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Preliminary observations on the *Echinacea*-induced lactoferrin production in goat milk*

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The study involved the use of Echinapur preparation, containing *Echinacea purpurea* extract, to promote milk lactoferrin secretion, and – as a result of its antibacterial properties – to reduce inflammatory changes in the goat's mammary gland. Ten goats with the highest somatic cell count (SCC) of milk were selected from the flock and treated for two weeks with Echinapur. In order to estimate treatment effectiveness, milk was examined for lactoferrin (Lf) concentration, SCC, bacteria counts as colony forming units (CFU) and yield and gross composition of milk, before and at the end of treatment, and then 2 and 4 weeks later. Milk Lf content was determined using the RP-HPLC technique, and milk SCC, CFU and chemical composition were determined instrumentally. Treatment resulted in a transient ($P<0.01$) decrease of milk protein content below the initial level, compensated by a gradual increase of milk yield (24% over the pre-treatment value). A substantial increase in milk Lf content, with a maximum response ($P<0.01$) at 2 weeks after the end of treatment, was accompanied by a gradual reduction ($P<0.01$) in SCC and CFU compared to initial values. This is the first report showing the feasibility of promoting Lf secretion in order to reduce inflammatory changes in the goat mammary gland.

KEY WORDS: Echinacea purpurea / hygienic milk quality / lactoferrin / mastitis

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Producers and consumers are both becoming increasingly interested in goat's milk. Consumers are attracted by its high digestibility, low allergenicity and beneficial chemical composition, more similar to human than to cow's milk. Producers are attracted by the hope of easier access to the market of goat than of cow's milk, production of the latter being restricted by quotas, as well as by the possibility of making luxury cheeses sold at increased prices.

The main problem for goat milk producers is the difficulty in following the hygiene standards, particularly as regards somatic cell count (SCC) – Zeng and Escobar [1996]. Due to the different mode of milk secretion [Paape 2000], the goat milk SCC can be much higher than that of cows' milk. Despite increasing the legal limit of milk SCC from $500 \times 10^3/cm^3$ to 1 mill./cm^3, many producers from various countries are unable to fulfil even this condition [Zeng 1996]. Meanwhile, the European market is setting ever higher standards for animal products. They should be not only fresh and tasty, but also safe and healthy. Programmes to improve the hygienic quality of milk are not fully effective, particularly in the case of goat milk. Simultaneously, improvement is hindered by a lack of a specific criterion for goats, to discriminate between milk from healthy or sick animals [Paape 2000, Wilson *et al*. 1995].

The over-use of antibiotics in humans and animals observed in recent years, has led to a rapid rise in the number of bacteria strains resistant to the majority of antibiotics, which makes infection even harder to control [Tan *et al*. 2000].

The withdrawal of antibiotics from animal production, which awaits us in the future, is a reason to seek alternative solutions. One of these is concerned with making use of the organism's own defence system. The secretory cells of the mammary gland produce and secrete casein and a range of whey proteins, only recently recognized as important functional components of dairy products. One of them – lactoferrin – is particularly noteworthy. Lactoferrin is present in most body fluids of mammals, and has long been known for its ability to bind iron [Wilce and Aguilar 1991]. At present, lactoferrin is known to have anti-bacterial, anti-viral, anti-fungal, anti-carcinogenic, and immuno-stimulating properties [Bellamy *et al*. 1992, Bezault *et al*. 1994, Dial *et al*. 1998, Vorland 1999, Shimazaki 2000, Tsuda *et al*. 2000, Gardiner 2001] and is used for treatment of various conditions. The activity of lactoferrin is, however, not high, and commercial preparations available are expensive [Shimazaki 2000].

The present experiment aimed at establishing the possibility of promoting milk lactoferrin production with Echinapur preparation, and improving the health status of the mammary gland by stimulating the goats' own defence system. The preparation contains an extract of *Echinacea purpurea,* which has proven ability to activate the immune system [Burger *et al*. 1997, Chang 2000]. In studies performed on healthy volunteers the *Echinacea purpurea* enhanced phagocytic activity of polymorphonuclear neutrophils [Chang 2000]. Macrophages in the presence of *Echinacea purpurea* produced significantly more cytokines and interferone than did unstimulated cells [Burger *et al*. 1997].

Material and methods

The experiment was carried out at the Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec, on Polish White Improved (upgraded) goats. Out of a flock of 30, 10 animals in the initial stages of lactation were chosen with the highest somatic cell count (SCC) of milk. During the experiment, all animals were kept in pens, and fed indoors. The diet was composed of 1.2 kg of concentrates, 2.5-3.0 kg of haylage and 1 kg hay. Echinapur pills (HERBAPOL, Poland), containing *Echinacea purpurea* extract were given over two weeks in starch capsules, twice daily, during milking. The daily dose of Echinapur applied was suitable for an adult man, *i.e*. 6 pills, each containing 100 mg of extract, being equal to 2 mg active substance (polyphenol acids) per pill. The effectiveness of Echinapur was established on the basis of its effect on the SCC/cm^3 milk, total number of milk bacteria, expressed as colony forming units (CFU/cm^3), and lactoferrin (Lf) content of milk.

The SCC, CFU and Lf content were determined in milk samples taken four times: before the start of treatment (initial level), immediately after administration the last dose (final level), and then two and four weeks after termination of the treatment. Milk samples were subjected to routine analysis for protein, fat, lactose and dry matter using Milkoscan FT 120 (FOSS ELECTRIC). The SCCs were determined with the Somacount 150 (BENTLEY) and CFUs using spiral inoculation in the WASP (BENTLEY) and automatic scanning with the CounterMat Flash 4.2 (BENTLEY).

The Lf content of milk was measured using reverse-phase liquid chromatography (RP-HPLC), with Agilent 1100 chromatograph, equipped with a UV detector and Supelcosil RP-C18 column, 4.6 × 250 mm and pore diameter of 5 μm (SUPELCO). The separation of whey proteins was based on methods described by Romero *et al*. [1996] and Wilce and Aguilar [1991]. Standard bovine Lf of 90% purity (SIGMA) was used as reference protein.

The results were assessed with the least squares method using the general linear model – GLM, according to SAS procedure [SAS 1992]. In one goat, with the highest SCC, clinical *mastitis* developed one day after the start of the experiment and antibiotic treatment was necessary. The animal was not included in the statistical evaluation of results.

The results were assessed with a least squares method according to SAS [1992] procedure using the general linear model (GLM) and taking into account the effect of the time of sampling, regression on the day of lactation, and random error.

Results and discussion

Administering of Echinapur did not affect significantly milk yield, as well as lactose and dry matter content. A small transient but significant decrease in the protein and an increase in fat content were observed. However, 14 days after the last dose of Echinapur has been administered, the protein content of milk increased (Tab. 1) and finally reached the level higher ($P<0.01$) than the pre-treatment value. Additional compensation of the transient decrease of protein content was provided by continuous

Table 1. Means and their standard errors (SE) for daily milk yield and composition in goats (n=9)

Traits	During treatment				After termination of treatment			
	initial level		final level		2 weeks		4 weeks	
	mean	SE	mean	SE	mean	SE	mean	SE
Milk yield (kg)	2.434^{ab}	0.117	2.442^{cd}	0.101	2.967^{ac}	0.098	3.032^{bd}	0.114
Protein (%)	2.508^{A}	0.101	2.218^{ABa}	0.089	2.589^{B}	0.090	2.603^{a}	0.104
Fat (%)	2.888^{A}	0.114	3.022^{bc}	0.100	2.605^{ab}	0.102	2.227^{Aac}	0.117
Lactose (%)	4.355	0.057	4.459	0.050	4.458	0.051	4.515	0.059
Dry Matter (%)	10.465	0.214	10.391	0.189	10.353	0.192	10.138	0.221

$^{a..A}$ Means in rows bearing different superscripts differ significantly at: small letters – P<0,05; capitals – P<0.01.

rise of milk yield compared to initial level (P<0.05).

During the two weeks while the goats were receiving Echinapur, the SCC of milk fell to 53% of the initial level (Tab. 2), showing that *Echinacea purpurea* extract stimulated

Table 2. Means and their standard errors (SE) for milk SCC and CFU levels of goat milk (n=9)

Trait	During treatment				After termination of treatment			
	initial level		final level		2 weeks		4 weeks	
	mean	SE	mean	SE	mean	SE	mean	SD
SCC	1641.01^{abc}	291.0	761.48^{A}	256.8	386.35^{a}	260.7	175.14^{c}	300.3
LnSCC	7.497^{abc}	0.31	6.366^{aA}	0.27	5.803^{b}	0.28	5.071^{Ac}	0.32
CFU	178.17^{ab}	22.62	57.08^{a}	19.97	57.80^{b}	20.27	95.41^{A}	23.35
Ln CFU	5.184^{abc}	0.18	4.032^{a}	0.16	3.818^{b}	0.16	4.264^{c}	0.19

SCC – somatic cell count × 10^{3}.
Ln – natural logarithm.
CFU – total number of bacteria /cm^{3} expressed as colony forming units.
$^{a..A}$ Means in rows bearing different superscripts differ significantly at: small letters – P<0.05, capitals – P<0.01.

the goats' immune system effectively, acting specifically on the secretion of Lf, instead of increasing production of leukocytes. After terminating the treatment with Echinapur, SCC/cm^3 of milk continued to decrease and a month later was by 89.3% lower than before treatment. A similar prolonged effect on immune system was observed after stimulating hens over three days with water containing high doses of *Echinacea angustifolia* extract [Świerczewska *et al*. 2003] where a significant increase in the activity of lysozyme was noted in the egg protein. In the present experiment milk lysozyme was not determined, since ruminant milk (in contrast to human milk) contains only traces of this protein [Heine *et al*. 1991]. On the other hand, in studies carried out on cows

[Dymnicka *et al*. 2003] given daily 300 g of dried whole plant *Echinacea purpurea* per animal over three weeks, significant changes were observed neither in milk SCC nor in the level of milk immunoglobulins. However, the effectiveness of *Echinacea purpurea* immuno-modulating activity may vary according to environmental conditions of plant growth, as well as to the method of herb conservation. No information was available concerning the level of active components in *Echinacea purpurea* administered to cows by Dymnicka *et al*. [2003].

The positive results of this study on Echinapur administration to goats at doses recommended for humans allow to anticipate that *Echinacea purpurea* extract may be used effectively in treating the sub-clinical *mastitis* in ruminants. However, although *Echinacea* has a proven effect on the immune system [Burger *et al*. 1997, Chang 2000] and its active constituents have been identified, its mechanism of action still remains unknown [Percival 2000].

Beneficial effect of administering Echinapur to cows has also been observed on CFU/cm^3 of milk (Tab. 2). As a result of the two-week therapy the decrease (67.9% of the initial amount) in the total CFUs in milk was found ($P<0.01$). It can, therefore, be anticipated that *Echinacea* is capable of effectively stimulating the organism's immune system, and thus lowering the milk CFU level. In this study, after finishing the course of Echinapur, its effect was maintained for a certain time, but the CFUs started rising earlier than SCC. Despite this, the CFU measured a month after the end of treatment was still by almost 50% lower than the initial CFU level ($P<0.05$).

SCC in goat milk is not considered to be an appropriate measure of the mammary gland health status [Paape 2000]. The Lf content of milk is regarded as a good indicator of inflammatory changes [Vorland 1999]. This immuno-active iron-binding protein is

Table 3. Lactoferrin content of milk (mg/L) in individual goats receiving Echinapur

Goat number	During treatment		After termination of treatment	
	initial level	final level	2 weeks	4 weeks
422	30.87	34.09	51.71	41.62
430	10.27	16.51	23.46	23.62
437*	32.77	45.39	45.26	29.68
463	12.30	27.00	29.76	18.75
477	13.58	21.34	49.13	42.56
525	32.16	41.77	48.63	30.97
505	14.55	16.32	27.19	19.86
519	31.74	33.99	35.36	28.82
545	13.82	22.22	28.30	20.28
586	11.97	18.72	23.19	22.98
Mean**	17.90^{A}	24.63^{b}	34.07^{aB}	26.37
±SE	3.0	3.0	3.0	3.0

*Clinical *mastitis*.

**Results of goat 437 (treated with antibiotic) are excluded.

^{aA} Means bearing different superscripts differ significantly at: small letters – $P<0.05$, capitals – $P<0.01$.

synthesized in the secretory cells of the mammary gland [Sanchez *et al*. 1992] and by leukocytes [Levay and Viljoen 1995]. Thus, in order to find out whether the immunity stimulation resulted from increased Lf production milk Lf level in the treated goats was measured (Tab. 3).

The initial Lf level of milk ranged from 10.27 to 52.77 mg/l what is similar to values reported by Schanbacher *et al*. [1993, 1997] for mature cow milk (10-100 µg/ml), but lower than those given by Palmano and Elgar [2002] or Hamann and Krömker [1997]. Contrary to colostrum, where the level of Lf exceeds even by 100% the values found in advanced lactation [Levay and Viljoen 1995], the milk Lf can reach very high level during involution of the bovine mammary gland [Schanbacher *et al*. 1993]. It should, however, be emphasized that all the authors mentioned refer to quite old information by Masson and Heremans from 1971 as cited by Recio and Visser [2000].

Mastitis provokes an immune response. The entry of bacteria into the gland through the teat canal evokes an increase in the number of leukocytes. Lf, as the main anti-bacterial whey protein, is produced both by the gland and the increased number of leukocytes, to fight infection. When infection has already been controlled, the milk Lf level decreases [Harmon 1994]. The changes of Lf concentration of milk of goat 437 (Tab. 3) strictly reflect the way the change occurs as described by Harmon [1994]. Goats receiving Echinapur (with the exception of no. 437, which had clinical *mastitis*) produced milk containing significantly more Lf than before treatment.

During 14 days after the treatment of Echinapur was terminated, the Lf content of milk kept increasing ($P<0.01$), while SCC and CFU decreased (Tab. 3), indicating an improvement of the mammary gland health status.

According to Iyer and Lonnerdal [1993] the lactoferrin of blood plasma is mainly produced by neutrophils. In the present study the rise in Lf content of milk in goats receiving Echinapur despite the significantly lower SCC and CFU values suggests that Lf is produced by secretory cells.

Echinapur continued to affect SCC and CFU in milk for about a month after termination of the treatment, pointing to a prolonged action of *Echinacea purpurea* extract on the immune system of goats. The milk samples taken four weeks post-treatment showed a slightly raised level of CFU, accompanied by not significant fall of Lf concentration. However, the final concentration of Lf still remained higher than the initial value.

The SCC did not correlate significantly with the CFU (Tab. 4) what corroborates the data of Park and Humphrey [1986] and confirms the opinion, that in goats the level of SCC in milk is not an appropriate criterion allowing the assessment of the mammary gland infection. Moreover, an association has not been found between SCC and the Lf content of milk (Tab. 4). On the other hand, despite the small number of animals, a correlation of 0.411 ($P<0.05$) was found between the CFU and the Lf content of milk.

The level of stimulation of Lf secretion varied. In goats with an initial Lf content of above 30 mg/l, the increase reached on average 43%, whereas in those with the content below 14 mg/l, it exceeded 135% (data not tabulated).

Despite Echinapur being a recommended, safe medicine when used in humans,

Table 4. Pearson's correlations between SCC, CFU and Lf content of goat milk

Trait	n	r	Significance at P
SCC × CFU	36	0.117	0.301
Lf × CFU	36	0.009	0.959
Lf × Ln SCC	36	-0.046	0.791
Lf × SCC	36	-0.393*	0.018
Lf × Ln CFU	36	-0.411*	0.013

n – number of determinations; r – correlation coefficient.
Ln – natural logarithm.

further studies on animals are necessary in order to establish the optimum period of treatment, as well as the safe and effective daily dose of *Echinacea purpurea*, in whatever form it may be. The easiest and cheapest would be the use of dried plants. However, establishing the level of active ingredients of *Echinacea purpurea* depending on the phase and conditions of growth and drying would be a problem.

It can be concluded that the efficiency of Echinapur preparation exceeded the expectations. A significant rise of Lf content of milk as a result of stimulating the immune system of goats with *Echinacea purpurea* extract may provide a model for production of milk with increased anti-bacterial properties. A significant lowering in SCC and CFU in milk of goats receiving Echinapur points to the feasibility of using the preparation to achieve beneficial changes in health of the mammary gland, and simultaneously an improvement in the hygienic and health-promoting quality of milk. The preparation does not induce negative changes in the content of basic milk components with the exception of a transient but significant decrease of the protein content.

Although the results presented here must be treated as preliminary, they suggest the potential feasibility of application of *Echinacea* as a immunostimulating feed additive.

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Wstępne obserwacje nad pobudzeniem produkcji laktoferyny w mleku kozim za pomocą ekstraktu z *Echinacea purpurea*

S t r e s z c z e n i e

Badano zastosowanie preparatu Echinapur zawierającego ekstrakt jeżówki purpurowej (*Echinacea purpurea*) do pobudzania sekrecji laktoferyny (Lf) w mleku i – w wyniku jej antybakteryjnych właściwości – zredukowania zmian zapalnych w gruczole mlekowym kóz. Ze stada wybrano 10 kóz z najwyższą liczbą komórek somatycznych (SCC) w mleku, którym przez 2 tygodnie podawano Echinapur. Jedno zwierzę wycofano z racji klinicznego *mastitis* i leczenia antybiotykiem. Dla oceny efektywności zastosowanego preparatu określano w mleku SCC, ogólną liczbę bakterii (CFU), poziom Lf oraz wydajność i podstawowy skład mleka przed i po zakończeniu podawania preparatu oraz po upływie dalszych 2 i 4 tygodni. Zawartość Lf w mleku oznaczano metodą RP-HPLC a SCC i CFU metodami instrumentalnymi. Stwierdzono przejściowe ($P<0,01$) obniżenie zawartości białka w mleku poniżej wartości wyjściowych, rekompensowane przez stopniowy wzrost wydajności mleka (24% powyżej poziomu początkowego). Nastąpiło znaczące zwiększenie zawartości Lf w mleku, z maksymalną reakcją ($P<0,01$) po 2 tygodniach od zakończenia podawania preparatu oraz stopniowe zmniejszenie ($P<0,01$) SCC i CFU w stosunku do wartości wyjściowych. Jest to pierwsze doniesienie o możliwości stymulowania sekrecji Lf w mleku i ograniczenia w ten sposób zmian zapalnych w gruczole mlekowym kóz.