

## EFFECTS OF REFRIGERATION, DEEP FREEZING-SPRAY DRYING AND PASTEURIZATION ON IGG BUFFALOCOLOSTRUM PRESERVATION

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### ABSTRACT

The aim of this paper was to evaluate the effects of refrigeration, several different methods of thawing, and pasteurization on the concentration of IgG in buffalo colostrum. Four different experiments were designed to analyse these effects. In the first of these, 50 samples of buffalo colostrum were stored in a cold-storage room at a temperature of 4 °C for a 3-month period. Nostatistically significant effects were observed within this time, although there was a reduction in IgG concentrations (51.05 and 25.11 mg/ml IgG at day 0 and 91, respectively). In the second experiment, 20 samples of buffalo colostrum were frozen and subsequently thawed using four different methods: spray drying (60 °C), refrigeration (4 °C), deep freezing (-20 °C) and freeze drying (55 °C). The process was carried out seven times for each of the four methods. The method of thawing did not affect the colostrum IgG concentration. However, the repetition of freezing and thawing tended to reduce IgG concentrations; albeit to no significant degree (15.50 and 10.73 mg/ml IgG at cycle 0 and 7, respectively). In the third experiment, 30 buffalocolostrum samples were used and a reduction of approximately 35% of IgG concentration after pasteurization was observed. Refrigeration, freezing and pasteurization are suitable methods for conserving buffalo colostrum.

**KEYWORDS:** Colostrum, Buffalo, Refrigeration, Thawing, Pasteurization

### INTRODUCTION

Colostrum is the nutrient rich, first secretion produced by mammals within 24 to 72 hours after parturition. It is a mixture of lacteal secretions and constituents of blood serum, notable immunoglobulin and serum proteins that accumulate in mammary gland during prepartum dry period which can be harvested immediately after parturition (Foley and Otterby, 1978). Buffalo Colostrum is the first milk secreted at the time of parturition which lasts for 2- 4 days which contains more lactalbumin and protein and rich in antibodies that confer passive immunity to the newborn.(Meena *et al.*, 2013). The colostrum can either be fresh (obtained directly from the mother) or preserved (either refrigerated or frozen). The latter system is of particular inter-est in areas where diseases such as CAEV are present, since colostrum is one of the direct means of trans-mission (Guerrault, 1990). Pasteurization can play a vital role in preventing such transmission. Several authors have confirmed this fact; Adams *et al.* (1983) demonstrated the inactivation of encephalitis arthritis virus in goats after a 60 min pasteurization treatment at 56 °C, while Moore *et al.* (1996) successfully in-activated the BIV virus with a 30 min pasteurization treatment at 47 °C, and Meylan *et al.* (1996) observed a reduction in levels of *Mycobacterium Para tuberculosis* in buffalo colostrum after pasteurization (62 °C for 30 min).

With respect to colostrum preservation methods, the most commonly cited forms are freezing (Voigtlander, 1981; Skrivanova et al., 1984; Morand-Fehr, 1989; Holloway et al., 2001), refrigeration (Valenta, 1982), lyophilizing (Klobasa et al., 1998), the addition of acidifying substances (Muller and Syhre, 1975) or sub-stances with a buffering capacity (Jenny et al., 1984). Chemical preservation methods are recommended for storage at room temperature in cases where freezing facilities are not available (Foley and Otterby, 1978).

The objective of this research was to determine the IgG concentration of colostrum refrigerated over a long period of time, and to evaluate the effects of different methods of thawing and pasteurizing techniques on IgG concentration in goat colostrum.

## MATERIAL AND METHODS

Four different experiments were conducted. In the first, the effect of cow colostrum refrigeration time on IgG concentration was evaluated. The study was conducted on 25 cross bred cows in Community Cattle Care Centre of CFDT, Koduvalli. Colostrum samples were collected for 7 days after parturition from cow. After collection, the colostrum samples were stored at  $-20^{\circ}\text{C}$  until further analysis. In the second experiment, the effect of the refrigeration method on IgG concentration was evaluated. In the first treatment, colostrum samples were filtered and packed in heat resistant laminated pouches and subsequently pasteurized for  $60^{\circ}\text{C}$  for 60 minutes and cooled to  $5^{\circ}\text{C}$ , stored in refrigerator for 7 days period and analyzed. The refined sugar, banana, cocoa powder were added and filled in pouches and subsequently pasteurized at  $63^{\circ}\text{C}$  for 30 minutes.

In the second experiment, the effect of the freezing method on IgG concentration was evaluated. In the second treatment, the colostrum is filtered and packed in heat resistant laminated pouches and pasteurized for  $60^{\circ}\text{C}$  for 60 minutes and cooled to  $5^{\circ}\text{C}$ , stored in deep freezer at  $-20^{\circ}\text{C}$ . During analysis the samples were thawed in hot water for 30 minutes at  $60^{\circ}\text{C}$ . After thawing refined sugar, banana, and cocoa powder were added and filled in another heat resistant laminated pouches and subsequently pasteurized at  $63^{\circ}\text{C}$  for 30 minutes. The third experiment used the sample was skimmed, pasteurized, spray dried and analyzed and stored at room temperature.

The IgG quantification was made according to Mancini et al. (1965) with some modifications. Briefly, the agar was prepared by adding 1 l of glycine buffer pH 7 (7.5 g glycine, 100 ml EDTA 0.4 M, in distilled water, and adjusting the volume to 1 l) to 20 g of agar. This suspension was placed in a boiling water-bath and stirred until all the agar had dissolved, after which it was cooled to  $60^{\circ}\text{C}$ . The antiserum (previously made by rabbit immunization with buffalo IgG) or a suitable dilution of it made in glycine buffer was heated to  $55^{\circ}\text{C}$ , after which both solutions were mixed thoroughly, avoiding bubbling, with the aid of a pipette preheated to  $60^{\circ}\text{C}$  in the water-bath. The antiserum-agar mixture was immediately poured into the petri dish with a final agar thickness of 0.3 cm. The petri dish was sealed and stored at  $4^{\circ}\text{C}$  until use. Circular wells were punched in the gel, using a 3mm bore needle. The small cylinders of gel cut out by the needle were removed by suction. Each of the wells received 10  $\mu\text{l}$  of antigen solution (standard curve) or blood serum. In each petri dish three wells received antigen solution for the preparation of the standard curve, this was prepared according to the method used by Catty and Raykundalia (1988).

With reference to statistical analysis, in experiment 1, a one-way analysis was used to test the effect of time on IgG colostrum concentration, in experiment 2, a GLM procedure was used, including number of cycle and thawing methods as

fixed effects, and in experiment 3, a one-way analysis was used to test the effect of pasteurization on IgG colostrum concentration and differences between means were tested by the Tukey t-test. All analysis were performed using the SPSS (v. 10.0) statistics program.

**RESULTS AND DISCUSSIONS**

Table 1 shows the evolution of IgG concentration in colostrum kept in refrigeration at 4 °C over a 91-day period. No significant statistical variation was observed (P = 0.579) in this concentration during refrigeration time, although it did tend to diminish at the end of the experiment (51%). The main reduction occurred during the first month of the test (30%), possibly because it was during this period that the natural fermentation process of the colostrum took place. It can be stated that refrigeration has a clear preservation effect on IgG concentration in goat colostrum, as was claimed by Valenta (1982) in his work with buffalo colostrum when he observed only slight variations of IgG colostrum concentration when this was refrigerated at -2 to +2 °C over a 14-day period. In contrast, Mbuthia *et al.* (1997) observed a significant reduction in IgG concentration in colostrum stored at 28 °C. In conclusion, after 3 months of preservation at refrigeration temperatures of 4 °C, IgG levels diminish by 30%, but the colostrum is still usable for new-born kids, although where possible it would be desirable to store colostrum for no longer than a month after collection.

The freezing of colostrum is a well-known method of preservation. Morand-Fehr (1989) stated that immunoglobulin's present in goat colostrum remain intact for 2 years, while Bilbao *et al.* (2001) ascertained that the maximum freezing time for buffalo colostrum is 15 years. In Table 2, the evolution of IgG concentrations in colostrum throughout the freezing-thawing cycles with different thawing methods can be observed. There is no statistical interaction between the two effects (cycle and thawing method), since the thawing method used shows no variation with respect to IgG concentration, although the number of times the colostrum was subsequently refrozen and thawed does have a reducing effect on IgG concentration.

**Table 1: IgG (Mean ± Standard Deviation) Buffalo Colostrum Concentration during Refrigeration Time**

Days	IgG (Mg/MI)	P
1	95.20±1.5 <sup>f</sup>	0.568
2	86.23±0.90 <sup>e</sup>	
3	71.1±1.25 <sup>d</sup>	
4	42.3±1.32 <sup>c</sup>	
5	21.3±0.17 <sup>a</sup>	
6	16.8±0.28 <sup>a</sup>	
7	12.5±0.27 <sup>a</sup>	

**Table 2: IgG (Mean ± Standard Deviation) Buffalo Colost Rum Concentration through Freezing-Thawing Cycles**

Day	Treatments				Effects*	
	Refrigerate d	Deep Freezing	Spray Drying	Freeze Drying		
0	85.52±0.5	81.43±0.2	80.56±0.2	81.22±0.8	0.069	0.959
15	81.13±2.1	78.26±3.1	76.27± 3.4	78.32±2.6		
30	77.56±3.4	73.46±1.4	72.56±3.32	73.46±1.4		

Table 2 Cont					
45	74.30±1.5	69.17±3.4	67.32±1.8	69.40±2.1	
60	71.26±1.2 <sup>a</sup>	64.25±2.6	62.26±1.7	64.16±1.8	
75	67.37±1.3	58.21±1.7	58.38±1.7	61.57±1.7	
90	62.34±2.1	53.34±1.5	52.16±3.2	56.24±3.1	

The reduction of IgG levels after several freezing-thawing cycles is probably due to the effect of temperature changes, though on no occasion did the temperature exceed 60 °C, the temperature at which globulins begin to break up, as has been demonstrated by Anema(2000). Similar results have been obtained by Jones et al. (1987) using cattle colostrum, observing that casein, IgG and IgM concentrations were not significantly affected by microwave thawing in reference to colostrum thawed for 25 min in water at 45 °C. With respect to the pasteurization of colostrum, Table 2 shows how this has a negative effect on IgG concentration, reducing it in the first case by 50.34% and by 37.84% in the second.

The CFU was strongly reduced by both pasteurization processes. Major significant differences between the IgG concentration in colostrum prior to and after pasteurization treatments was observed, while no statistically significant differences are observable between the two treatments carried out. The reduction observed in the colostrum IgG concentration after pasteurization is greater than that observed in the results of the research carried out on buffalo colostrum by Meylan et al. (1996). Incomplete contrast to this is the work carried out by Steinbach et al. (1981) in which no reduction in IgG concentration was observed after the pasteurization process. This apparent discrepancy could be due to the decreased exposure time to pasteurization of the colostrum used in the analysis (30 min) in comparison with our study.

## CONCLUSIONS

In conclusion, refrigeration is a good method of IgG preservation for goat colostrum for the first 3 months, and the methods of thawing used have less effect on IgG concentration than the number of times which colostrum is thawed and refrozen. Pasteurization has a negative effect on IgG concentration, although the effect of the latter on the transfer of passive immunity needs to be evaluated. The recommendations of this paper are to freeze in small quantities and to refrigerate left over thawed colostrum.

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