

Peculiarities Regarding the Testing of Milk Physicochemical and Cytology at a PIC Sows Sample

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Abstract. Testing the main physicochemical and cyto-morphological milk parameters during lactation debut represented the monitoring of breast health at a lot of PIC sows (n = 15) and its impact on health and growth indicators in suckling piglets. Investigations were carried out on samples of milk collected by manual milking, using stimulation of milk ejection by oxytocin injection (n = 10) and by breastfeeding 2-3 piglets (n = 5). Physicochemical tests were performed with semi-automatic EKOMILK analyzer, consisting in determining the main biochemical components (non-fat dry substance - NFS, fat, protein), density, freezing point and water added. Cyto-morphological investigations consisted in making the milk cytograms on the milk sediment smears, defatted with xylene or benzene and panoptic stained (MGG and Dia - Quick panoptic). The evolution of investigated compositional and cytological parameters revealed large variations of individual and average values, grouped in intervals comparable to those reported in consulted studies and with minor deviations and non-pathological connotation. Thus, the test results of milk samples outlined a physicochemical profile, characteristic on lactation debut in the sow, with the following individual oscillations around the average values: 5.37 - 13.3% (7.97%) for protein, 10.8 - 19.9% (13.67%) for non-fat dry substance, 1.71 to 14.5% (8.18%) for fat, 1.028 to 1.076 (1.043) for density, -0.954 to -0.744 °C (0.28 °C) for the freezing point. The cyto-morphological configuration of this sample of sows milk was characterized by increasing the proportion of macrophages with advancing lactation (42.86%, 30-60%), usually associated with PMN leukocytes decreasing (15.8%, 8-43%) and lymphocytes rarely (31.0%, 11-46%) or epithelial cells (10.4%, 3-20%). We have given a particular character for the onset of lactation in sows to the distribution and morphology of epithelial cells. Regarding the distribution, it was relevant the higher proportion of epitheliocytes (10.4%) in the milk of investigated sows to that found in milk of ruminant species. Most of the observed epitheliocytes had a glandular origin, and morphological were large cells, integral, uni- or binucleate, surrounded by a bulky cytoplasmic mass, low basophilic, with many fat microspheres, often gaining sparkling appearance and rarely ring.

Key words: PIC sows, milk, compositional parameters, cyto-morphology.

INTRODUCTION

To sow lactation status requests seem to be more important than in other mammals, and lactogenesis and lactopoiesis requiring higher energy and mineral consumption (Acie *et*

al., 1999). Viewed in this context, the monitoring of lactation, as a health surveillance of mother sows and piglets, should be a basic concern of veterinary medicine research and practice (Pârvu *et al.*, 2003). Increasing the progress in this area requires, however, diversification and improvement of methods of assessment and risk management during sow neonatal period, which was the objective and base of this study made on a sample of PIC sows, in early stages of lactation.

MATERIALS AND METHODS

Implementing a comprehensive research protocol and observations in a PIC (EUROHYB-Mures) multiplication farm, included as a first phase the investigation of a sample of lactating sows with piglets (55 nests), including physical, chemical and cytological investigations carried out on milk samples, collected randomly from 15 sows in the first week of lactation.

In order to harvest milk samples we tried several versions of the milking based method, recommended in the establishment of sow milk production. Considering the fact that the sow lactation characteristics significantly limit the amount of milk that can be harvested, we resorted to two manual milking procedures: the first preceded by injecting a dose of oxytocin ($n = 10$) and the second concurrent with lactation of 2 -3 piglets ($n = 5$). After collection, milk samples were processed quickly, without recourse to preservatives treatment, followed immediately to be physical, chemical and morphological investigated. Physicochemical tests were performed with semi-automatic EKOMILK analyzer, consisting in determining the main biochemical components (non-fat dry substance - NFS, fat, protein), density, freezing point and water added. Each of the samples were subjected to morpho-cytological examinations, consisting in making the milk cytograms on the milk sediment smears, defatted with xylene or benzene and panoptic stained (MGG and Dia – Quickpanoptic). Individual and average data processing was based on the use of existing programs of Biostatistics (Graph Pad Instant V3.0, V4.0 Graph Pad Prism, Microsoft Excel), which allowed a proper statistical analysis.

RESULTS AND DISCUSSIONS

Physicochemical test analysis. All average and individual values obtained to determine the basic physical and compositional milk parameters of the investigated sows shaped a physicochemical profile characteristic on the lactation debut in general, with some specific features in pigs. Components of this profile showed oscillations more or less important, outlining the wide range of variation of individual values around the mean, circumscribed within the limits of physiological species: 5.37 - 13.3% (7.97%) for protein content, 10.8 to 19.9% (13.67%) for non-fat dry substance, 1.71 to 14.5% (8.18%) for fat, 1.028 to 1.076 (1.043) for density, -0.954 - 0.744 °C (0.28 ° C) for freezing point (Table 1).

The relevance of values obtained by us was provided by comparing them with those reported by other researchers in the field of lactation authorized to sow. In this regard we mention only the data reported by Krider and Carroll (2002), showing different proportions of the main components of milk, specially the sow colostrum, in the dry substance (19.26, 25.27% respectively), protein (6.5, respectively 15.25%) and fat (6.75, 5.31% respectively). All the data on the chemical composition of sow milk reveals like in other mammals, the nutritional and biological value. In this regard there are relevant the differences in the composition of colostrum, whose content is richer in dry substance, protein, vitamins and

minerals and low in fat and lactose than milk. Another specific feature for the sow lactation composition evolution is given by quickly lowering of the colostrum content in gammaglobulins, which explains the importance of breastfeeding in the first hour after piglets birth (Ognean *et al.*, 2010, Persson *et al.*, 1989).

The early achieving of the first feeding is required by other metabolic and immune features of newborn piglets. That is the case of glycogen low reserve (271 kJ), which makes the piglets to be dependent on the energy from colostrum (586-628 kJ/100 ml) to prevent hypoglycemia. In the same context is the insurance of the predominantly passive immunity in suckling piglets, which makes the intake of colostrum and breast milk to be essential for IgG absorption (Pabst *et al.*, 1999, Rooke and Bland, 2002).

Cyto-morphological test analysis. Milk cytogram results can be assessed based on the data presented in table 2, which shows the first feature of the sow milk cytologic configuration, increasing the proportion of macrophages to measure progress of lactation, individual values ranging between 30% and 60%, with an average of 42.86%. Macrophage population growth was achieved mainly on account of falling share PMN leukocytes (15.8%, 8-43%), rarely of the lymphocytes (31.0%, 11-46%) or epithelial cells (10.4%; 3-20%).

The analysis of individual data showed that the PMN leukocyte population increased proportions in the case of four sows (20-43%), of which only one has been a considerable microbial load. A major deviation from the normal configuration of milk cytogram we have seen in a sow with granulocytosis (43%), who registered the lowest percentage of macrophages (30%).

Tab. 1.

Individual and average values of the main physicochemical milk parameters at a sample of PIC sows

Sample (no.)	Fat (%)	NFS (%)	Density (g/cm ³)	Added water (%)	Freezing point (°C)	Protein (%)
1	10.8	10.6	1.0280	4.81	0.571	5.37
2	4.75	11.2	1.0361	0.0	0.618	5.77
3	4.61	11.2	1.0360	0.0	0.621	5.81
4	1.71	19.9	1.0760	0.0	0.000	13.3
5	9.28	11.2	1.0319	0.53	0.597	5.83
6	14.5	12.1	1.0309	10.3	0.538	6.71
7	7.2	14.5	1.0475	0.0	0.744	8.58
8	8.98	10.8	1.0308	0.0	0.588	5.54
9	13.6	12.6	1.0340	6.68	0.560	7.15
10	6.15	18.5	1.0658	0.0	-0.954	12.1
11	8.35	17.8	1.0607	0.0	-0.835	11.5
12	6.19	14.7	1.0431	0.0	0.281	5.99
13	10.17	12.6	1.0437	4.1	0.280	9.95
14	7.19	11.7	1.0435	1.0	0.271	6.96
15	9.17	15.6	1.0433	3.1	0.292	8.98
Mean	8.18	13.67	1.0434	2.14	0.280	7.97
St.Dev.	3.374	3.039	0.0140	3.156	0.515	2.635

Note: NFS=non-fat dry substance.

A particular trend we noted in the distribution of subpopulations of PMN leukocytes in investigated sows milk, the proportion of neutrophils returned majority (75-100%), but not negligible representation of eosinophils (0-25%) and even basophils (0-5%). The distribution of individual values also revealed that 8 of sows lymphocytes share has exceeded 30%, considered as upper limit for cow's milk (Ognean *et al.*, 2010), drawing an increased representation (31%) of lymphocytes population in the sows milk of the investigated sample.

Macrophages population dynamics was characterized by important individual variation, its frequency ranging between 30-60%. The correlation of high average levels (42.86%) with high percentage of active cells (42.60%) show that macrophages are almost the majority cell population in sow's milk, providing an efficient local defense in various forms of mastitis.

Epithelial cell population showed the most relevant features, including both morpho-physiological and representative aspects, its share being higher (10.40%) in this sample of sows milk, compared with the proportions found in ruminant species, which rarely reaches 7 - 9%. As the overview and detail images are showing in Figure 1, we have assigned a special significance to the morpho-physiological features captured at cellular subpopulations examinations on stained panoptic smears.

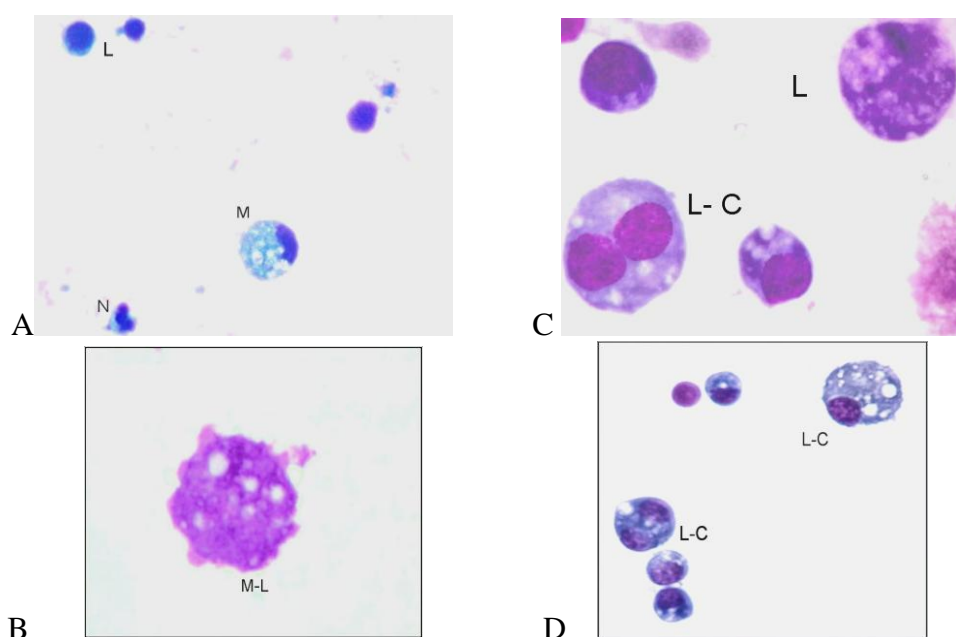


Fig. 1. WBC (A and B) and epithelial cells (C and D) reported in sow colostrum and milk: PMN leukocytes (N), macrophages (M), some with lipophage character (M-L), lymphocytes (L), alveolar epithelial cells in various stages of activity (L-C), containing one or two spherical nuclei, basophilic cytoplasm and fat spherules at the apical pole (sow milk sediment, MGG stained, x100).

Thus, the overall cytological configuration was characterized by the presence of: PMN leukocytes, mainly mature neutrophils, multi-core; small and large lymphocytes in close proportions, some of them gaining atypical aspects; macrophages in different states of activity, more or less typical, with lipophage character; epitheliocytes, predominantly with glandular origin, being integrity lactocytes derived from the alveolar epithelium. In terms of

cytology, we have considered the characteristic aspects of the sow lactation onset, the abundance of acinar lactocytes and the low frequency of those derived from the epithelial milk ducts or other epithelial cell types. Thus, most reported epithelial cells were presented as high integrity cells, uni- or binucleate, bulky cytoplasmic mass surrounded, weak basophilic, with many fat microspheres, gaining sparkling look frequently and rarely ring (fig. 1).

Tab. 2.
Individual and average values of milk cytogram at a sample of sows during the first week of lactation

Sample (no.)	PMN Leukocytes (%)				Lymphocytes (%)	Macrophages (%)			Epithelial cells (%)
	Total	N	E	B		Total	Inactive	Active	
1	12	90	10	0	34	48	58	42	6
2	20	85	10	5	26	51	48	52	3
3	8	75	25	0	29	52	36	64	11
4	9	100	0	0	21	57	46	54	13
5	10	100	0	0	22	60	47	53	8
6	29	93	7	0	31	30	60	40	10
7	14	100	0	0	44	34	56	44	8
8	11	90	10	0	46	31	68	32	12
9	6	100	0	0	41	43	60	40	10
10	43	93	4	3	11	38	47	53	9
11	15	100	0	0	35	38	45	55	12
12	12	100	0	0	38	41	80	20	9
13	9	100	0	0	31	38	68	32	22
14	8	100	0	0	34	38	74	26	20
15	31	97	3	0	22	44	68	32	3
Mean	15.80	94.86	4.60	0.53	31.00	42.86	57.40	42.60	10.40
St.dev.	10.564	7.376	6.978	1.457	9.517	9.078	12.495	12.495	5.235

Note: N=neutrophils; E=eosinophils; B=basophils.

Reduced availability of data on sow milk cytology causes many researchers to resort to extrapolation and their comparison with those on cows, sheep or goats milk, which are much better represented and updated (Durward *et al.*, 1980). Analyzed in a general clinical context, testing milk for correlative monitoring procedure of lactating sows and suckling piglets health, can give good results in detecting and diagnosing various syndromes expressed by mastitis, or even of general pathological conditions.

The main feature of mammary infections in pigs is that they usually triggers immediately after birth and are associated with agalactia and metritis, with the predominant effect of reduction and significant change in milk secretion (Persson *et al.*, 1989). Without having the data in using the test diagnosis of mastitis in general and specially sow, we believe that determining the electrical conductivity and cytology may give conclusive results at swine species. During galactopoiesis, the risk of mastitis in sows is associated with the milk production level, being increased in the first 3 weeks of lactation, when milk production is

growing. Follows a gradual decrease during the fourth week, when the milk production remains constant. Still slightly reduces milk production until the sixth week, following its decline decision by the end of lactation (Einarsson and Rojkittikhun, 1993).

CONCLUSIONS

- The physicochemical and cyto-morphological investigations performed on milk samples (n = 15), collected from a sample of lactating sows, were the basis for formulating the following conclusions:
- Testing the milk samples has outlined a physicochemical profile characteristic onset of the sow lactation, individual values ranging between 5.37 and 13.3% (7.97%) for protein, 10.8 and 19.9% (13.67%) for non-fat dry substance, 1.71 and 14.5% (8.18%) for fat, 1.028 and 1.076 (1.043) for density, -0.954 and 0.744 ° C (0.28 ° C) for freezing point;
- The piglets were breastfed within the first hour after birth and received an optimal intake of colostrum and breast milk throughout the lactation period (28 days), decreasing the risks due to rapid decrease of colostrum gamma globulins concentration, colostrum passive immunity prevalence and low reserves of glycogen, which makes newborn piglets to be dependent on the energy from colostrum;
- The milk cytogram configuration at the sow was characterized by increasing the proportion of macrophages with advancing lactation (42.86%, 30-60%), usually associated with PMN leukocytes decreasing (15.8%, 8-43%) and rarely lymphocytes (31.0%, 11-46%) or epithelial cells (10.4%, 3-20%);
- We have given a particular character to the distribution of PMN leukocyte subpopulations in investigated sows milk, being predominant neutrophils (75-100%), but without the representation of eosinophils (0-25%) and even basophils (0-5%) to be neglectable;
- PMN leukocyte proportions were increased in 4 sows (20-43%), only one of them showing considerable microbial load associated with significant granulocytosis (43%);
- Macrophage population was characterized by almost the majority share (42.86%), with significant fluctuations (30-60%) and high percentage of active cells (42.60%), which may be associated with efficient local defense against mastitis in sows general;
- Epithelial cells have characteristic aspects outlined by milk cytogram at sow, given by their increased share data (10.40%) and their morpho-physiological characteristics, presenting often as large cells, integral, uni- or binucleate, with voluminous cytoplasmic mass, weak basophilic and with many fat microspheres.

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