Functional state of mammary gland of sows dried off by different techniques

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ABSTRACT: Sows were dried off by a traditional method (control – C, restricted feeding before, during and after weaning) or by a modified one (experimental – E, intensive feeding before, during and after weaning). On day 5–6 after weaning of the 3rd or 6th litter, in the sows lactating for the period of 6 weeks (experiment I) or 4 weeks (experiment II), histomorphological and caryometric evaluation of mammary alveoli was carried out. The cellular infiltrations (plasmocytes, eosinophils, histiocytes and neutrophils) in the mammary gland showed no significant differences between groups dried off by different techniques. The alveolar epithelial cell DNA index (*DI*) and nucleus volume (V_N) were higher in sows lactating for 4 weeks than in those lactating for 6 weeks and in sows after 3rd lactation than in those after 6th lactation. In experiment I, after weaning of the 3rd or 6th litter, absolute *DI* values in groups C and E were as follows: 2.092, 1.520, 1.409, 2.443, and V_N 42.679, 28.060, 38.064, 48.185. In experiment II, they were 2.072, 3.039, 2.890, 3.208, and V_N 52.274, 63.213, 57.980, 63.337, respectively. After weaning of the 3rd litter, the domination of group E compared to group C was found during 4-week lactation ($DI - P \leq 0.01$, $V_N - P \leq 0.01$). In 6-week lactation, more favourable conditions were obtained in group C compared to group E ($DI - P \leq 0.001$, $V_N - P \leq 0.001$). After weaning of the 6th litter, in 4-week lactation, the domination of group E in relation to group C was insignificant: *DI* + 11.0%, V_N + 9.24%, and during 6-week lactation, it was statistically confirmed ($DI - P \leq 0.01$, $V_N - P \leq 0.01$). The results justify the need of intensive feeding during the weaning time in sows with longer lactation compared to those with the shorter one, with the performance period prolonged till the 6th reproduction cycle.

Keywords: sows; drying off technique; mammary gland; functional state; DNA index; nucleus volume

The restriction of feed intake in sows is a commonly employed procedure during drying off the mammary gland. It is also assumed that an equally effective method for lactation termination and drying off the mammary gland is to maintain a high level of the sow's feeding after weaning of piglets, with a free access to water. An increase in the pressure inside alveoli in the mammary gland and a decrease in the blood flow throughout the gland inhibit the process of milk synthesis and secretion. The pressure of milk on the epithelial cells of the secreting alveoli induces an apoptotic reaction (Quarrie et al., 1994, 1995; Banes et al., 1995; Motyl et al., 2000, 2001). This phenomenon is accompanied by a decline in the secretion of prolactin, growth hormone, oestradiol and progesterone, leading to involution (Travers et al., 1996; Wilde et al., 1997).

The aim of the study was to determine the functional state of the mammary gland of multiparous sows during the involution period after weaning of 3 or 6 litters staying with their mothers for 6 weeks (prolonged lactation) or 4 weeks (shortened lactation). A modified technique of drying off, consisting in intensive feeding before, during and after the weaning time and a traditional technique of drying

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off the sows with feeding restriction during the weaning time were employed.

MATERIAL AND METHODS

Material

The study included 48 crossbred sows of Polish Landrace × Polish Large White breed, nursing their piglets for 42 days (experiment I) or 28 days (experiment II). During the whole performance period, the animals were individually managed in uniform environmental conditions. In both experiments, the sows after weaning of 3 and 6 litters were used in *post mortem* tests.

Sow feeding during the weaning time – techniques of drying off

The sows were fed according to the recommendations of Polish Swine Nutrition Requirements (Anonymus, 1993). During the pregnancy period, and in the period of waiting for oestrus, after the litter weaning, the sows were individually fed full-ration mixtures for pregnant sows – LP (the composition: 13.34 MJ ME, 145.6 g of crude protein and 6.3 g of lysine per 1 kg). Until the 90th day of pregnancy, the daily ration amounted to 2.3 kg per sow and between day 91 and 110 to 3.2 kg/sow. During lactation, the feed for suckled sows (LK) with the content of 13.98 MJ ME, 165 g of crude protein and 7.8 g of lysine per kg was administered. The rations of 2.0 kg for sows and 0.45 kg for each piglet/day were employed. The level of feeding the sows in control (C) and experimental (E) group was the same in experiment I and II.

Two techniques of the sow drying off were used: a traditional method (group C) and a modified one (group E). Feeding the sows of group C and E before weaning, during weaning and after weaning was differentiated. It was restricted in group C before weaning. Two days before the weaning of piglets the control sows received a half of the ration of lactation diet, one day before weaning the sows were fed a half of the ration from the previous day and on the day of weaning they were fasted. After weaning, i.e. on day 1, 2 and 3, the sows were fed 1.0; 2.0 and 3.0 kg of LK mixture, respectively; during the successive days they received 3 kg of the mixture per sow/day till the day of oestrus onset and mating. In group E, the sows were intensively fed before weaning, analogically like in the period of complete lactation, according to the number of piglets in the litter (Anonymus, 1993). A high level of feeding was maintained on the day of weaning and during the successive days till the oestrus onset but not longer than till the 10th day after piglet weaning. In the absence of oestrus, the feed ration was reduced to 3 kg per sow/day from the 11th day after weaning. The onset of oestrus and service led to a change in the feed (from LK to LP) and to a reduction in the ration to 2.3 kg per sow/day.

Histopathological and caryometric tests

After the completion of lactation, during the period of mammary gland involution, i.e. on day 5 to 6 after piglet weaning, 48 sows were slaughtered according to the obligatory procedures. Twenty-four sows, i.e. 12 sows from group C and 12 sows from group E, were slaughtered in experiment I and II, respectively (after the weaning of 3 or 6 litters, 6 sows from each group). The samples of mammary gland collected *post mortem* from each sow were fixed in 4% buffered formalin solution; the samples included a mammary tissue. The research material was taken from mammary glands 2, 3, 4 or 5 on the left and right side. After embedding in paraffin wax serial sections 5 μm in thickness were made and stained with haematoxylin-eosin (H-E) and by PAS – Alcjan method (Alcjan Blue) in order to detect alkaline and acid mucopolysaccharides. The frequency of occurrence of changes in the examined samples: + (very low ~ 1/6), ++ (low ~ 1/3), +++ (medium ~ 1/2), ++++ (quite high ~ 2/3), +++++ (high ~ 5/6), ++++++ (very high ~ 1).

In the slides stained with H-E, under 200 × magnification, the profiles of the nuclei of epithelial cells of the alveoli were separated in the regions of the preparation chosen at random and their size (area), volume (V_N) and densitometric parameters were determined.

The objects in which the mean level of brightness was found within the spectrum of 420 to 480 nm and in which the outline of the profile (nucleus membrane) was a linear structure of low brightness were considered as nuclear profiles. The outlines of the objects were detected and highlighted, using a composite gradient filter of Sobel and Roberts. The application of this filter enables to

obtain the effects similar to those obtained in the observations using phase contrast microscopy.

The content of DNA of the nuclei of epithelial cells was determined by static cytophotometry (image cytometry ICM-DNA) in the slides stained by the Feulgen method, which was modified according to Gaub (Dooley and Allison, 1992; Zeiger et al., 1997).

The system was calibrated and the determinations were performed according to the indications contained in the Report of Working Group of the European Society of Analytical Cytopathology (Bocking et al., 1994; Elavathil et al., 1996; Smolle, 1996).

The slides were illuminated with monochromatic light of the wavelength 560 nm and 590 nm (for parafuchsin and thionine – components of Schiff's reagent) (Anonymus, 1988; Aubelle et al., 1994; Gimenez-Mas et al., 1995; Kline et al., 1995; Leo et al., 1995; Murotsuki et al., 1996). The variability of IOD (integrated optical density = mean optical density of profile × area of profile) in the samples of stained nuclei is recognized as a measure of ploidy (quantity of genetic material – amount of DNA in the cell nucleus dependent on the number and length of chromatids). The system was calibrated according to the standard quantity of DNA being found in the nuclei of control cells. The reference value for parameters was the optical density of nuclei of lymphocytes of the cortical layer of lymphatic node in pigs (internal standard) and nuclei of erythrocytes of rainbow trout (external standard). The preliminary calibration of the stained preparations was carried out according to the external standard. The difference between the IOD median of diploid cells of the studied material and of the control cells was used as a calculation coefficient for the calibration of the results of measurements. The results were treated as reliable when the variability of IOD index for the population of control diploid cells did not exceed 0.06.

The level of IOD index for 1 000 nuclei of epithelial cells of alveoli chosen at random was determined using a 40 × objective (the interval of the focusing depth was equal to 3 μm).

The auxiliary index which characterized the distribution of ploidy was created by DNA index (*DI*) being defined as follows:

$$DI = \frac{\text{measure of the central tendency (IOD) of the population of examined cells}}{\text{measure of the central tendency (IOD) of the population of standard cells}}$$

Table 1. Histomorphological evaluation of the occurrence of cellular infiltrations in mammary glands of sows during the involution period

Examined traits	Experiment I		Experiment II	
	C	E	C	E
After weaning of the 3rd litter				
Plasmocytes	++	+ (i)	+++	++++
Eosinophils	++++ (i)	++ (i)	++++ (i)	+++ (i)
Histiocytes	–	++ (i)	–	+ (i)
Neutrophils	–	+	–	–
After weaning of the 6th litter				
Plasmocytes	++	+	+++ (i)	+++++
Eosinophils	+++++ (i)	+++ (i)	+++++ (i)	++++ (i)
Histiocytes	–	–	+	+
Neutrophils	–	–	–	–

The sows lactating for 6 weeks (Experiment I) and for 4 weeks (Experiment II) were dried off either by a traditional method (control group – C, restricted feeding before, during and after weaning) or by a modified method (experimental group– E; intensive feeding before, during and after weaning). Mammary tissue was collected on day 5–6 after weaning

+ (very low ~ 1/6); ++ (low ~ 1/3); +++ (medium ~ 1/2); ++++ (quite high ~ 2/3); +++++ (high ~ 5/6); ++++++ (very high ~ 1) – frequency of occurrence of changes in the examined samples

(i) – infiltrations of mononuclear cells

C – control group; E – experimental group

In this study specialist packages for the analysis of biomedical image Lucia 4.21 (NIKON) were used. Before the commencement of measurements, the system was calibrated with the aim to inform it about the real dimensions of the image. To this end, Bürker chamber for the counting of blood cells (Fein-Optic Jena) was employed, using sections of 50 μm.

Statistics: The results were statistically analysed by one-factor analysis of variance with the application of the least-squares method (SPSS 10.0).

RESULTS

In histological evaluation, changes in the mammary tissue in the form of cellular focally-massive infiltrations were found. In the intraparenchymal connective tissue of mammary gland, infiltrations of mononuclear cells with the focal domination of acidophilic granulocytes or with the participation of plasmatic cells were observed. The connective tissue stroma was abundant. The hyperplasia of the intraparenchymal connective tissue and defoliation of epithelial cells of secreting vesicles indicated a moderate number of changes, not being differentiated in the groups dried off by different techniques in experiment I and II (Table 1).

Histological evaluation of the sow mammary gland showed a moderate number of changes, not being differentiated in the groups of animals dried off by different techniques in both experiments (Table 1).

The functional state of epithelial cells of the final intralobular duct of mammary gland, being expressed as *DI* and V_N with consideration of differences between the groups C and E dried off by different techniques, is illustrated in Table 2. A higher value of DNA index and higher mean volume of cell nuclei were found in the slaughtered sows after the nursing of 3 litters in experiment II for group E compared to group C while the relationships in group E and C in experiment I were reverse (Table 2). A higher value of DNA index and higher mean volume of cell nuclei in group E compared to group C in both experiments were found in the sows the mammary gland of which was examined after the nursing of 6 litters. Statistically significant differences were determined in the sows with 6-week lactation (experiment I) after the completion of the 6th reproduction cycle (Table 2).

Table 2. Functional state of mammary gland cells after weaning of piglets from the 3rd or the 6th litter in experiment I and II

Examined traits	Experiment I (after weaning of the 3rd litter)				Significance of difference	Experiment II (after weaning of the 3rd litter)				Significance of difference
	C		E			C		E		
	$\bar{x}$	SD	$\bar{x}$	SD		$\bar{x}$	SD	$\bar{x}$	SD	
DI	2.092	0.588	1.520	0.416	***	2.072	0.577	3.039	1.520	*
V_N	42.679	19.525	28.060	14.797	***	52.274	22.730	63.213	28.455	**
	after weaning of the 6th litter					after weaning of the 6th litter				
DI	1.409	0.579	2.433	0.013	**	2.890	1.242	3.208	1.960	NS
V_N	38.064	15.807	48.185	24.030	**	57.980	40.357	63.337	31.002	NS

The values in the lines marked with symbols differ statistically: * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; other details are given in Table 1

Table 3. The rate of changes in the functional state of mammary gland cells in the sows dried off by different techniques in experiment I and II

Examined traits	E/C (%)		Experiment II /experiment I (%)		E/C in experiment I and II (%)	Total – experiment II/ experiment I (%)
	Experiment I	Experiment II	C	E		
Functional state of mammary gland cells after nursing of the 3rd litter						
DI	–27.34	+46.67	–0.96	+99.93	+9.49	+47.50
V_N (μm^3)	–34.25	+20.93	+22.48	+125.28	–3.88	+63.26
Functional state of mammary gland cells after nursing of the 6th litter						
DI	+73.39	+11.00	+105.11	+31.31	+31.45	+58.31
V_N (μm^3)	+26.59	+9.24	+52.32	+9.24	+16.12	+40.66

Other details are given in Table 1

Table 4. Rate of changes in the functional state of mammary gland cells in the sows dried off by different techniques after the nursing of 3 or 6 litters

Examined traits	Experiment I (%)		Experiment II (%)		Experiment I and II (%)		After nursing of 6 litters/after nursing of 3 litters (%)
	C	E	C	E	C	E	
DI	–32.65	+60.72	+39.48	+5.56	+3.24	+23.95	+14.06
V_N	–10.81	+71.72	+10.92	+0.20	+1.15	+22.19	+11.46

Other details are given in Table 1

The rate of the changes (E/C) for the examined indices, i.e. *DI* and V_N, was positive in the majority of the compared cases (Table 3). The rate of the changes in comparison with the results of experiment II and I for *DI* and V_N of the mammary gland cells of the sows, as examined after the completion of the 3rd reproduction cycle was high in the experimental group and that of the mammary glands examined after the completion of the 6th cycle was high in the control group (Table 3). The rate of the changes E/C in experiment I and II in total was positive for *DI* and V_N evaluation of the studied glands, examined during the drying off period after the 6th reproduction cycle.

The positive rates of changes in *DI* and V_N were obtained for the majority of the comparable variants, considering the experiment (I – lactation of 42 days, II – lactation of 28 days), performance time (3 or 6 reproduction cycles) and group (C, E) (Table 4). The difference was extremely large in experiment I, with the performance of sows for 6 or for 3 reproduction cycles in group E compared to C.

DISCUSSION

The histological evaluation of the deviation with respect to the standard value and its intensity pointed to the lowered local resistance of the gland in older sows compared to the females after 3 reproduction cycles. Similar relationships were found in other studies (Rekiel, 2002; Rekiel and Bielecki, 2004).

Higher values of V_N in the examined sections of mammary gland, being collected from females after the completion of 28-day period of feeding the piglets from 3 litters and after the weaning of 6 litters in 6- and 4-week lactation, were found in group E compared to group C. The differences in the mean volume of epithelial cell nuclei of the final intralobular duct of mammary glands in the sows indicate that there is a positive influence of feeding in the time around weaning on V_N. It points to a positive effect of intensive feeding during the weaning time on V_N. The measurement of the size of cell nuclei allows an indirect evaluation of the functional state of cells; however, it does not permit the differentiation of the cells remaining in a rest phase and in phase G_1, so it is not sufficient to determine their life cycle. On the other hand, an increase in the mean volume of cell nuclei may reflect the intensification of the processes of DNA synthesis in phase S of the cell cycle. Kim et al. (2001) showed that the unsuckled mammary glands

of the sows fed a high energy and protein level were still very well developed on the 5th day of lactation. Their weight as well as dry matter, protein, fat, ash and DNA content per one gram of the tissue were higher compared to the unsuckled mammary glands of the sows fed the mixture with a low energy and protein level.

The state of the unsuckled mammary gland five days after the beginning of lactation and that five days after the end of lactation is probably different. The differences may arise due to differences in hormonal activity in these two periods. In the former period the hormonal activity is oriented on lactation development while in the latter on its decrease. The levels of milk production in the last phase of 4-week and 6-week lactation are different, which may also indicate the differentiation of metabolic transformation.

The size of the cell nucleus is determined by its DNA content and degree of metabolic activity of the nucleus. The intensity of metabolic changes in the cell is related to the process of transcription, and then to the protein synthesis and it is correlated with the increase in the volume of cell nucleus.

The rate of the changes in *DI* and in V_N was more favourable in the females nursing their piglets for 4 weeks compared to the sows nursing their piglets for 6 weeks (Table 3). The results show the domination of a shorter, i.e. 28-day, period of nursing the piglets over the longer, 42-day, nursing period; it supports the correctness of earlier weaning of piglets from their mothers (Table 3). The increase in V_N may indicate the intensification of processes of DNA synthesis and may be a signal for inducing the proliferation. The domination of this trait (V_N) in the sows with a shorter lactation (experiment II) compared to the sows with a longer lactation period (experiment I) amounted to more than 63% after the nursing of 3 litters, and after the nursing of 6 litters it was equal to more than 40%.

The calculated rates of changes in *DI* and V_N indices for the sows nursing 6 or 3 litters of piglets point to the need of the intensive feeding of sows during the weaning time, in lactation lasting for 6 weeks if the females are kept in the herd for 6 reproduction cycles. When considering different criteria for the evaluation of the mammary gland of sows dried off by different techniques, i.e. physical and cytological properties of milk, involution degree and histomorphological and caryometric studies as well as the level and change of body weight, fattening and muscling of the sows, a possibility of employing the technique of drying off consisting in the intensive feeding of sows during the weaning time was confirmed (Rekiel, 2002). The results of the studies of other authors (Atwood and Hartman, 1995; Wilde et al., 1999; Ford et al., 2000; Kim et al., 2001; Rekiel, 2002) indicate a possibility of introducing the changes in the reconstruction of mammary gland via the change in the level or intensity of feeding after weaning. The results of reproduction and piglet nursing proved that there was no contraindication of using a different not traditional drying off technique in the peri-weaning time (Rekiel, 2003). The comparisons of the efficiency of sows' drying off and the reproductive results revealed the advantage of drying off technique with intensive feeding for sows with prolonged lactation (weaning in 42 days vs. 28 days), and for primiparous sows in comparison with multiparous ones.

The mammary gland involution was evaluated morphologically 21 or 42 days after the suckling of one udder half was prevented in crossbred beef cows (Akers et al., 1990). DNA content was reduced by 50% and 64%, respectively, after 21 and 42 days of involution. However, the percentage of tissue occupied by the epithelium was similar in suckled and unsuckled glands. The presence of alveoli after 42 days indicates that the redevelopment of udder in subsequent lactations is less dramatic than suggested by the study of other species.

The effect of the experimental factor, i.e. the level of feeding during the weaning time, although being incompletely univocal, indicates the rightness of employing a different technique of weaning, i.e. the method consisting in the intensive feeding of sows during the studied period. The increase in V_N index may point to the intensification of processes of DNA synthesis. In shorter, 4-week lactation and shorter, 3-cycle performance of the sows, the modified technique caused an increase in the values of both studied indices – *DI* and V_N. The results justify also the need of more intensive feeding – during the weaning time – the sows with longer lactation than those with the shorter one, with the prolonged (up to the 6th reproduction cycle) period of performance.

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