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# THE EFFECT OF INCOMPLETE COLOSTRUM MILKING ON ITS CONTENT AND ON TRIPSIN INHIBITOR LEVEL

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## **ABSTRACT**

Analyses on variability of trypsine inhibitor content in cow colostrum and its composition during first 5 milkings after parturition, in dependence with method of milking were performed. Cows were distributed into 3 groups: I -

complete colostrum milking in successive milkings after parturition - control (18 cows); II - leaving approx. 30% of colostrum (more complete milking) in first 5 milkings after parturition (18 cows); III - leaving approx. 60% of colostrum (less complete milking) in first 5 milkings after parturition (18 cows).

After analysis, it was stated that incomplete milking of cows during first milkings after parturition supports keeping higher level of G immunoglobulin in consecutive milkings. Leaving more colostrum in mammary glands restricts trypsine inhibitor production and causes decreasing of its level in colostrum from successive milkings. There is highly significant positive dependence between trypsine inhibitor and other colostrum components. Incomplete milking lowers intensity of colostrum components production, including immunoglobulins mobilized in organism, what may positively affect relieve when trespassing from drying off to intensive milk production and keeping cows healthy after parturition.

Key words: colostrum milking, content of cows colostrum, tripsin inhibitor level

### INTRODUCTION

Decreasing of assimilability of colostrum immunoglobulins in calves during consecutive hours after parturition is correlated with level of enzymes in colostrum and in calf's alimentary truct, permeability of intestine enterocytes and activity of gastric juices (6). Pedersen et al. (8) stated the presence of 3 trypsine inhibitors in cow colostrum. It was showed that inhibitors in 1 ml of colostrum inhibited degradation of 500-600 mg of trypsine (Veselsky et al., 1978). According to Quigley et al. (9). Trypsine secretion in digestive tract of newborn calves decreases absorption of G immunoglobulins (Ig) in colostrum and enhances deficiency of passive immunity, especially in calves sucking first time hours after parturition. Quigley et al. (10) stated that addition of trypsine inhibitor increases assimilability of G and M immunoglobulins of 16 and 30% and also increases total concentration of proteins in calves serum. The highest activity of trypsine inhibitor (CTI- Colostral Trypsin Inhibitor) was found in colostrum from the first milking after parturition (301 mg/1). Statistically significant correlation (r = 0.93) was estimated between CTI activity and concentration of whey proteins in colostrum on 1<sup>st</sup> and 2<sup>nd</sup> day after parturition. Positive relation between trypsine inhibitor and G Ig level (+ 0.54) in colostrum was also found. Positive relations were also found for fat content, total proteins content and dry matters content in colostrum. Correlation between total activity of trypsine inhibitor and concentration of crude proteins in whey from 1 st, 2nd, 5th and 7<sup>th</sup> milking after parturition was 0.97 (1).

# THE AIM OF THE STUDY

The study was undertaken to determine changes of trypsine inhibitor level in cow colostrum, in dependence on its milking <u>degree</u> during first 3 days after parturition and to estimate its interdependences with other colostrum components.

### MATERIALS AND METHODS

The studies were conducted on milking cows farm in Rychnów. Fifty four Black and White cows with mean yield of 6 000 litres of milk per year, were under analyses. Cows were distributed into 3, depending on their colostrum amount in consecutive milkings after parturition, groups:

I - complete colostrum milking in successive milkings after parturition - control (18 cows);

II - leaving approx. 30% of colostrum (more complete milking) in first 5 milkings after parturition (18 cows);

III - leaving approx. 60% of colostrum (less complete milking) in first 5 milkings after parturition (18 cows).

Cows were collected, basing on analogues, considering their age, milk yield during previous lactation, health state of their mammary glands and general condition.

Colostrum samples were collected from 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> milking after parturition. All samples were analysed for fat, protein, lactose, dry matter, whey protein, G immunoglobulins and trypsine inhibitor content.

Basic colostrum components were determined using Milcoscan 133B A/SN Foss Electric (Denmark). The level of immunoglobulins in whey was estimated using immunodiffusion tests The Binding Site (Great Britain).

Antiproteolytic activity of colostrum was estimated by incubation of 185 ml of colostrum with 10 ml of trypsine (of concentration 6.6 mg/ml) in 1.5 ml of buffer (10mM Tris, 20mM CaCl<sub>2</sub>, 0.01% Triton X-100, pH 8.3) for 10 minutes. Then 10 ml of BApNA (4-nitroanilide N-benzoic of L-arginine) substrate was added to mixture and its increasement of absorbance was recorded for 5 minutes. Analogue mixture without colostrum addition was considered as control. Consequently, the increase of absorbance was estimated in inhibitor concentration g/l. The measurements were performed using HP 8452A spectrophotometer (Hewlett-Packard, Palo Alto, USA). BapNA, 4-nitroanilide N-benzoic of L-arginine, Tris, CaCl<sub>2</sub> Triton X-100 were supplied by Sigma Chemical Company (St. Louis, USA).

The results were statistically analysed using Duncan test and correlation method.

### **DISCUSSION**

It was stated, that fat content was significantly increasing together with increasing of milking colostrum amount, what is connected with content of final colostrum fractions, which contain more fat (Table 1). Similar relation was observed by Szulc et al. (11). No statistically significant effect of milking on total protein and whey protein content in colostrum of consecutive milkings was observed, though decrease of protein content was slower in experimental groups. Incomplete colostrum milking highly significantly affected changes in G immunoglobulin content in consecutive milkings. Cows completely milked in 2<sup>nd</sup> milking had significantly lower G Ig content. Milking of only 30% of colostrum caused higher, of approx. 18% for group II and 71.9% for group III, G immunoglobulins content in colostrum whey from 3<sup>rd</sup> milking. The above results indicate, that completely milked cows did not refill their loss of immunoglobulins. Simulating analyses show that complete milking mobilize more immunoglobulins to consecutive colostrum portions, what meaningfully burdens cow's organism and decreases immunoglobulins reserves. The results are similar to relations observed by Szulc et al. (11) who considered differentiate milking of separate mammary gland quarters. Lack of cows' negative reactions to incomplete milking shows possibility of using this method to keep higher colostrum quality in consecutive milkings, what may support calves health improving (12).

Table 1. Changes in colostrum composition on milking ratio on the following milking after parturition.

Group	I Control-completely milked			II More completely milked			III Less completely milked		
Following milking after parturition	1	3	5	1	3	5	1	3	5
Fat, (%)	5.9*	4.7 <sup>A</sup>	5.9 <sup>A</sup>	4.4	4.0 <sup>a</sup>	7.0 <sup>A</sup>	5.1	3.4 <sup>B</sup>	3.7 <sup>B</sup>
	2.9**	1.74	2.59	1.73	1.38	2.65	1.86	2.68	1.90
Crude protein, (%)	16.7	6.4	4.9	15.6	7.3	4.6	14.6	7.2	5.2
	2.93	3.30	2.97	1.54	0.68	2.06	0.59	0.58	1.50
Lactose, (%)	1.6	3.8	4.0	2.0	3.7	3.7	2.1	3.8	4.0
	0.98	0.78	0.50	0.54	0.43	0.45	0.35	0.42	0.70
Dry matter, (%)	22.9	15.9	15.5 <sup>a</sup>	22.7	15.8	15.9 <sup>a</sup>	22.5	14.4	13.5 <sup>b</sup>
	4.15	2.75	3.75	1.70	1.71	2.85	1.55	2.84	2.16
Whey matter, (%)	10.6°	3.0	1.8	8.5 <sup>b</sup>	3.5	2.2	9.3	2.8	2.3
	2.32	2.51	1.60	1.14	1.13	1.01	0.33	0.79	0.82
Immunoglobulin, (g/l)	135.4 <sup>A</sup>	59.0 <sup>A</sup>	16.1	113.1 <sup>B</sup>	70.0 <sup>A</sup>	16.8	132.5 <sup>A</sup>	101.4 <sup>B</sup>	16.3
	22.11	19.63	11.12	20.06	18.14	22.64	5.86	5.41	6.59
Trypsin inhibitor, (g/)	0.64	0.36	0.39	0.64	0.47	0.34	0.63	0.26	0.14
	0.10	0.25	0.22	0.12	0.21	0.16	0.22	0.10	0.09

A, B – means with the same letter are not significantly different. Capitals P $\leq$ 0.01, small letters P $\leq$ 0.05

Concentration of trypsine inhibitor in cows colostrum did not show direct relations with method of milking. Its the lowest ratio in colostrum collected from cows of group III during 3<sup>rd</sup> and 5<sup>th</sup> milkings, indicates that leaving bigger amount of colostrum in mammary gland causes restriction of trypsine inhibitor secretion by lactigenous epithelium of mammary gland. It is difficult to explain why part of colostrum left in mammary gland did not increased of inhibitor content in consecutive milkings.

Analysis of dependency (<u>Table 2</u>) between trypsine inhibitor and colostrum components indicates high and positive relations, although lower that estimated by Honkanen-Buzalski and Sadholm (4). The differences observed in successive groups may be caused by

<sup>-</sup> X<sub>śr</sub>

insufficient number of animals, but also by changes in trypsine inhibitor secretion. That is the reason why studied relations were not estimated for whole population of analysed cows.

Tabela 2. Interdependence between trypsin inhibitor concentration and colostrum composition in groups and following milking (r).

Group	Following milking	Fat (%)	Crude protein (%)	Laktose (%)	Dry matter (%)	Ig G (g/l)	Whey protein (%)
I	1	0.80	0.38	0.53	0.57	0.32	0.94*
	3	0.73	0.33	0.31	0.34	0.52	0.69
	5	0.34	0.38	0.64	0.39	0.35	0.75
II	1	0.72	0.42	0.30	0.50	0.37	0.50
	3	0.54	0.29	0.42	0.36	0.46	0.42
	5	0.13	0.21	0.77	0.33	0.40	0.80
III	1	0.90	0.70	0.20	0.77	0.53	0.91
	3	0.86	0.85	0.31	0.90	0.59	0.41
	5	0.77	0.69	0.76	0.64	0.60	0.93

<sup>\* -</sup> corelation coefficient significant at P≤0.05

# **CONCLUSIONS**

It should be stated that incomplete milking of cows during first days after parturition supports keeping higher level of colostrum G immunoglobulins in consecutive milkings. Leaving bigger amount of colostrum in mammary glands inhibits production of trypsine inhibitor and causes its decreasing in successively milking colostrum. There is highly significant relation between trypsine inhibitor and the other colostrum components. Incomplete milking decreases intensity of colostrum components production, including amount of mobilized immunoglobulins, what may positively affects relieve, when trespassing from drying off to intensive milk production and keeping cows healthy after parturition.

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