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COMPARATIVE ANALYSIS OF CELL POPULATION PRESENT IN THE MILK AND THE COLOSTRUM OF ALPINE AND CARPATHIAN GOATS

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Abstract
The analysis of the cell population present in the milk and colostrum of goats represents basic morphological characteristics of milk, through which the health of mammary gland and of the milk intended for the consumers can be determined. The purpose and the goals of these determinations consisted in comparative, quantitative and qualitative evaluation of the cellular content from raw milk and colostrum of goat from two lots of goats, clinically healthy, the breeds being French Alpine (n = 10) and Romanian Carpathian (n = 10). Morpho-physiological investigations were conducted during March-July 2015, on samples of raw milk and colostrum, using Squash technique, panoptic colored (Dia-Quik-Panoptic), and milk cytogram method. Microscopic examination revealed that the milk cytogram of both breeds' colostrum shows a higher frequency of epithelial cells, lactocytes in various forms of activity, lymphocytes, macrophages. The highest frequency was for neutrophils. The milk cytogram in raw mixture milk indicates that the cell population is very similar for both races, with same increase or decrease in those studied months. The neutrophils reached an average of 45.7% for the Alpines, respectively 46.17% for the Carpathians. There were no significant differences of interest regarding cell population of raw milk and colostrum of both races. In conclusion, the results support the need for correlation between quantitative and qualitative microscopic cytological tests on smears because there are no standards in automated system for goat milk in terms of health assessment of the mammary gland and of safety of the milk meant for consumers.

Key words: *goat milk, colostrum, cell population.*

INTRODUCTION

The analysis of the cell population present in the milk and colostrum of goats represents basic morphological characteristics of milk, through which the health of mammary gland and of the milk intended for the consumers can be determined The research on goats must quickly progress to reach an advanced level of knowledge especially in terms of milk production (Arguello, 2011).

As Mahe said in 1997, milk is a complete physiological liquid, secreted by the mammary gland, valid for all female mammals, for the raising of the newborn and also as a main aliment for humans. Being a constitutive and essential element of a diet (Park and col., 2007, Ksontini and col., 2011,

Yadav and col., 2014) is formed of proteins, fats, carbohydrates, vitamins and minerals. The quality of milk is considered essential for the well being and the safety of the consumers (Nandhini and Palaniswamy 2013). It is a well known fact that unfortunately, milk is a proper environment for the development of many pathogenic microorganisms (Michel, 2001, Yadav and col., 2014). Goat's milk is an essential element for plenty diets, used in the prevention and treatment of some diseases in man because of it being a complete aliment with a balanced content of proteins, fats, carbohydrates, vitamins and minerals (Hanzen, 1994, Michel, 2001, Park and col., 2007, Ksontini and col., 2011). Cellular content is also a major component of goat milk. The cell population is one of the most relevant hygienic-sanitary parameters used to assess the health of the mammary gland hygienic and also to assess the hygienic quality of milk as a product intended for public consumption (Sabău and Rotaru, 2006). Cell population is derived from the fund of the cell body and can be classified into four main types: macrophages, lymphocytes, neutrophils, polymorpho-nuclear leukocytes and somatic cells. Together they have a foremost role in maintaining hygienic health of milk, firstly through their phagocytic action, but also through preparing the specific immune reaction, facilitating the contact between lymphocytes and pathogenic agents in order to trigger the immune response (Rotaru and Ognean 1998). In the cytomorphology of milk

can also be found extramammary cellular structures represented by microbes, yeasts, fungi and parasites (Ognean 2001).

MATERIALS AND METHODS

Smears were made, using the Squash technique, colored panoptic (Dia-Quik-Panoptic), afterwards following the Milk cytogram method on samples of mixed raw milk. There were two determinations per month for mixed raw milk during April-July from two groups of goats clinically healthy at their third lactation, respectively French Alpine (n = 10) and Romanian Carpathian (n = 10). Next was the milk cytogram on individual samples of colostrum, analyzing the samples from the 20 studied goats, 10 for every race: in March for Carpathian breed, respectively in April for Alpine race. For a high performance of milk production and for avoid infections, milking is done twice a day (X2) (Capote and col., 2009). There have been previous studies on the same lots of goats in terms of the number of somatic cells (NSC), these was between physiological parameters. In the last two years in the micro-farm, it was not recorded cases of mastitis. (Nasalean and col., 2015). The determinations consisted of the comparative quantitative and qualitative evaluation of the cellular content of goat's raw milk and colostrum, recording the average data for all determinations.

RESULTS AND DISCUSSIONS

After carefully analyzing all smears, made through milk cytogram method, the data was corroborated and the statistic analysis of cellular population from the analysed milk was made. During qualitative examinations, the milkcytogram from the colostrum of the Carpathian breed revealed a high frequency of epithelial cells, of lymphocytes (21.9%), of macrophages in activation was (9.4%) and inactive macrophages (21.7%). Neutrophils have the highest frequency (41.2%). The qualitative examinations reveal a high level of heterogeneity between active or hyperactive cells, large, slightly edematiated cells represented by macrophages and lymphocytes, mainly present in colostrum. Therefore, for the colostrum milk from the Carpathian goats we have registered the following statistical data (Table1).

Table 1. Milk-cytogram of colostrum Carpathian breeds

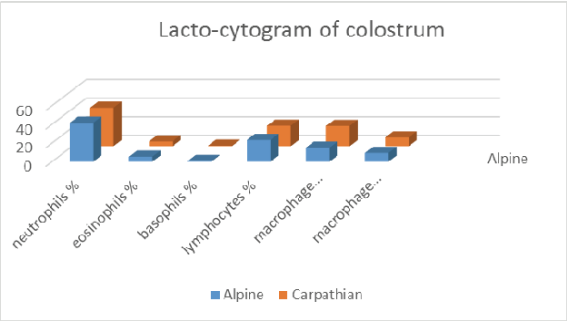
Colostrum Carpathian breeds	No. goats	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Macrophage. Inactive %	Macrophage. in activation progress %
	1	42	2	1	21	20	14
	2	38	2	1	20	29	10
	3	43	8	0	24	17	8
	4	39	3	2	26	20	10
	5	45	4	1	18	16	16
	6	40	10	0	22	20	8
	7	43	6	0	23	22	6
	8	41	8	0	21	22	8
	9	45	5	0	18	26	6
	10	36	4	1	26	25	8
Average		41.2	5.2	0.6	21.9	21.7	9.4

Table 2. Milk-cytogram of colostrum Alpine breeds

Colostrum Alpine breeds	No. goats	Neutrophiles %	Eosinophiles %	Basophiles %	Lymphocytes %	Macrophage. Inactive %	Macrophage. in activation progress %
	1	44	4	1	26	15	10
	2	38	2	2	30	20	8
	3	42	7	0	23	18	10
	4	40	4	2	22	22	10
	5	41	8	1	25	15	10
	6	43	4	0	22	22	9
	7	40	6	1	23	18	12
	8	45	6	0	20	23	6
	9	41	8	1	19	21	10
	10	43	4	0	22	23	8
Average		41.7	5.3	0.8	23.2	19.7	9.3

The colostrum milk from the Alpine goats registered the following statistical data, represented in (Table2).

For the qualitative examinations, the milkcytogram from the colostrum of the Alpine breed presented, just as for the Carpathian breed, a high frequency of epithelial cells, of lymphocytes (23.2%), of macrophages in activation was (9.3%) and of inactive macrophages (19.7%). The proportion of neutrophils was significant (41.7%). The comparative analysis of milkcytogram from the colostrum of both studied goat breeds (Graphic1).



Graphic 1. Milkcytogram of colostrum both breeds

The comparative analysis of lactocytogram on samples of mixed raw milk from the studied Alpine goats lot and Carpathian goats lot made during April – July 2015 shows that cytomorphologicaly, in the Alpine goats' milk the preponderance of PMN neutrophils, lymphocytes and macrophages was observed, this being a probable activity for the phagocytosis of possible germs.

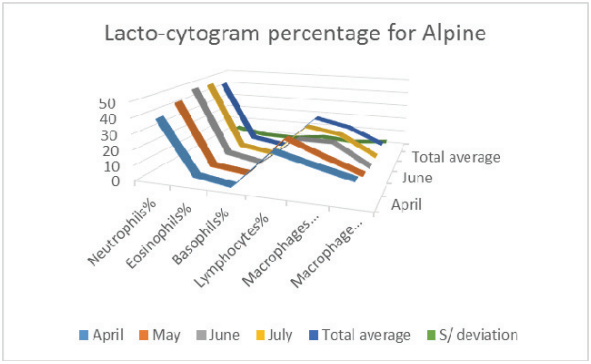
The cell population is numerous, with an intense phagocytic activity, the inactive and in activation progress macrophages cells being in different stages of activity. Therefore it can be observed a variation in the neutrophils percentage, it being 38.8%, registered in April, followed by a notable increase in May to 44.2%, reaching the highest percentage of 49.1% in June. Afterwards, the amount starts to slowly decrease, having a value of 48.2% for July and registering an average for those 4 months of 45.7% (Table 3; Graphic 2).

From a cytomorphological point of view, for the Carpathian breed the preponderance of PMN neutrophils cells and of macrophages was observed, this being a probable activity for the microphagocytosis of possible germs. The cellular population is numerous, with an intense phagocytic activity, the inactive and in activation progress macrophages cells being in different stages of apopthosis. Therefore it can be observed a variation in the neutrophils percentage, it being 41.2% in April, followed by a slight increase in May and in June reaching the highest percentage, 50.1%. Afterwards, the amount starts to slowly decrease, having a value of 48.8% for July and registering an average for those 4 months of 46.17% (Table 4; Graphic 3).

Comparing both breeds we notice a more intense activity of neutrophils in milk for the Carpathian goats (Graphic4).

Table 3. Percentage of the average of the lactocytogram for the studied months for Alpine breed

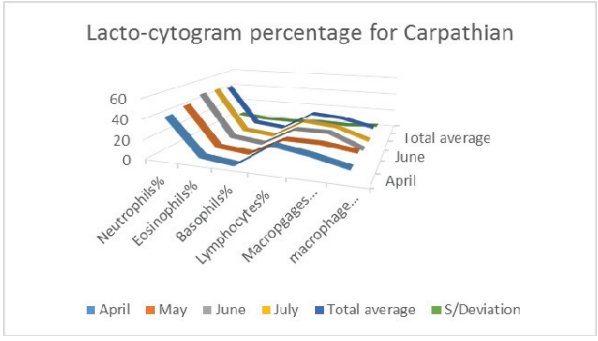
Alpine Monthly average/ 2 determinations	Neutrophils%	Eosinophils%	Basophils%	Lymphocytes%	Macrophage inactive%	Macrophage. in activation progress %
April	38.8	4.3	0.6	24.8	18.6	12.9
May	44.2	3.6	0.8	26.4	16.8	8.2
June	49.1	5.3	0.8	19.7	19.6	5.5
July	48.2	4.4	0.6	22.6	18.6	5.6
Total average	45.07	4.4	0.7	23.37	18.4	8.05
St. deviation	4.69	0.6	0.115	2.9	1.16	3.46



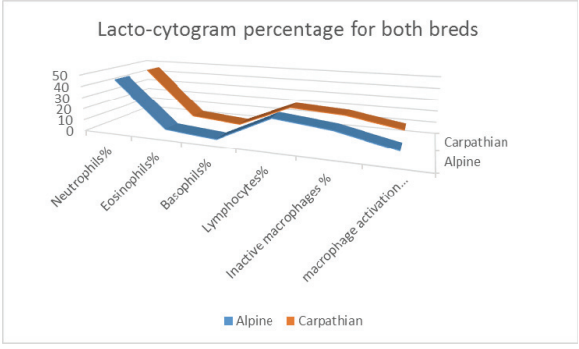
Graphic 2. Percentage of the average of the lactocytogram for Alpine

Table 4. Percentage of the average of the lactocytogram for the studied months for Carpathian breed

Carpathian Monthty average / 2 deteriminations	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Macrophage inactive%	Macrophage. in activation progress %
April	41.2	4.3	0.4	23.4	17.8	9.9
May	44.6	3.9	0.6	18.8	18.4	13.7
June	50.1	4.8	0.8	19.7	19.6	5.5
July	48.8	3.6	0.2	19.7	16.6	5.1
Total average	46.17	4.15	0.5	20.5	17.97	9.95
S/Deviation	4.06	0.51	0.25	2	1.05	3.6



Graphic 3. Percentage of the average of the lacto-cytogram for Carpathian



Graphic 4. Lacto-cytogram percentage for both breeds

CONCLUSIONS

The presence of cells PMN, (polymerphonuclear) in mammary cistern ensure a barrier against infection and initiates a fast infiltration in the infected milk (Paape, and col., 2003). It is known as lymphocytic population in normal healthy milk reaches values between 10-27%, neutrophils 45% and macrophages to 20% (Lee and col., 1980., Rotaru and Ognean., 1998). Carpathian goat is an indigenous breed is believed to be better adapted to the environmental conditions in our country, but there were not important differences between milk and colostrum of this two studied breeds. There were not registered important differences regarding the cell population from colostrum milk of both breeds. The lacto-cytogram in mixed raw milk reveals the fact that the level of neutrophils is very similar for both breeds, registering the same increases and decreases for the studied months, reaching an average of 45.7% for Alpines, respectively 46.17% for the Carpathians. It can't be recommended the qualitative evaluation of cellular content with automatic systems because there are no standards for goat milk. Therefore we recommend the microscopic qualitative evaluation on a smear because while examining the smears there can also be made quantitative estimations about the evaluation of the health of mammary gland and estimations about the safety of milk intended for consumers.

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