the neutrophils was independent of IgG levels in foals [23,24] or in neonatal kittens [25]. Contrary to the results obtained in serum IgG levels, colostrum treated by heat had no influence on phagocytic activity of neutrophils. Neutrophils from neonatal kids were functional, since they could phagocyte xenogeneic opsonized bacteria (*E. coli* from the kit) and this activity increase with the time, as Menge et al. [26] showed in neonatal calves.

The intradermal injection of the mitogen PHA produces a local tissue reaction with the same characteristics that DTH, being a rapid and practical method to assess the cellular immunity in vivo [27] and has been used to measure the ability of the livestock to generate CMIR [12]. Results of the DTH test indicate that colostrum treated by heat could have destroyed some of the cell immunity components of the colostrum, altering cell-mediated immunity in kids. Mammary secretions contain a large variety of immunological components, such cytokines and cells, and are rich in T lymphocytes being the most prevalent and active of the lymphocytes subpopulations, contributing to the cellular defense of the neonate [28,29]. Cell immunity is an effector function of T lymphocytes, and our results show cell immunity was impaired. Also, colostrum cytokines are important in developing of immune function in the newborn because they are transferred to neonatal circulation as was demonstrated in calves by Yamanaka et al. [30], showing that IL-1β, TNF-α, and IFN-γ cytokines from colostrum to circulation enhanced the mitogenic response of neonatal PBMC. The heating of the colostrum at 56 °C, 30 min, would have destroyed the colostral cytokines and their function, being these proteins functional in the mammary secretions transferring immunity to neonate [28,30].

In conclusion, the results of the present work indicate that goat colostrum treated by heat (56 °C, 30 min) could impair some immunological functions of kids, specially serum IgG concentration and DTH response. However, no adverse effects were observed on the growth and health of the kids.

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