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Influence of *Prototheca* mastitis on milk quality.

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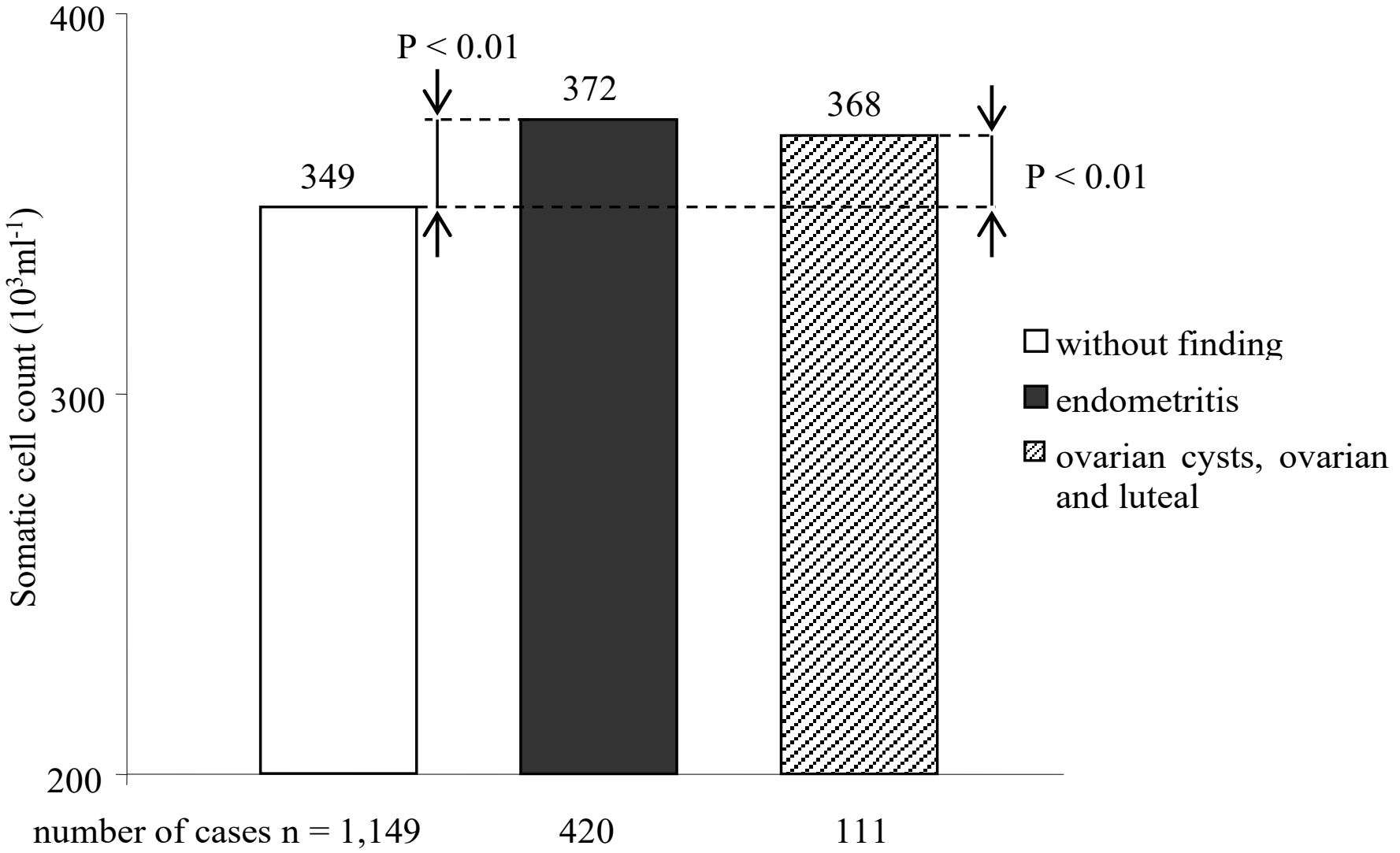
Milk secretion disorders, or mastitis, according to their degree of classification (clinical, subclinical, non-specific, latent infection, etc.), I:

- permanent problem;
- milk yield losses according to somatic cell count (SCC) up to 20%, normally 10% and milk discards 5% (for the farmer);
- milk quality losses (for the farmer, the dairy and the consumer);
- increases the risk of residues of inhibitory substances (RIS);
- increases the SCC;
- reduces the content of lactose and potassium;
- increases the concentration of salts in milk (chlorides and sodium);
- increases the milk electric conductivity;

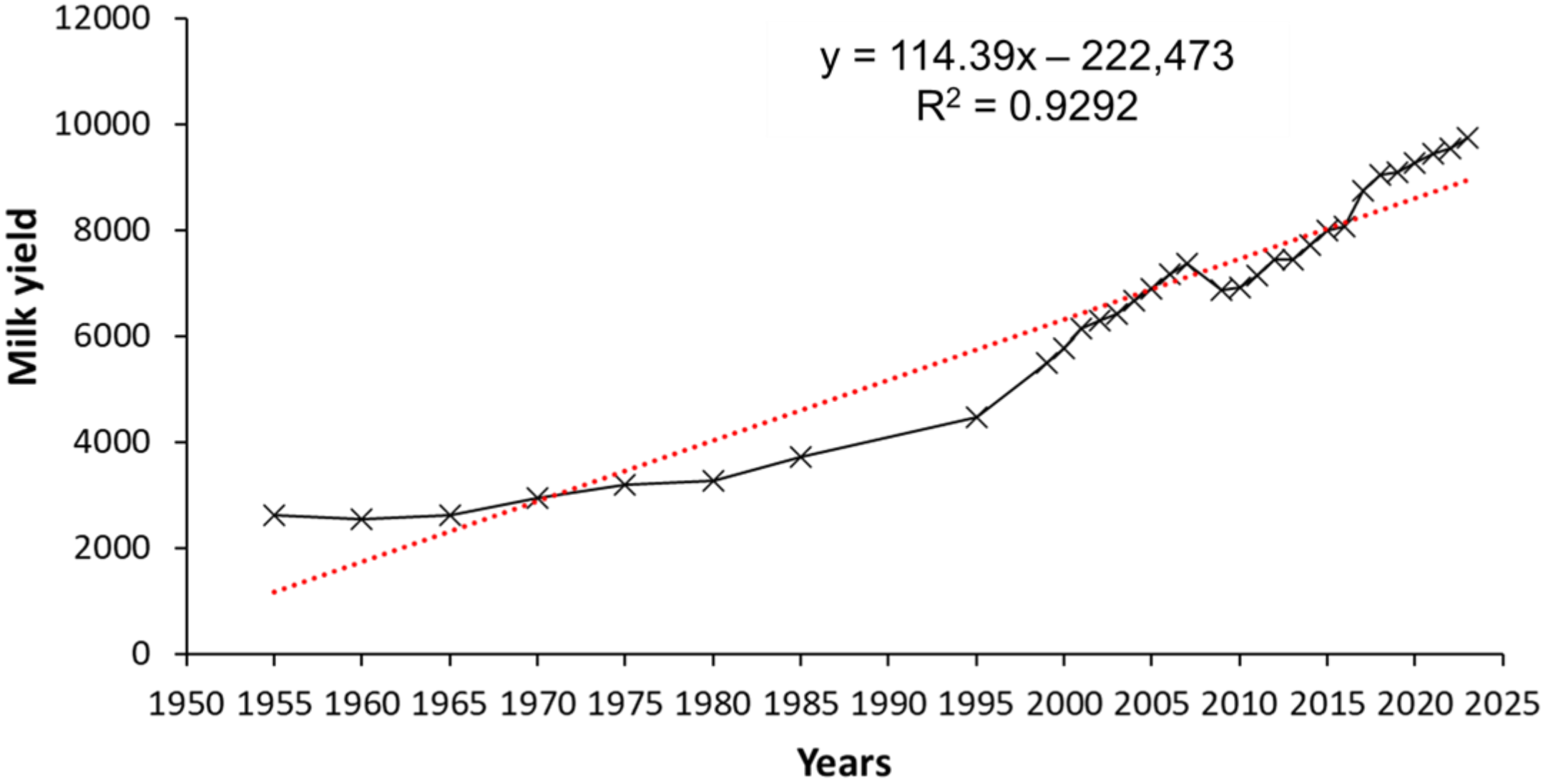
Milk secretion disorders, or mastitis, according to their degree of classification (clinical, subclinical, non-specific, latent infection, etc.), II:

- **increases the content of gammaglobulins and other antibodies;**
- **increases the content of free fatty acids (FFAs) in milk fat (threat to shelf life);**
- **worsens the milk fermentationability in terms of the production of fermented milk products;**
- **worsens the cheese-making properties of milk and cheese yield;**
- **worsens the thermostability of milk proteins for all heat treatments;**
- **only the milk freezing point can be sporadically improved by mastitis as a result of the physiological activity of the regulatory mechanisms of the mammary gland to maintain osmotic pressure and to maintain own lactation.**

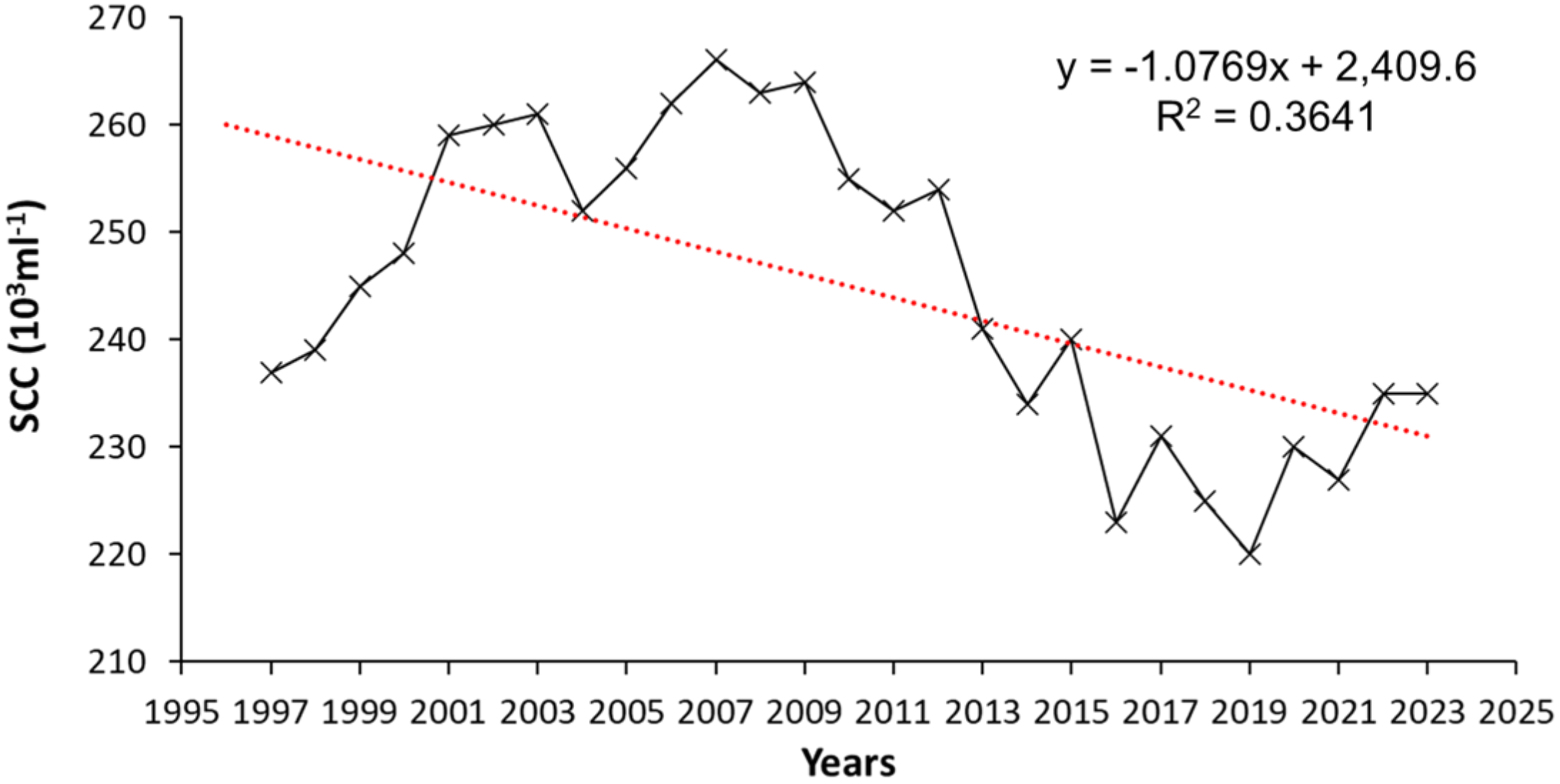
Relationship between somatic cell count (SCC) in milk in the first half of lactation and reproductive complications in cows.



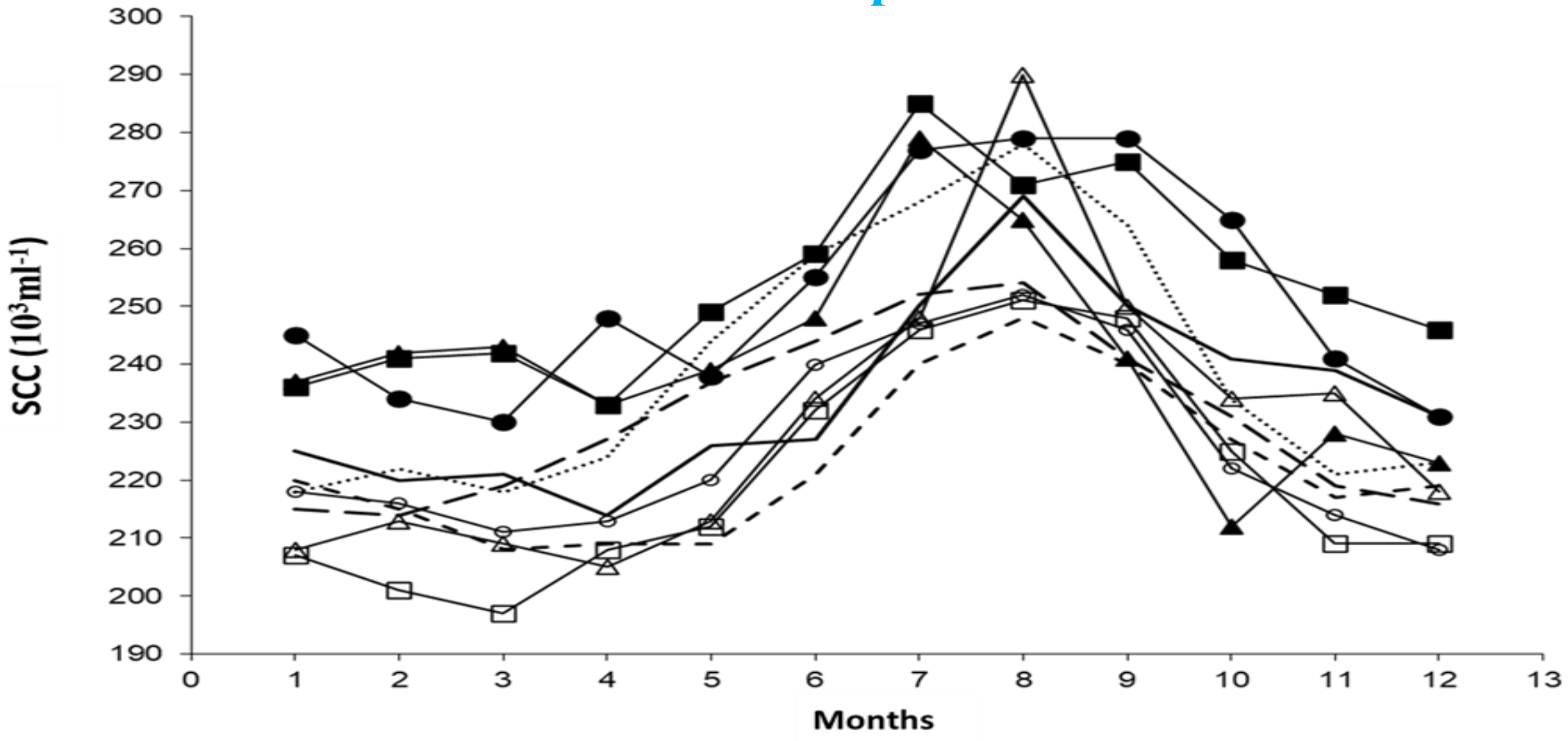
Dynamics of development of average milk yield (kg) during standard lactation in milk recording in the Czech Republic (r = 0.964; P < 0.001; 69 years).



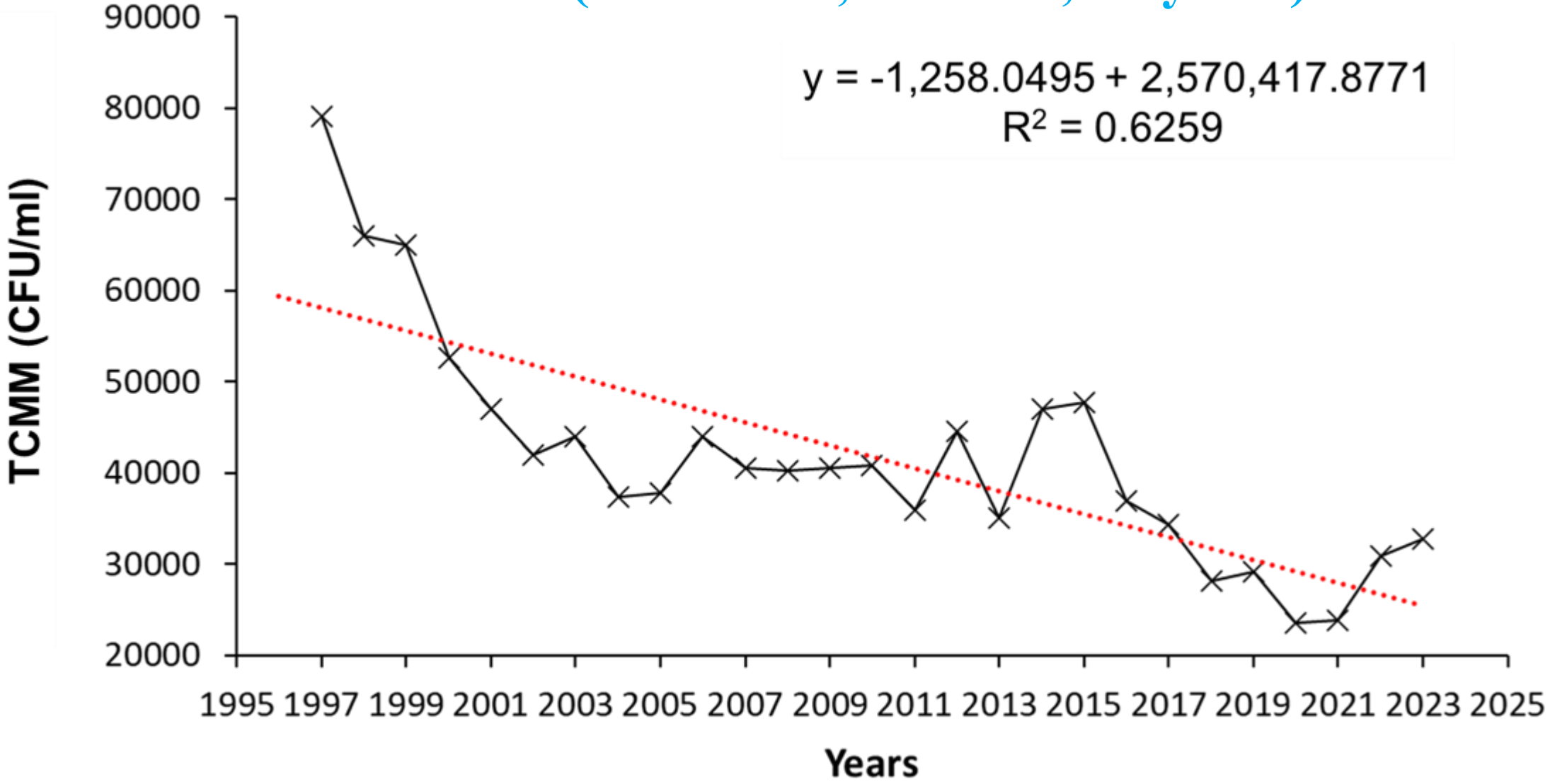
Dynamics of SCC development in supplied milk (r = -0.60; P < 0.05; 27 years, 1997 - 2023), (2011 – 2020, Sládek, 2024, ČMSCH, a.s.).



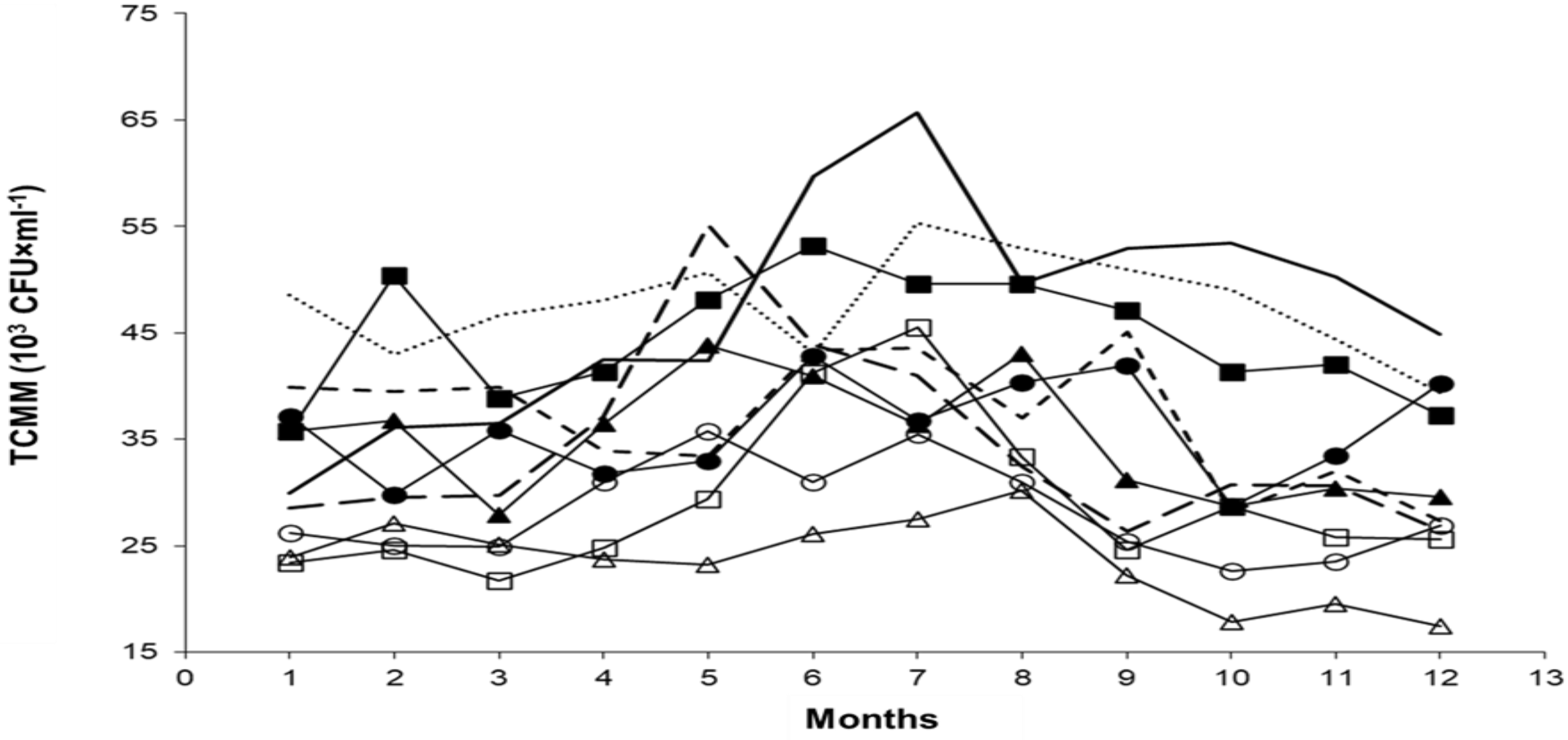
Significant recurrence of the seasonal effect in the SCC ($10^3 \times ml^{-1}$) in months during years (10 years) in raw milk quality control in the Czech Republic.



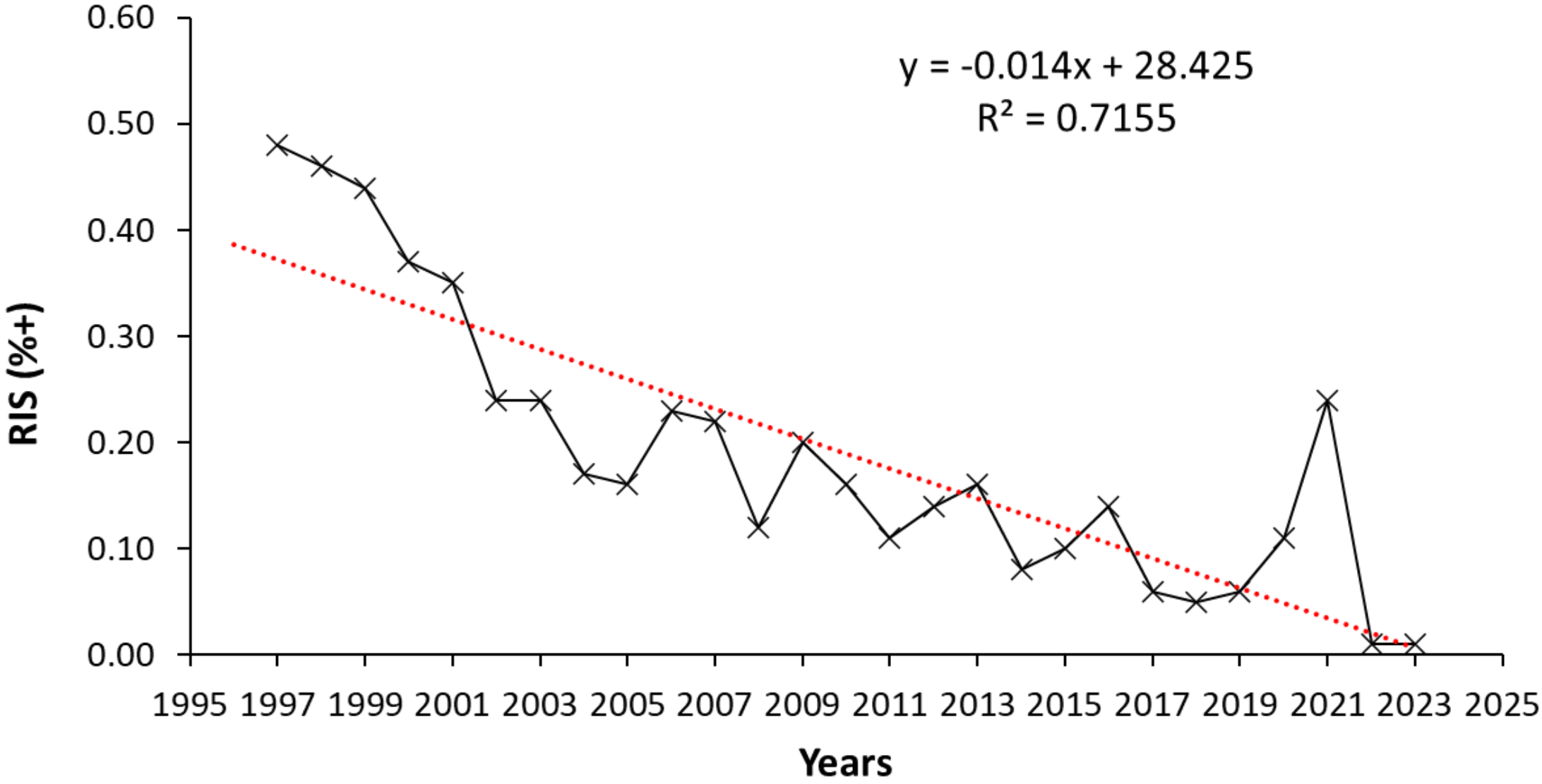
Dynamics of development of the total count of mesophilic microorganisms (TCMM, 10^3 CFU×ml^{-1}) in the delivered raw cow milk (r = −0.791; P < 0.01; 27 years).



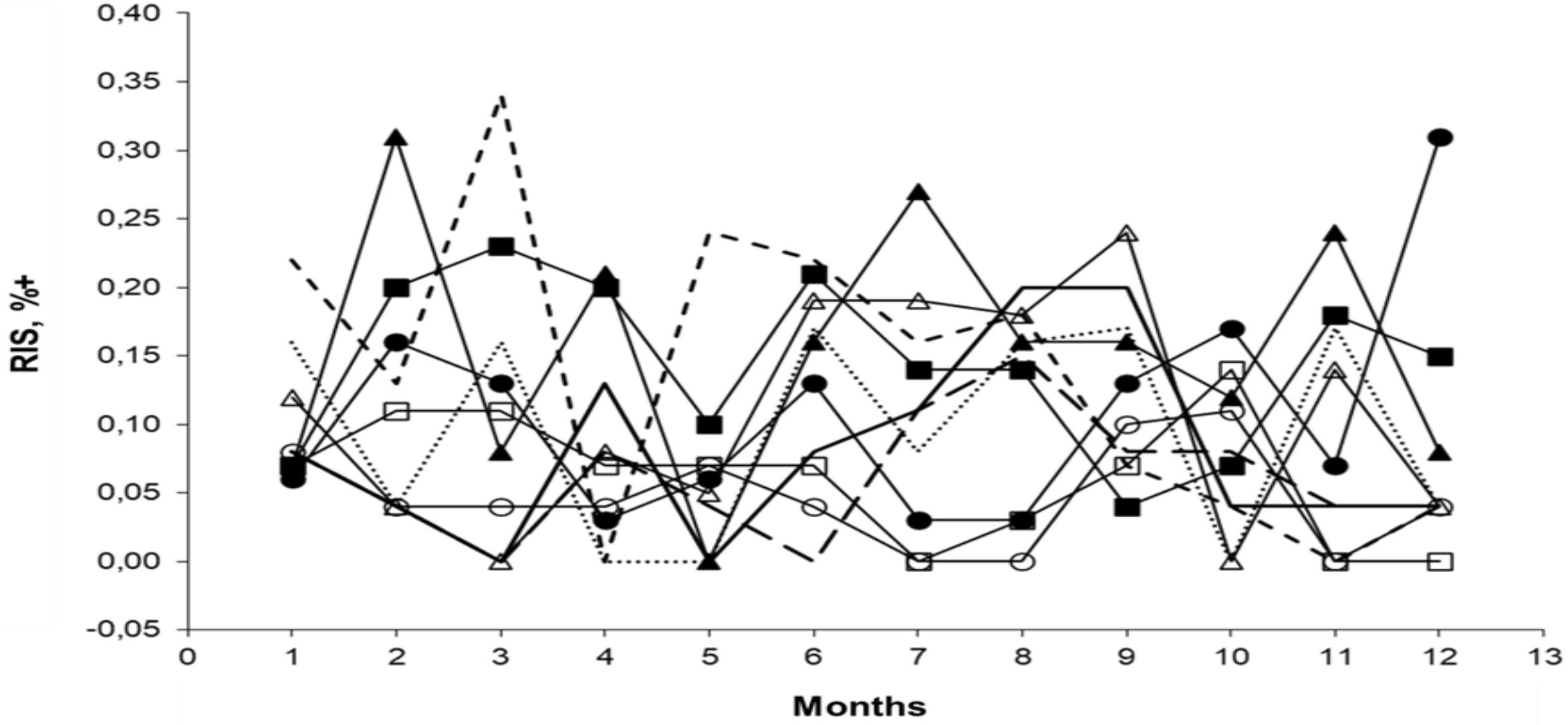
Visible repetition of the seasonal effect in the TCMM (10^3 CFU×ml^{-1}) in months by years (10 years) in the quality control of raw milk in the Czech Republic.



Dynamics of development of inhibitory substance residues (RIS, %+) in supplied raw cow milk (r = −0.846; P < 0.001; 27 years).



No repetition of the seasonal effect in the RIS (%+) in months by years (10 years) in the quality control of raw milk in the Czech Republic.



Tracking *prototheсae* (P), A.

The issue of subclinical mastitis, especially of non-bacterial origin (P), is now coming to the fore with the development of housing and milking technologies. This is especially true in warmer, subtropical and tropical countries (Argentina, Chile, Brazil, Colombia, Turkey; Zarora et al., 2011; Ely et al., 2019; Libisch et al., 2022**), however, the increasing incidence is also starting to be detected in European countries (Ukraine, Poland, Hungary, Croatia, Sweden, Italy;** Kwiecinski 2015; Morandi et al., 2016; Libisch et al., 2022**) or Japan, including the Czech Republic (**Seydlová et al., 2009, 2019, 2022; Bzdil, 2013**).**

Tracking *protothecae* (P), B.

The spread of P mastitis is probably not the result of the climatic effect of warming, but rather an increased analytical attention to the decrease in SCC in connection with other pathogens. The situation has been going on for about 15 years, yet this fact has been unjustifiably underestimated by dairy practice in our country and this problem is practically very persistent, with a significant impact on the economy of production and milk quality. Similarly, as with bacterial mastitis, this fact is related to the increase in SCC, high negative selection of supplier milk, reduced marketability and losses due to reduced milk yield on average by 10 to 20% (37% of the total loss per case of mastitis; Kvapilík et al., 2015).

Monitoring of *prototheceae* in the Czech Republic and elsewhere.

In the Czech Republic, 22,688 quarter milk samples (2008 - 2012) were examined for the presence of environmental potential agents of bovine P mastitis (green achlorophyllous algae) previously (Bzdil, 2013). 109 strains of three species of the genus Prototheca were detected (prevalence 0.48%). These were *Prototheca zopfii* (*bovis*) gen. 1 and 2, *Prototheca stagnora* and *Prototheca wickerhamii*. The highest sensitivity was found in vitro to nystatin (100%).

It was further stated (Shave et al., 2021) that knowledge of how P infections respond to treatment is insufficient and in vitro susceptibility tests are a poor predictor of the success of existing antiprototheca treatments. Notable cases of treatment failure when isolates appeared susceptible, or success when isolates appeared resistant, have been recorded.

Monitoring of *prototheceae*, Czech Republic, pilot project, A.

A pilot, area-based, multi-year monitoring of the occurrence of a non-bacterial microorganism, a unicellular non-green algae, *Prototheca bovis*, in bulk tank milk samples from the collection areas of 21 dairies was carried out in the Czech Republic. 1,051 samples were taken - typical representatives of yeast, mold and algae were diagnosed and their quantification was performed (Seydlová et al., 2022).

Of these, a total of 45 suspected isolates of *Prototheca* spp. 2019-2021 were identified by two multiplex qPCR systems for the identification of selected species of *Prototheca* (P; *P. bovis*, *P. blaschkeae*, *P. ciferrii* and *P. wickerhamii*) with internal inhibition control (Morávková et al., 2022). Susceptibility to amphotericin B (0.008-16 mg/l), nystatin (0.008-16 mg/l) and fluconazole (0.062 – 128 mg/l) was tested in 15 selected *P. bovis* isolates.

Monitoring of *prototheca*e, Czech Republic, pilot project, B.

Only 3.6% of bulk tank milk samples (BTMS) were free of non-bacterial pathogens. Yeasts were isolated in 96% and representatives of the genus *Prototheca* in 4.37%. When checking for mold, 6.66% of the samples from 961 BTMS were detected (Seydlová et al., 2022). According to molecular analysis (Morávková et al., 2022), 91% of isolates from BTMS belong to the species *P. bovis*, which is the most common cause of P mastitis in cattle. Although P infection in cattle is currently considered an incurable disease, human protothecosis is treated with surgical intervention and antifungal agents such as amphotericin or azoles, with varying effectiveness. Susceptibility testing showed: most *P. bovis* isolates had high MICs to fluconazole with MIC50 >128 mg/l, MIC50 for amphotericin was 1 mg/l and MIC50 for nystatin was 2 mg/l.

Field experiment, case study.

Samples (individual dairy cows) from the milk recording of herd CF 90% and H 10% from November 2019 were divided according to the mycological examination (VEDIA) of dairy cows positive for algae into groups *Prototheca* (positive finding, P+, n = 57) and without *Prototheca* (negative finding, P-, n = 215).

Data from the milk recording (individual samples) and experimental separate milking of groups P+/P- animals (n = 6 and 6 P+ and P-, bulk tank milk samples) were evaluated. Estimates of the effect of positivity on milk yield and milk quality were made.

Mean results, milk recording, individual milk samples.

Indicator of lactation and milk, milk recording	P+	P-	Notice (explanation, x arithmetic and xg geometric mean)
All dairy cows, n	57	215	
Mean of lactation number	3.25	2.31	more infected longer utilized animals
Days in milk	145	155	small difference, infection in maximal milk production
Milk yield, kg	23.5	25.3	significant losses, 7.1%
Fat content, %	4.06	4.17	small difference
Crude protein content, %	3.97	3.87	mastitis can cause more whey protein
Lactose monohydrate content, %	4.75	4.82	this copy the mastitis and possible damage of secretion tissues
Solids non fat content, %	9.31	9.29	small difference
Somatic cell count, $10^3 ml^{-1}$	525	100	large difference in xg and x in sense of mastitis
	840	270	

Mean results, field experiment, bulk tank milk samples, 1.

Milk indicator, experiment	P+	P-	Notice
Bulk tank sample, November, n	6	6	
Contagious pathogens	-	-	*Str. agalactiae* and *Staph. aureus*
Environmental pathogens	3 cases	-	*Str. uberis*
Residues of inhibition substances	-	-	in P+ 4 suspected cases
TCMM, CFU/ml	55,119	1,886	large difference xg and x
	248,667	2,333	
Count of algaes and yeasts	10 039	60	*Prototheca* and *Candida*, xg and x
	11 050	596	
SCC, $10^3 ml^{-1}$	777	114	large difference in xg and x in the sense of mastitis
	814	127	

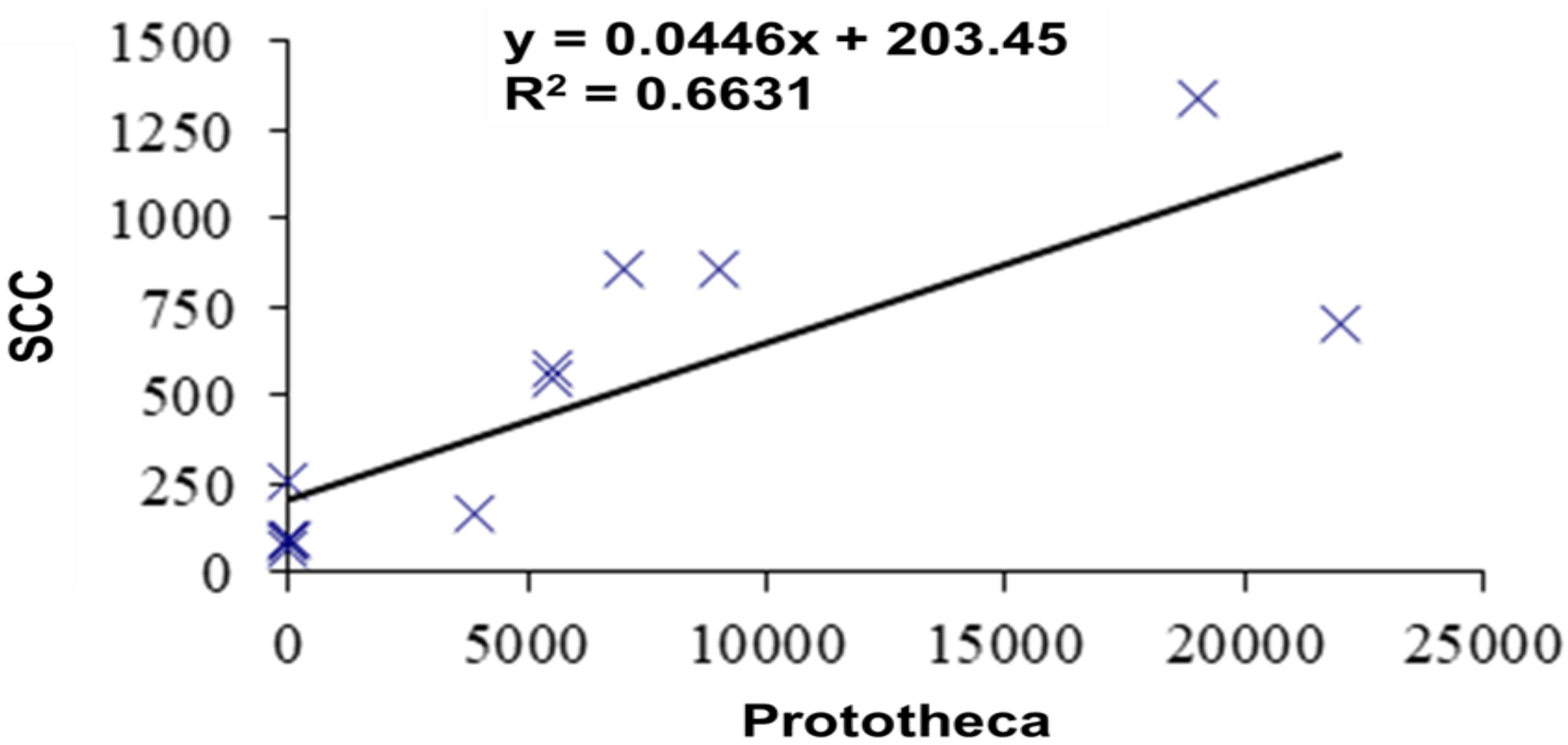
Mean results, field experiment, bulk tank milk samples, 2.

Milk indicator, experiment	P+	P-	Notice
Bulk tank sample, November, n	6	6	
Fat content, %	3.47	4.06	insignificant, in terms of mastitis pathogenesis
Crude protein content, %	3.96	4.06	insignificant
Casein content, %	3.18	3.24	in terms of mastitis pathogenesis
Lactose monohydrate content, %	4.75	4.87	insignificant, in terms of mastitis pathogenesis
Solids non fat content, %	9.32	9.53	significant, in terms of mastitis pathogenesis

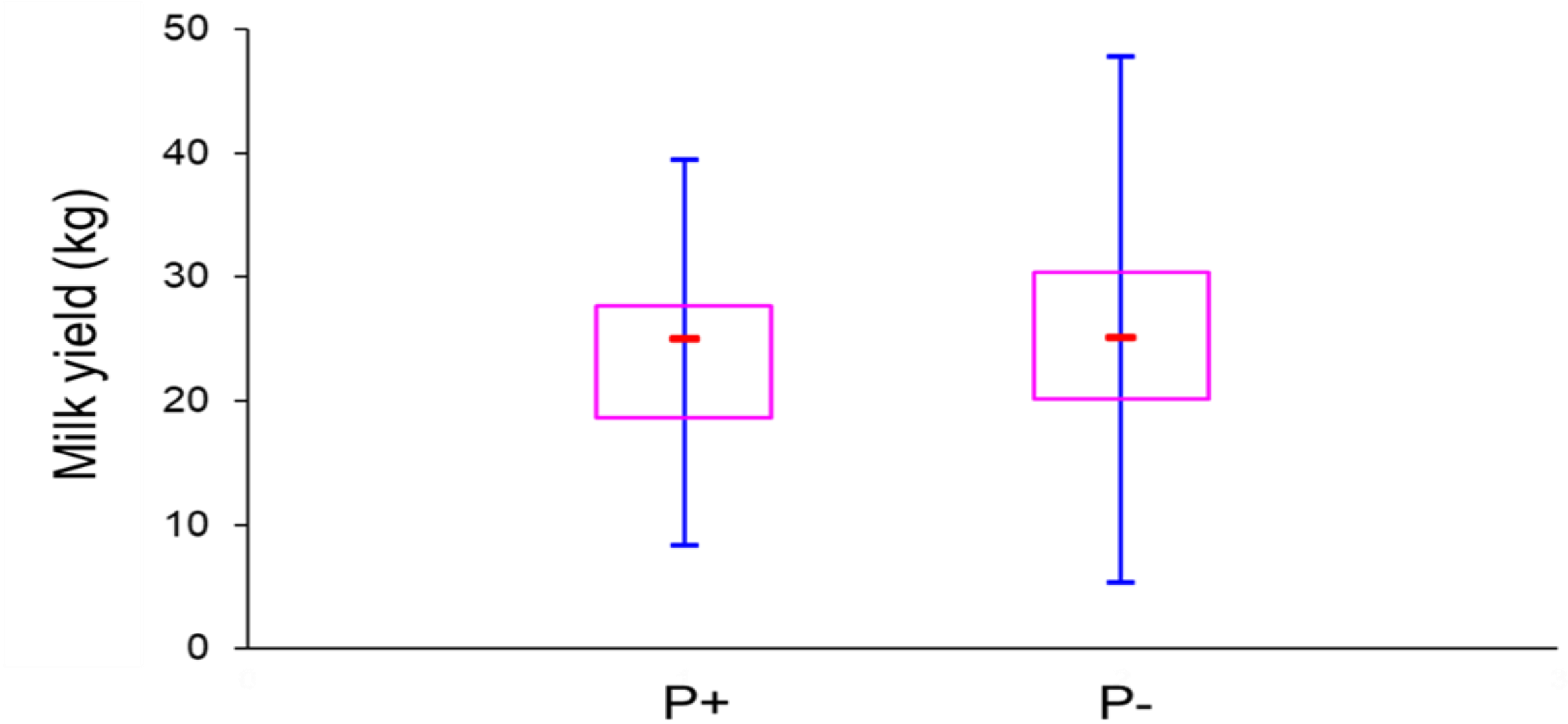
Mean results, field experiment, bulk tank milk samples, 3.

Milk indicator, experiment	P+	P-	Notice
Bulk tank sample, November, n	6	6	
Milk freezing point, °C	-0.536	-0.53	in terms of mastitis pathogenesis
Electrical conductivity, $mS.cm^{-1}$	4.31	3.98	in terms of mastitis pathogenesis
Active milk acidity, pH	6.64	6.61	buffering capacity
Free fatty acids of fat, mmol/100g	0.61	1.985	surprising, the opposite was expected
Alcohol stability of milk proteins, ml	5.05	6.2	P+ significantly worse
Titration acidity, °SH	7.14	7.9	mastitis alkalization, less protein and casein for P+
Time of enzymatic coagulation (cheeseability), seconde	55.3	60.7	coagulation of proteins by rennet, surprising
Fermentationability (yoghurt test), °SH	23.3	25.1	P+ worsening

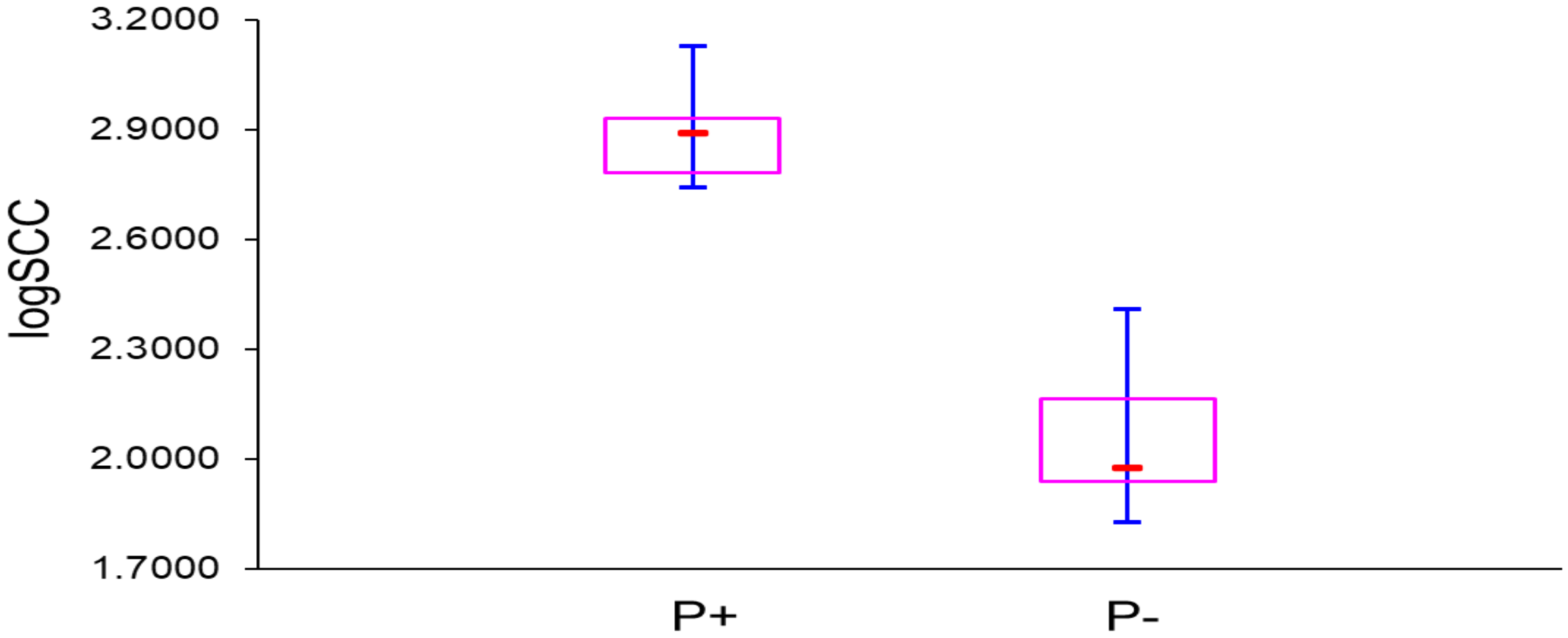
Relationship between *Prototheca* frequency (CFU/ml) and somatic cell count (10^3ml^{-1}) in bulk tank milk n = 12; r = 0.814**.



Difference in kg of milk of P+ and P- animals in milk recording (arithm. avg. 23.5 and 25.3) per day (loss 7.1%) – median, variation range, 2nd and 3rd quartile.



Difference in SCC (log SCC) in P+ and P- animals in bulk tank milk samples, n = 6 and 6, xg = 777 and 114 $10^3 ml^{-1}$ ($P < 0.001$) – median, range of variation, 2nd and 3rd quartile.



FACTS:

- **sources of contamination of animals and milk with *Prototheca* infections are practically very difficult to identify, they may be biofilms from the internal surfaces of milking equipment;**
- **treatment is relatively ineffective, often cases end with healthy culling of cows;**
- **the situation is still generally underestimated by dairy practice in the Czech Republic, although the risk increases over time, which needs to be changed;**
- **economic losses are very similar to other etiologies of mastitis, i.e., economically serious;**
- **hygiene and prevention are of considerable importance;**
- **only acidic disinfectants are effective in eradicating this microbial burden (on selected surfaces of the environment and milking equipment), alkaline ones have no effect on vitality, however, they also contribute to the elimination of biofilms.**

CONCLUSION 1:

- ***Prototheca*** **(P) infection is more common in older cows around the 3rd lactation and is more a matter of the beginning of lactation when susceptibility to mastitis in general is increased;**
- **P infection, probably in combination with other pathogens (*Str. uberis*), significantly reduces the productivity of dairy cows, about 7% and perhaps more;**
- **P infection, probably in combination with other pathogens (*Str. uberis*), significantly increases SCC, as well as electrical conductivity, while improving MFP;**
- **from the technological properties, alcohol stability is impaired in P and milk is also alkalized (lower titration acidity);**
- **another slight deterioration of technological properties in P can be detected in the sourness of milk.**

CONCLUSION 2:

It follows from the above that the effective implementation of all known effective anti-mastitis measures in herds of milked animals aimed at the gradual elimination of clinical and subclinical bacterial and non-bacterial mastitis and the reduction of the somatic cell count will increase the quality of milk and the milk yield of animals, thus improving the economics of cow rearing.

CONCLUSION 3:

The newly obtained results and knowledge leading to the analysis of the causes, sources of variability and possibilities of eradication of the negative phenomenon of non-bacterial mastitis are very important for dairy practice, with the possibility of a positive contribution to the economics of milk production and health issues in dairy farming.

Another important item are the hygienic studies of the behavior of these non-bacterial pathogens in technological biofilms, which allow for effective modification of the necessary sanitation of the technology to eliminate the spread of these pathogens, therefore it is necessary to continue these studies as effectively as possible.

NON-BACTERIAL MASTITIS IN DAIRY FARM - ABSTRACT.

Milk secretion disorders are consistently one of the main sources of losses in milk production and quality. At the same time, the influence of non-bacterial mastitis algae *Prototheca bovis* (P) is increasing. P was isolated in 4.37% of bulk tank milk samples in the Czech Republic. The prevalence of P+ dairy cows in the problem herd was determined to be 26.5%. There were ($P < 0.01$) correlations (0.81 to 0.86) between the somatic cell count (SCC) and the P count. Higher SCC ($P < 0.001$) were in P+ (xg 777 vs. 114 $10^3 ml^{-1}$). A decrease in milk yield in P+ animals by 7.1% was determined. The total number of microorganisms was lower in P- (2.3×10^3 vs. 2.5×10^5 CFU/ml; $P < 0.01$). P+ had a better milk freezing point (-0.536 vs. -0.530 °C; $P < 0.01$), which is related to a higher content of osmotically active ions due to mastitis. This was confirmed by a higher electrical conductivity in P+ (4.31 vs. 3.98 $mS.cm^{-1}$; $P < 0.01$) and a lower titration acidity (7.14 vs. 7.9 °SH; $P < 0.01$). Treatment of P with antibiotics is usually ineffective. The only way to eliminate mastitis in P is prevention with good hygiene of rearing and milking technology.

„Thank you for your attention"