

# Technology of obtaining cow colostrum aseptic concentrates and their application to postpartum calves<sup>\*)</sup>

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### Summary

The aim of the study was an analysis of the possibility of bacterial flora reduction in cow colostrum using the thermal method, ultraviolet waves, focused electromagnetic field, pulse electric field and filtration on ceramic membranes, as well as an assessment of immunoglobulin availability from dried and lyophilized colostrum protein concentrate obtained as a result of filtration. It was noted that total reduction of *E. coli* and coliforms from cow colostrum may be attained as a result of filtration on ceramic membranes. Other methods appeared to have little effect and not to be useful in this range. Calves in the first days of life, instead of colostrum, were fed with dried or lyophilized colostrum protein concentrate obtained as a result of membrane filtration. It was found that an application of dried and lyophilized cow colostrum concentrate instead of maternal colostrum caused significantly higher absorption of class G immunoglobulins in calves with regard to maternal colostrum. This was established as a result of the study conducted that dried or lyophilized cow colostrum concentrates, obtained as a result of filtration, can be used in supplementation and support of calves' immunity, while their aseptic and concentrated form (dried material) allow better storage and distribution, as well as application in various herds.

**Keywords:** colostrum, calves, membrane filtration, colostrum concentrate

Calves immunity deficiency in the first period of life can result from: low concentrations of immunoglobulins in colostrum, too small dose of colostrum applied, its late administration or deficient infant alimentary tract. The result is an increase in the occurrence of alimentary and respiratory system diseases (2, 4, 5). Attempts at the supplementation of infants with preparations containing immunoglobulin do not always produce profitable results, and the cost of obtaining globulins often does not balance the gains from the conducted supplementation (17, 18). Often the proposed formulations do not contain adequate amounts of immunoglobulins to ensure a normal neonatal immune status. There are cases in animal breeding that

maternal colostrum is characterized by a low level of immunoglobulins and a decreased level of their availability (4, 17). Then the preserved colostrum may be applied – frozen or in colostrum preparations – which would allow an increase in newborn immunological status and ensure its protection in the first period of life (1, 8, 10).

A substantial problem is, however, the considerable infection of cow colostrum, which limits the possibility of its application in a variety of facilities, as pathogenic bacterial flora can infect fed newborns. Therefore, the search for methods of the elimination of bacteria from colostrum along with maintaining its biological value is an important problem. This will allow the use of the enormous immunological potential of cow colostrum and its biological value in supplementing of suckling calves and other animal species (2, 6, 10, 20). Temperature pasteurization, ultraviolet irradiation, and an activity of a pulsated and focused electromagnetic field or colostrum separation

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on microfiltration ceramic membranes can be used in this process. Another problem is dehydration of colostrum or colostrum preparations in order to reduce its volume, thus facilitating its storage and distribution.

The aim of the study was to examine the possibility of bacterial flora reduction in cow colostrum and colostrum preparations and an evaluation of immunoglobulins absorption by calves from dried and lyophilized colostrum protein concentrate obtained as a result of microfiltration.

### Material and methods

Cow colostrum was subjected to chemical analysis, and then to temperature pasteurization, an activity of focused and pulsed electromagnetic field and microfiltration. The presence of *E. coli* in colostrum and preparations was determined by culturing in Petri dishes (14, 15).

The pulsed activity of the electromagnetic field used the SU-1 reactor. The process involved 100 impulses of a width of 90 ms and a voltage of 30 kV.

Spray drying was performed using a spray dryer B-290 of BUCHII. An inlet air temperature in the drying process was 140°C and the outlet one was 60°C. The assumed parameters caused the dried concentrate particles to be heated up to a temperature of approximately 40°C, which minimized the possibility of the damage of proteins, vitamins and enzymes contained in the dried substance. The process of lyophilization was carried out using a laboratory freeze dryer CHRIST Alpha 1-4 LSC with a sublimation temperature of 18°C.

Colostrum protein concentrate obtained as a result of microfiltration was treated with pulsed electromagnetic field, and then was subjected to a dehydration process in a spray drier or lyophilizer.

Twenty-four calves of Polish Holstein-Friesian breed of black-and-white variety were selected for the study and were assigned to 3 groups. Two subgroups with different levels of IgG were created in group II (receiving dried concentrate) and in group III (receiving lyophilized concentrate): group I – control, calves fed with maternal colostrum, group II – dried cow colostrum protein concentrate covering the estimated calves immunoglobulin demand: A – to about 100%, B – to about 120%, group III – lyophilized cow colostrum protein concentrate covering the estimated calves' immunoglobulin demand: A – to about 100%, B – to about 120%.

Calves immunoglobulin demand was estimated based on the amount of their body water space (24). It was assumed that the amount of body water space accounts for 44% in the calf organism, and the amount of IgG required ensuring an adequate immune response is 15 g of immunoglobulins per 1 l of liquid.

Dried and lyophilized colostrum concentrates were dissolved prior to feeding in whole cows' milk, so that the calf received the same amount of liquid as in the control group (5 liters in the first day).

Blood from the right jugular vein was collected from calves at 2, 7, 14, 21 and 28 day of life. Animals were subject to constant observation by breeder and veterinary care.

**Tab. 1. Composition of colostrum, dried and lyophilized cow colostrum protein concentrate (after microfiltration) administered to calves**

| Composition   | Dry matter<br>% | Protein<br>% DM | IgG<br>% DM | Casein<br>% DM | Lactose<br>% DM |
|---|-----------------|-----------------|-------------|----------------|-----------------|
| Cow colostrum   | 23.98           | 58.63           | 23.07       | 24.58          | 9.81            |
| Dried and lyophilized cow colostrum protein concentrate | 98.13           | 83.19           | 58.79       | 2.63           | 12.25           |

### Results and discussion

The composition of control group colostrum did not differ from the values reported by other authors (4, 11, 20). The protein concentrate in turn, contained a 142% higher amount of protein, 255% more immunoglobulins and 10% casein in dry matter compared to the colostrum (Tab. 1).

The content of *E. coli* and coliforms in colostrum indicates a large diversity of its infestation with these bacteria strains, similar to those reported by other authors (6, 12). Mean content of *E. coli* in 1 ml was 284.00, while for coliforms this value was 68 834.00. The highest values were 1 300.00 and 1 070 000.00 for *E. coli* and coliforms, respectively. This indicates that colostrum or colostrum preparations not void of this flora can be a source of infection of the neonates that they are administered to.

Pasteurization appeared to be the least useful among the methods of bacterial flora reduction, since total reduction of the bacteria was observed at a temperature of 80°C with 5 minutes of heating, or at a temperature of 75°C with 10 minutes of temperature activity. These temperatures and their impact over time result in a substantial loss of biological colostrum features as well as colostrum immunoglobulin damage (6, 9, 12, 21). Focused microwave field and pulsed electromagnetic field caused a 50% bacteria reduction. Equally significant microflora reduction, by more than 6 units on a logarithmic scale with respect to *E. coli*, and almost 4 units for *L. innocua*, was noted by Dutreux et al. (3), using the parameters of pulsed electromagnetic field (PEF), which does not change the temperature of the test substance. It is suggested that a pulsed microwave field causes damage to cell membranes and an inactivation of bacterial enzymes, although their activity mechanism has not been fully recognized yet (7). In our study there was no reduction in the level of immunoglobulins, lysozyme and lactoferrin after an application of a focused microwave field.

It was found that during the second day of life, the mean value of IgG in calves serum in subgroup IIA (25.97 g/l) was significantly lower ( $p < 0.05$ ) from the content of the group IIIA, which was 27.77 g/l (Tab. 2). A statistically significant difference ( $p < 0.05$ ) was noted at the 28<sup>th</sup> day of life between the content of immunoglobulins in the serum of the calves from the control group and subgroup IIA, receiving a lower dose of the dried colostrum protein concentrate, while the values were 16.44 g/l and 16.03 g/l, respectively.

**Tab. 2. IgG content in blood serum of calves depending on the type and dose of colostrum preparation ( $\bar{x} \pm SD$ )**

| Groups | Days         |                           |              |              |                           |                           |
|--------|--------------|---------------------------|--------------|--------------|---------------------------|---------------------------|
|        | 2            | 7                         | 14           | 21           | 28                        |                           |
| I      | 26.11 ± 1.81 | 21.35 ± 1.05              | 20.05 ± 2.11 | 17.00 ± 1.77 | 16.44 <sup>a</sup> ± 1.18 |                           |
| II     | A            | 25.97 <sup>a</sup> ± 1.16 | 21.41 ± 0.93 | 20.06 ± 0.76 | 16.76 ± 1.29              | 16.03 <sup>a</sup> ± 1.20 |
|        | B            | 26.02 ± 1.35              | 21.64 ± 1.94 | 20.11 ± 1.55 | 17.16 ± 1.12              | 16.21 ± 1.08              |
| III    | A            | 27.77 <sup>a</sup> ± 1.72 | 21.83 ± 1.64 | 20.68 ± 0.74 | 17.39 ± 1.32              | 16.32 ± 1.33              |
|        | B            | 26.12 ± 1.19              | 21.41 ± 0.45 | 19.86 ± 1.12 | 17.21 ± 1.21              | 16.10 ± 1.24              |

Explanation: a, b – values in columns marked with different letters differ significantly ( $p < 0.05$ )

**Tab. 3. Availability of immunoglobulins from colostrum and colostrum preparations, assuming that their supply in the colostrum of cows is 100% ( $\bar{x} \pm SD$ )**

| Groups | IgG content    |  |                                |                                 |                               |
|--------|----------------|--|--------------------------------|---------------------------------|-------------------------------|
|        | Provided       | Content obtained in one liter of blood serum | Availability                   | In respect of colostrum         |                               |
| I      | 440.10 ± 50.52 | 26.11 ± 1.51                                 | 19.92 <sup>ABCDab</sup> ± 1.26 | 100.00 <sup>ABCDab</sup> ± 7.77 |                               |
| II     | All            | 330.00 ± 30.00                               | 26.08 <sup>a</sup> ± 1.49      | 135.07 <sup>Ac</sup> ± 14.49    |                               |
|        | A              | 300.00 ± 0.00                                | 26.06 <sup>b</sup> ± 1.61      | 28.48 <sup>B</sup> ± 2.64       | 147.24 <sup>B</sup> ± 9.11    |
| III    | All            | 360.00 ± 0.00                                | 26.11 ± 1.35                   | 23.50 <sup>Aad</sup> ± 1.33     | 122.90 <sup>ad</sup> ± 6.37   |
|        | A              | 330.00 ± 30.00                               | 27.03 ± 1.50                   | 27.40 <sup>c</sup> ± 3.48       | 140.39 <sup>c</sup> ± 18.23   |
| III    | A              | 300.00 ± 0.00                                | 27.86 <sup>ab</sup> ± 1.32     | 30.67 <sup>Dcde</sup> ± 1.20    | 157.39 <sup>Dcde</sup> ± 7.43 |
|        | B              | 360.00 ± 0.00                                | 26.21 ± 1.19                   | 24.13 <sup>Bbe</sup> ± 1.21     | 123.39 <sup>be</sup> ± 5.62   |

Explanations: a, b, c, d, e – values in the columns marked with different letters differ significantly ( $p < 0.05$ ); A, B, C, D – values in the columns marked with different letters differ significantly ( $p < 0.01$ )

For other days there were no significant differences in the level of serum IgG, both between the groups and subgroups.

No significant differences were also noted in the content of immunoglobulins in the serum of calves with regards to the colostrum preparation drying method. The content of immunoglobulins in calves fed with lyophilized colostrum concentrate, in the first four samplings, slightly exceeded the concentration of IgG in the serum of calves fed with dried protein concentrate, and the calves fed with maternal colostrum, which was confirmed in the study by Szulc and Zachwieja (20), indicating that the lyophilized colostrum is slightly better absorbed compared to dried colostrum. The differences noted between the groups were not significant statistically in any of the subsequent samplings.

It was also found that the dynamics of changes in immunoglobulin levels within each group was typical for the changes observed by other authors; moreover, the values between subgroups within the groups did not differ significantly (13, 16). However, considering the amount of IgG given in the first day of life, in terms of 1 kg of body weight, there were significant differences ( $p < 0.05$ ) between the control group and the groups II and III. This was due to the high supply of globulins in maternal colostrum – 440.10 g. Immunoglobulins in colostrum of cows significantly exceed the amount

specified as minimum protection for the immune status of the calf in colostrum preparations. Additionally, differences in the level of IgG content between subgroup IIIA and subgroup IIIB were noted in group III, however this result was not confirmed statistically.

On the basis of immunoglobulins provided (440.10 g for colostrum, and 300 and 360 g for subgroups A and B, respectively) and their concentration in the blood serum of calves, the percentage of IgG bioavailability was calculated, assuming that at the second day of life serum constitutes 8.59% of their body weight (18) (Tab. 3). Statistically significant differences ( $p < 0.05$ ) were noted in serum IgG concentration between group IIIA – 27.86 g/l (lyophilized concentrate), and II and IIA (dried concentrate), 26.08 and 26.06 g/l, respectively.

Analyzing the results of availability, it was found that immunoglobulins from preparations were absorbed to a much greater extent than maternal colostrum immunoglobulins. Highly significant differences ( $p < 0.01$ ) were observed between the control group and the following groups: II – 25.99%, IIA – 28.48%, III – 27.40% and IIIA – 30.67%. However, significant differences ( $p < 0.05$ ) occurred between the control group and IIB – 23.50% as well as IIIB – 24.13%. Also, differences were noted between the availability level within the experimental groups: between group IIA and IIB, and IIIA and IIIB, as well as IIB and IIIB. These differences result from various levels of immunoglobulins in preparations given in these subgroups. They also indicate that maternal colostrum with high amounts of immunoglobulins exceeds the calves' immunoglobulin demand even at a dose of 5 liters in the first day.

Similar results were recorded when assessing availability of colostrum preparations assuming that colostrum availability was 100% (Tab. 3).

The differences in availability level between the control group and experimental ones may also result from a higher concentration of trypsin inhibitor in the protein concentrate (19). The difference in the amounts of immunoglobulins assimilated in the control group with regard to experimental ones results from a much higher immunoglobulin supply which calves in the control group received in maternal colostrum. The results obtained cannot be confronted with the results of studies by other authors, since no similar

research in this range has been found in the literature available.

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