

Original study

Effects of calf separation and injection of oxytocin on milk performance and milk composition of the Polish Red cows

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Abstract

The aim of this study was to analyse the yield and composition of milk from Polish Red cattle after 12-h isolation of calves. Sixty six Polish Red cows were kept together with calves. Control milkings were carried out once a month after 12-h isolation of calves. Five subsequent milkings after calving were taken under consideration. In the season (May-July 2012) oxytocin (1 ml, 3 min before control milking) was injected. The milk of cows milked mechanically after 12-h isolation of calves was characterized by a very low fat content (0.47-0.58%) depending on the month after calving. There were no differences in protein and lactose content compared to the standard composition of cow's milk. The milk yield was 6.16 kg in the first milking after calving and 3.55-4.01 kg in the four further milkings. After administration of oxytocin a significant increase of milk was observed in the first (12.9 kg) and subsequent months of lactation (8.5-12.4 kg). Milk fat content was significantly higher (4.14% in the first and 3.39-3.86% in the further milkings).

Keywords: Polish Red cattle, suckling, low fat content, milk yield and composition

Abbreviations: CST: calf suckling technique, PR: Polish Red

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Introduction

Polish Red (PR) cattle were acknowledged as the only indigenous breed in Poland. Towards the late 1960s, the PR cattle population consists of approximately two million cows. Because of agricultural intensification, this breed has been consistently removed from most of their original area since the 1970s. At the moment there are about 3000 cows under milk recording and in herd-books. About 2300 cows are involved in the genetic resources conservation programme (Sosin-Bzducha *et al.* 2012). The aim of this programme is to restore and maintain the old type population of PR cattle and to preserve the existing genetic variation.

The farmers who keep PR cows and take part in the conservation programme and the Rural Development Programme are largely dependent on subsidies. Adamczyk *et al.* (2008) reported that in the case of lifted or even limited subsidies, as many as 80 % of the farmers wanted to abandon cows of this breed in favour of breeds that are more efficient and economically profitable (Boonbrahm *et al.* 2004b). Many farmers who keep dual-purpose cows, such as the PR, are interested in the suckling system because of relatively low milk yield and production profitability. Gajos & Dymnicki (2012) stated that this system in case of PR cattle is profitable.

The mixed nursing and suckling system remains popular in many parts of the world (Hernandez *et al.* 2006, Negrao & Marnet 2002, Combellas & Tesorero 2003), especially in ecological or organic farms where calves are kept with their dams for a certain period of time (De Passille *et al.* 2008). Early separation of the calf from the cow is the basis of the modern industry, but the cow-calf rearing system is useful in organic and ecological farms where healthy rearing and production is required. There are a number of studies reporting on higher weight gains during the suckling period for restricted suckling calves compared to the artificial rearing system (Fallon & Harte 1980, Boonbrahm *et al.* 2004a, 2004b, Shamay *et al.* 2005, Fröberg *et al.* 2007). Bar-Pelled *et al.* (1997) reported on a better cow and calf health for the nursing system, higher height at the withers, an earlier age at calving and a tendency towards higher milk production during the first lactation.

The number of ecological farms in conservation programmes is growing every year and the most farms are small family businesses. In the mixed nursing and suckling systems it is difficult to quantify lactation yield. The nursing or suckled cows do not release all their milk during machine milking. Pegram *et al.* (1991) hold that two methods are most frequently used to measure the milk production of a nursing cow. The first is called Calf Suckling Technique (CST) and involves weighing a calf before and after suckling and the second utilizes oxytocin. The authors consider that both methods give the same results.

The aim of the study was to examine the effects on milk production and fat content of nursing PR cows, milked once a month after 12-h separation of calves, before and after injection of oxytocin.

Material and methods

Sixty-six cows of the PR breed were kept outdoors at a private farm and housed, fed and treated according to the appropriate guidelines. The cows were fed hay and silage *ad libitum* in the winter and were pastured in the summer. In the winter season the cows received about 2 kg concentrate per animal. Water was available *ad libitum* from float activated water bowls placed outdoors. The cows nursed calves immediately after calving. Control machine milking

was performed once a month (over the five subsequent months after calving) after 12-h separation of calves. Calves were not present during the control milking. The milk samples were taken immediately after milking by the officer of the Polish Federation of Cattle Breeders and Milk Producers (PFCBMP). The analysis of milk samples was done at the Laboratory of the PFCBMP in Bydgoszcz (Fossomatic 5000 Milkoscan 6000, Combifoss). The milk components analysed were fat content, protein content, lactose content and solids. In the next season, all cows were injected intramuscularly with 1 ml of oxytocin (3 min before milking).

The data were analysed with one-way variance analysis using ANOVA procedure (Statistica 9.1, StatSoft, Inc. Tulsa, OK, USA), with parity or successive milkings as the main factor. Cows for which no information about successive milkings was available were removed from further analysis.

Results

A significant influence of oxytocin injection on fat content in milk (Table 1) was observed. The average fat concentration in milk of control cows was 0.55 % while in the experimental group it was 3.66 %. Also both groups differed in terms of fat content variation. Variation coefficient of the control group was more than twice as high as the variation coefficient of the oxytocin group. Table 2 presents the milk yield (milking after 12 h of calf separation) and milk composition for successive monthly milkings after calving of control cows. To compute daily yield, this amount should be multiplied by two. Milk yield was the highest during the first month after calving with significant differences in relation to the other months. There were no differences in milk yield between milkings 2-5 ($P \geq 0.05$). Similar relations were observed in terms of fat concentration changes across the milkings. The highest concentration of milk fat was reported at first month (0.83 %). There were significant differences between first and other milkings. Additionally, the protein concentration varied across the milkings. The lowest protein level was observed in the 2nd month. The most significant differences were stated between the 2nd and 4th milking. The pick of the lactose concentration was reported at 2nd milking. Significant differences were observed between the 1st and other milkings.

Table 1
Statistics for fat variable in milk of PR cows in five subsequent milkings after calving (control and oxytocin test)

Variable	Milking (subsequent monthly milkings)				
	1	2	3	4	5
Control	N=29	N=29	N=28	N=16	N=10
Mean \pm SD, %	0.69 \pm 0.42	0.48 \pm 0.37	0.47 \pm 0.2	0.52 \pm 0.36	0.58 \pm 0.3
Minimum	0.06	0.1	0.09	0.2	0.09
Maximum	1.68	1.85	0.83	0.93	1.09
Variance	0.17	0.14	0.04	0.04	0.11
Var. Coeff.	60.6	77.1	43.1	40.2	56
Oxytocin test	N=50	N=50	N=53	N=60	N=54
Mean \pm SD, %	4.14 \pm 1.31	3.39 \pm 0.65	3.5 \pm 0.55	3.41 \pm 0.71	3.86 \pm 0.8
Minimum	2.52	2.31	2.59	2.1	2.12
Maximum	7.99	5.07	5.16	6.73	5.8
Variance	1.72	0.42	0.3	0.5	0.66
Var. Coeff.	31.67	19.15	15.7	20.74	21.0

Table 2

Milk yield and milk composition of suckled PR cows in five subsequent milkings after calving

	Milking (subsequent monthly milkings)					SE	P
	1 N=37	2 N=37	3 N=36	4 N=24	5 N=13		
Milk yield, kg	6.16±3.83 ^A	4.82±1.96 ^B	4.01±1.45 ^B	3.64±1.4 ^B	3.55±1.51 ^B	0.21	≤0.01
Fat, %	0.83±0.55 ^A	0.47±0.36 ^B	0.48±0.29 ^B	0.58±0.37 ^B	0.58±0.3 ^B	0.04	≤0.01
Protein, %	3.4±0.3 ^A	3.07±0.42 ^B	3.25±0.35 ^{AB}	3.58±0.44 ^{AC}	3.55±0.39 ^{AC}	0.04	≤0.01
Lactose, %	4.35±0.68 ^A	4.87±0.41 ^B	4.84±0.51 ^B	4.74±0.45 ^B	4.57±0.42 ^{AB}	0.05	≤0.01
Solids, %	9.31±0.83 ^{ac}	8.95±0.56 ^b	9.13±0.46 ^{ab}	9.46±0.57 ^c	9.31±0.55 ^{abc}	0.05	0.02

SE: standard error of the mean, $P>0.05$: no statistically significant differences, ^{ABC} $P\leq 0.01$, ^{abcd} $P\leq 0.05$

Daily milk yield was twice as high as after oxytocin injection (Table 3). Also milk composition was improved significantly. The biggest changes were observed in fat concentration. Oxytocin injection had an effect on milk yield and fat content. Fat percentage in the first milking with oxytocin was similar to that of »standard« milk (4.14 vs. 4.2 %) and was much lower at the 2nd and 3rd milkings. In all cases fat percentage was much lower in milk obtained at milking after 12-h calf separation compared with case of oxytocin injection (0.47-0.8 % and 3.51-4.14 %). In the oxytocin group the highest differences were also reported between the 1st and the 2nd milking. At 1st milking protein level in the milk of the experimental group was lower than in the control group. Excluding the 2nd milking protein concentration was similar in both groups. The tendencies in lactose changes in both groups were also similar.

Table 3

Milk yield and milk composition of PR suckled cows in five subsequent milkings after calving—oxytocin test

	Milking (subsequent monthly milkings)					SE	P
	1 N=50	2 N=50	3 N=53	4 N=60	5 N=54		
Milk yield, kg	12.92±4.04 ^A	12.4±2.75 ^A	9.32±2.7 ^B	9.98±3.32 ^B	8.49±2.86 ^B	0.22	≤0.01
Fat, %	4.14±1.31 ^A	3.39±0.65 ^B	3.51±0.55 ^B	3.41±0.71 ^B	3.86±0.81 ^A	0.05	≤0.01
Protein, %	3.34±0.66 ^A	3.29±0.27 ^A	3.29±0.23 ^A	3.53±0.34 ^B	3.54±0.41 ^B	0.03	≤0.01
Lactose, %	3.92±0.63 ^A	4.9±0.31 ^B	4.95±0.22 ^B	4.6±0.42 ^C	4.78±0.27 ^B	0.03	≤0.01
Solid, %	12.4±1.46	12.21±0.67	12.48±0.64	12.4±0.84	12.73±0.93	0.06	0.08

SE: standard error of the mean, $P>0.05$: no statistically significant differences, ^{ABC} $P\leq 0.01$

The milk yield of multiparous cows was higher than that of primiparous cows ($P\leq 0.01$) regardless of oxytocin injection (Table 4 and 5). There were no differences in milk composition between cows at the 1st and 2nd lactation in the control group (Table 4). However, the average percentage of milk fat was much lower than breed average (4.2 %), with no difference between primiparous and multiparous cows.

After the oxytocin injection there were no differences in milk components between primiparous and multiparous cows ($P\geq 0.05$) in the 1st to 3rd milkings except milk yield, which was higher for multiparous cows in the 1st ($P\leq 0.04$), 2nd ($P\leq 0.09$) and 3rd ($P\leq 0.15$) milkings (Table 5). Differences in most of milk components between primiparous and multiparous cows were observed in the 4th and 5th milkings.

Table 4
Milk yield and milk composition of suckled PR cows according to lactation number (control group)

Item	Milk yield, kg	SE	Fat, %	SE	Protein, %	SE	Lactose, %	SE	Solids, %	SE
Milking 1*										
I, n=17	4.54±1.59 ^A	0.39	0.74±0.42	0.1	3.38±0.3	0.07	4.41±0.53	0.13	9.24±0.68	0.17
II, n=12	8.52±5.58 ^B	1.61	0.63±0.43	0.12	3.39±0.43	0.12	4.19±0.97	0.28	8.96±0.76	0.22
P	0.009		0.46		0.98		0.44		0.31	
SE, N=29	0.78		0.07		0.07		0.14		0.13	
Milking 2*										
I, n=17	3.94±1.07 ^A	0.26	0.45±0.29	0.06	2.97±0.46	0.11	4.84±0.29	0.07	8.81±0.57	0.14
II, n=12	5.93±2.23 ^B	0.64	0.53±0.48	0.14	3.12±0.34	0.09	5.05±0.38	0.11	9.13±0.63	0.18
P	0.003	0.7	0.59		0.34		0.1		0.16	
SE, N=29	0.35		0.04		0.08		0.06		0.11	
Milking 3*										
I, n=17	3.22±0.71 ^A	0.17	0.51±0.18	0.04	3.16±0.36	0.08	4.78±0.37	0.09	9.05±0.3	0.07
II, n=11	5.31±1.58 ^B	0.48	0.4±0.22	0.07	3.22±0.32	0.1	4.89±0.73	0.22	9.03±0.48	0.15
P	0.001		0.15		0.64		0.6		0.89	
SE, N=28	0.29		0.09		0.06		0.09		0.07	
Milking 4*										
I, n=17	3.21±1.09 ^A	0.31	0.56±0.14	0.04	3.49±0.52	0.15	4.77±0.4	0.12	9.38±0.34	0.1
II, n=7	5.35±1.18 ^B	0.59	0.41±0.35	0.17	3.69±0.28	0.14	4.44±0.76	0.38	9.16±0.16	0.8
P	0.001		0.23		0.47		0.28		0.22	
SE, N=24	0.36		0.05		0.11		0.13		0.08	
Milking 5*										
I n=9	2.77±1.03 ^A	0.42	0.72±0.31	0.12	3.44±0.41	0.17	4.4±0.42	0.17	9.23±0.63	0.26
II n=4	5.35±0.85 ^B	0.43	0.37±0.22	0.11	3.78±0.25	0.12	4.81±0.3	0.15	9.52±0.28	0.14
P	0.001		0.09		0.19		0.13		0.43	
SE, N=13	0.51		0.1		0.12		0.13		0.17	

*subsequent monthly milkings, I: primiparous, II: multiparous, SE: standard error of the mean, P>0.05: no statistically significant differences, ^{ABC}P≤0.01

Table 5
Milk yield and milk composition of primiparous and multiparous (oxytocin test)

Item	Milk yield, kg	SE	Fat, %	SE	Protein, %	SE	Lactos, %	SE	Solid, %	SE
Milking 1*										
I, n=18	11.34±3.35 ^a	0.79	3.98±1.5	0.35	3.38±0.55	0.13	3.97±0.81	0.19	12.33±1.49	0.35
II, n=32	13.81±4.18 ^b	0.74	4.24±1.21	0.21	3.32±0.73	0.13	3.90±0.51	0.09	12.44±1.62	0.26
P	0.04		0.51		0.75		0.71		0.79	
SE, N=50	0.57		0.19		0.09		0.09		0.21	
Milking 2*										
I, n=21	11.52±2.43	0.53	3.45±0.78	0.18	3.32±0.32	0.07	4.87±0.33	0.07	12.3±0.79	0.17
II, n=29	12.89±2.91	0.57	3.34±0.54	0.16	3.27±0.23	0.04	4.91±0.32	0.05	12.09±0.63	0.12
P	0.09		0.38		0.52		0.66		0.06	
SE, N=50	0.75		0.1		0.04		0.04		0.1	
Milking 3*										
I, n=21	9.87±3.4	0.73	3.56±0.84	0.18	3.33±0.22	0.05	4.93±0.22	0.07	12.54±0.77	0.17
II, n=32	8.81±2.06	0.35	3.25±0.67	0.11	3.29±0.23	0.04	4.99±0.23	0.16	12.25±0.77	0.13
P	0.15		0.13		0.51		0.38		0.17	
SE, N=53	0.36		0.1		0.03		0.03		0.1	
Milking 4*										
I, n=24	7.98±1.7 ^a	0.35	3.63±0.87 ^a	0.18	3.63±0.44 ^a	0.09	4.58±0.43	0.09	12.68±1.1 ^a	0.22
II, n=36	11.31±3.48 ^b	0.58	3.25±0.53 ^b	0.09	3.46±0.23 ^b	0.04	4.65±0.41	0.06	12.21±0.6 ^b	0.09
P	≤0.01		0.04		0.05		0.53		0.03	
SE, N=60	0.43		0.09		0.04		0.05		0.11	
Milking 5*										
I, n=26	7.13±2.98 ^A	0.55	3.62±0.68 ^a	0.13	3.46±0.34	0.09	4.71±0.26	0.05	13.02±1.0 ^a	0.2
II, n=28	9.76±2.06 ^B	0.35	3.25±0.67 ^b	0.11	3.29±0.23	0.06	4.85±0.27	0.05	12.46±0.8 ^b	0.14
P	≤0.01		0.02		0.14		0.07		0.02	
SE, N=54	0.39		0.11		0.06		0.04		0.13	

*subsequent monthly milkings, I: primiparous, II: multiparous, SE: standard error of the mean, P>0.05: no statistically significant differences, ^{ABCD}P≤0.01, ^{abcd}P≤0.05

Discussion

Bar-Pelled *et al.* (1995) stated that blood oxytocin levels are higher during suckling than during milking. Therefore, such low daily milk yield (12.32 kg after calving and 7.1-9.64 kg in the next monthly milking) can be affected by low concentration of oxytocin. Elevated oxytocin concentrations are necessary during milking for complete milk removal (Bruckmaier *et al.* 1994, Manteuffel 2002). Wall & McFadden (2012) stated that oxytocin is involved in elevated milk production elicited by increased milking frequency or suckling. De Passillé (2008) stated that oxytocin levels during the milking of nursing cows (twice daily 2 h after milking) were much higher ($P \leq 0.03$) than for machine milked cows (31.7 vs. 18.0 pg/mL). The same study confirmed a significant interaction between the type of cow (nursing or machine milked) and phase of lactation. Before nursing the level of oxytocin was the same in both groups (about 14.15 pg/mL) and during nursing it increased three-fold in the control group (35.5 vs. 11.0 pg/mL). The same significant interactions were noted for prolactin. When suckling is not limited the cows may have reduced oxytocin and prolactin release at milking (Akers & Lefcourt 1984, Lupoli *et al.* 2001, Mayntz *et al.* 2006). Oxytocin is released in response to teat stimulation and prolactin and cortisol are induced both by machine milking or suckling (Bruckmaier & Blum 1998, Tančin *et al.* 1995, Williams *et al.* 1993).

Mao & Caruolo (1973) reported that mammary blood flow was inversely related to the amount of milk accumulated in the gland and that decreased milk secretion during milk stasis may be mediated by a decreased availability of nutrients for the mammary gland. Farr *et al.* (2000), noted that extended milk stasis in lactating goats resulted in a 50 to 75 % decrease in mammary blood flow and capillary permeability, as well as a marked regression of vasculature. Twelve-hour separation is probably too short but with factors like stress the mechanism could be the same. Some stress situations, for example, relocation or separation can inhibit oxytocin release and increase prolactin and cortisol concentration (Bruckmaier *et al.* 1993). Kraetzl *et al.* (2000) observed no stimulation effect on oxytocin release in cows during suckling by other cow's calf. The positive effect of exogenous oxytocin on milk production was reported in previous studies (Ballou *et al.* 1993, Lollivier & Marnet 2005) but Linnerud *et al.* (1966) claimed that in the absence of milk removal exogenous oxytocin had no effect on milk yield. Bar-Peled (1998) found greater plasma concentrations of growth hormone, prolactin and IGF, and lower insulin concentrations in nursing cows.

The higher milk production of nursing cows may result from hormonal changes.

Low milk fat in milk obtained during a mixed system (suckling and machine milking) was observed in ewes (McKusick *et al.* 2001, McKusick *et al.* 2002b, Fuertes *et al.* 1998) and in cows (Combellas & Tesorero 2003). No publications on this subject over the last few years have been found. McKusick *et al.* (2002b) consider that one or all of the following mechanisms are responsible for low fat content in the milk of nursing or suckled ewes at machine milking:

1. milk ejection during machine milking does not occur,
2. milk fat synthesis is inhibited,
3. milk fat transfer from the alveoli to the cistern between milkings does not occur.

Combellas & Tesorero (2003) consider that more than 80 % of milk is stored in udder alveoli and transferred to the cistern by a neurohormonal reflex initiated by the contact of the calf's mouth with the udder. The same authors presented the results of studies carried out to

evaluate the cow-calf relationship during milking and its effect on milk fractions. Without calf presence, 1-2 kg less milk was obtained compared to cows stimulated by the calf. Fat content was lower too (1.2 % vs. 2.4-2.7 % respectively). It is well known that the fat content of milk increases during the milking process. Dobicki *et al.* (1993) reported that fat content was about 3 % in the first litre of milk and about 7 % in the last litre. Similar results were obtained by Dymnicki *et al.* (2012) (unpublished data). Whittlestone (1953) reported on fat content to be 1.6 % in fore milk and 8.9 % in the fore-strippings. In ewes, McKusick *et al.* (2002a) stated that the cisternal milk fat but not milk protein percentage was lower than in alveolar milk (4.49 % vs. 7.92 %). The suckled cows do not release all of their milk during machine milking, so that the low fat percentage can be partly attributed to the fact that it is only part of the milk stored in the cistern (Boonbrahm *et al.* 2004a).

Levy (1964) observed a decrease of about 90 % of fatty acid synthesis in rats by 24h of weaning. The synthesis of fatty acids was restored when pups were returned to the mother for suckling. Heesom *et al.* (1992) suggested that fat synthesis may be regulated by a negative feedback mechanism involving medium-chain fatty acids.

Ewy & Barowicz (1977) reported that primiparous cows were more responsive to incremental doses of 0.05 to 0.2 IU of oxytocin than multiparous cows. In the present study that was not observed.

The growing interest of farmers in ecological production has increased the interest in native dual-purpose breeds of cattle included in the conservation programme. In Poland the main emphasis was placed for many years on milk production, while beef production was less important. When milk production is uneconomic, especially for small farms, farmers are looking for additional opportunities to improve the profitability. One way is to rear calves with mothers and to produce veal meat on pastures. This method affects the yield and chemical composition of milk, especially milk fat content. Twelve-hour isolation of calves from their mothers is not enough for milk to return to »normal« parameters. The administration of oxytocin restored the standard composition of milk and in part explained the altered composition of the milk of cows nursing their calves.

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