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Original article

The presence of anti-*Hypoderma* antibodies in udder milk samples, and correlation with selected parameters of dairy performance

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Abstract

Hypodermosis is responsible for significant economic losses and remains a current problem in animal husbandry. This study determined the extent of *Hypoderma* spp. invasion in north-eastern Poland in dairy herds. The extent of the invasion, determined on the basis of positive results of an ELISA assay (Bovine Hypodermosis Milk Screening of Institut Pourquier), varied in herds. Statistical analysis performed on the results of milk productivity and content of basic components revealed a decrease in the milk yield and changes in milk protein in animals revealing positive, doubtful and negative results. Statistically significant differences were absent as regards the content of milk fat or milk total solids. The milk yield and the content of particular milk components were determined using the AT4 method, as part of a control program carried out by the Polish Federation of Cattle Breeders and Dairy Farmers (PFHBiPM).

Key words: ELISA, hypodermosis, dairy cows, milk yield, milk protein

Introduction

Bovine hypodermosis is a parasitic disease caused by the larvae of insects of the *Hypoderma bovis* and *Hypoderma lineatum* species (Diptera, Oesttidae). Diptera invasions pose a serious problem in dairy cattle living in the northern hemisphere (Haine et al. 2004, Otranto et al. 2005, Karatepe and Karatepe 2008). Since the mid-20th century, hypodermosis control programmes have been developed and implemented in many regions and countries of Europe, numerous countries recorded the effectiveness of the pro-

gramme, including: the Czech Republic, Denmark, France, Holland, Ireland, Germany, Switzerland and Great Britain (Boulard 2002). In Poland, research carried out by researchers of the National Veterinary Research Institute in Puławy found that 14 years after the end of the warble fly control campaign, the occurrence of hypodermosis reached the level present before the campaign (Ziomko and Cencek 1994). The disease causes significant economic losses. The direct results of hypodermosis involve reduction of growth and milk production, which is the effect of the activity of adult forms of warble flies as well as of their larvae

in the animal body. Indirect effects result from the activity of migrating larvae, which cause a reduction of slaughter and technological value of meat, skin damages and reduction of milking capacity in cows (Boulard 2002, Khan et al. 2006, Karatepe and Karatepe 2008, Cicek et al. 2011). Many countries have calculated the losses resulting from the occurrence of hypodermosis. In Italy, annual losses have amounted to approximately \$11.5 m, in the USA about \$600 m and in Canada around \$14 m (Hassan et al. 2010). One of the above-mentioned adverse effects of hypodermosis in dairy cattle herds is the reduction of milk yield. Advances in diagnostics and application of methods based on detecting anti-*Hypoderma* antibodies have enabled detection of animal infestation at an early stage. Current assessment of cow performance in herds facilitates control of the yield and content of milk components. The possibility of detecting the presence of anti-*Hypoderma* antibodies and constant control of milking capacity of cattle became the basis for carrying out the research.

The major objective of this study was the comparison of the milk yield and the content of basic nutritional components in the milk of seropositive, doubtful and seronegative animals. The paper also determines the extent of warble fly invasion in dairy cattle herds.

Materials and Methods

Selecting dairy cattle herds for serological examinations

Five dairy herds of various sizes from the area of north-eastern Poland were selected for examination purposes. Selected herds were composed of the following numbers of animals: herd B – 201, G – 44, K – 33, S – 43, and W – 84 animals. Herd K was kept in the indoors system, while the other herds were grazed on pastures. All herds were subject to a dairy cattle performance assessment carried out by the Polish Federation of Cattle Breeders and Dairy Farmers (PFHBiPM).

Collecting and storing milk samples

The material for serological examination were milk samples. The samples were collected in February 2011 from all cows in the period of lactation, according to applicable good practice principles. Altogether 341 samples of milk were collected from Polish Holstein-Friesian cows younger than 8 year (from herd B – 159, G – 41, K – 31, S – 34 and from herd W – 76).

After transporting them to the laboratory the samples were centrifuged (12.100 x g), and then frozen and stored for maximum two months at -20°C.

Serological examination

Udder milk samples, after defrosting at room temperature, were examined with the use of an ELISA assay (ELISA Bovine Hypodermosis Milk Screening, Institut Pourquier, Montpellier, France). The serological examination was performed according to the manufacturer's instruction. A sample was considered positive if the S/P ratio (sample-to-positive control) was higher than or equal to 115%, a negative – if the S/P ratio was lower than 85% and a S/P ratio between 115% and 85% was considered to be doubtful.

Assessment of the milk yield and the content of basic milk components

The milk yield of animals and the content of individual milk components, i.e. protein, fat and total solid, were determined using the AT4 method, pursuant to the decision of the EU Commission (Commission Decision No 2006/427/EC of 20 June 2006). The parameters were calculated for 305 day of milking period. Parameters were determined in the PFHBiPM Laboratory.

Statistical analysis

The extent of the invasion in herds was calculated on the basis of positive results of the serological assay and was expressed as a percentage value.

The obtained results of the serological examination of milk samples and values of milk yields and the content of the selected milk components were subject to statistical analysis with the application of the Kruskal-Wallis test. The analysis was carried out using STATISTICA 9.0 (StatSoft Inc., USA).

Results

The mean extent of *Hypoderma* spp. invasion, determined by a serological assay of milk samples, was 28.2%. The extent in individual herds varied and amounted to, respectively, in herds B – 3.1%, G – 51.2%, K – 9.7%, S – 58.8%, and W – 18.4%. A comparison of the milk yield and the content of fat, protein and total solids in milk of cows revealing

Table 1. Mean values of the productivity and the content of selected nutritional components in cow's milk, depending on the results of the serological assay. N – number of samples, Mean – mean value, STDM – standard deviation of the mean value.

Hypoderma	N	Yield [kg]		Fat [%]		Protein [%]		Total solids [%]	
		Mean	STDM	Mean	STDM	Mean	STDM	Mean	STDM
Total	341	6485	172	4.52	0.04	3.35	0.02	13.35	0.04
Positive	63	5730	388	4.61	0.10	3.25	0.04	13.34	0.10
Negative	272	6625	194	4.50	0.04	3.38	0.02	13.35	0.05
Doubtful	6	8097	947	4.51	0.25	3.47	0.08	13.42	0.38

seropositive, doubtful and seronegative results is presented in Fig. 1. Statistically significant differences were found only between the mean values of milk productivity and the content of milk protein of seropositive and doubtful animals. The probability values amounted to $p = 0.0346$ and $p = 0.0467$, respectively. On the basis of the values of descriptive statistics presented in Table 1, it can be stated that the mean decrease of milk production in cows with a positive result of serological assay was about 14% and a decrease in the milk protein originating from seropositive animals was about 4%. No statistically significant differences were found as regards the content of milk fat and milk total solids.

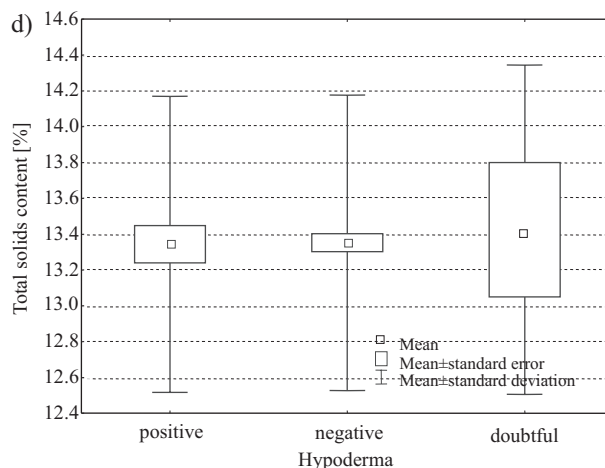
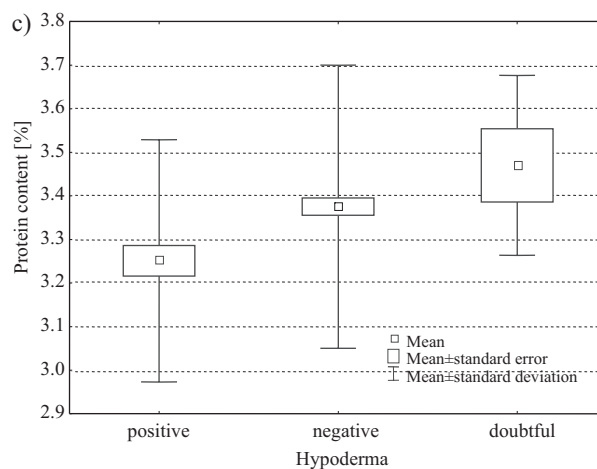
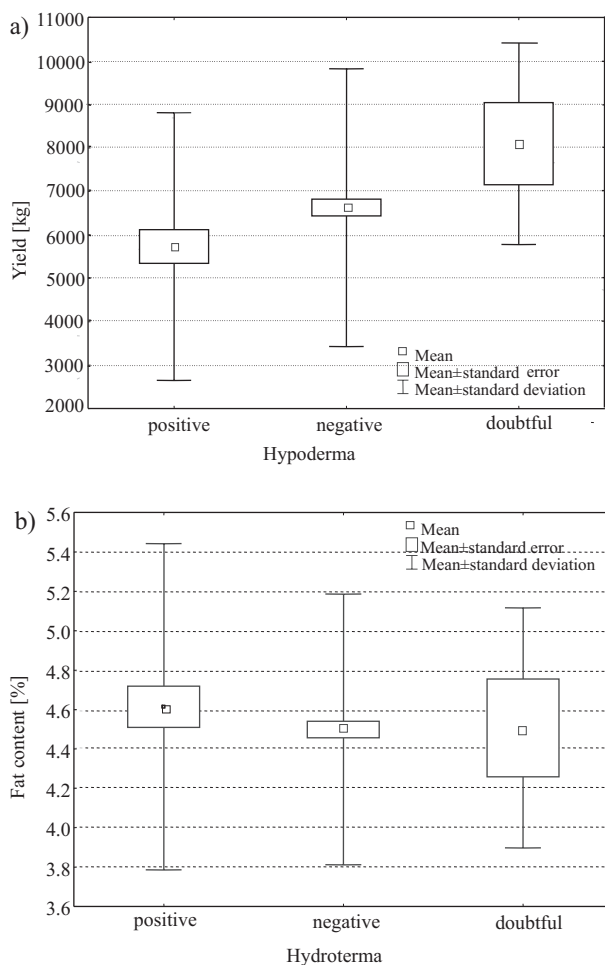


Fig. 1. Interrelations between milk yield (a); and the content of fat (b); protein (c); total solids (d), and the result of serological assay of cow milk samples towards *Hypoderma* spp. $P < 0.05$ indicate statistically significant difference.

Despite the lack of statistically significant differences, an increase in the milk yield and the content of protein was observed in milk of cows showing doubtful reactions as compared to the productivity and the content of protein in the milk of seronegative and seropositive animals.

Discussion

The prevalence of bovine hypodermosis caused by infestation of larvae of insects of the *Hypoderma bovis* and *Hypoderma lineatum* species is the reason for which the studies on the extent of the invasion, particularly in the areas where the climate favours the development of those parasites, are still of current interest. Examinations carried out at the end of the 20th century in cattle herds in the area of north-eastern Poland revealed the occurrence of hypodermosis in 25 to 100% of animals (Cencek and Ziomko 2001). The results obtained in the research presented in this paper show a reduction in the extent of *Hypoderma* spp. invasion in this area. There may be several causes for reducing the extent of the invasion in dairy cattle herds. One of them is a significant improvement of animal breeding conditions and their adjustment to meet hygienic requirements for milk producing farms (Regulation 852/2004, Annex I point II 4 f). According to legal regulations, the places where milk is obtained (thus those in which cows stay) are to be protected against access of pests. Numerous authors point to a relationship between the weather and the presence of free-living stadium (Haine et al. 2004, Karatepe and Karapete 2008). Mature insects need sunny, windless weather and temperatures above 18°C to take a mating flight and lay eggs (Cencek and Ziomko 2001). The occurrence of such conditions – and they actually occurred in the summer of 2010 – affects the time when subsequent developmental stages of those parasites emerge. The differences in the extent of the invasion can also be explained by the method of herd management. Most herds in north-eastern Poland are kept on pasture during the summer and some of them in an indoor system. The cattle that do not graze on pastures are less exposed to infestation by larvae. Herd K was kept in the indoors system, while the other herds had access to pasture in summer.

Many other factors affecting the extent of *Hypoderma* spp. invasion have been described, including: origin of animals, local breeds or hybrids, gender or age (Kara et al. 2005, Simsek et al. 2008, Cicek et al. 2011). It was also found that the cattle infested with warble flies in one season were less susceptible to invasion in the next season (Evstaf'ev 1980, Gingrich 1980).

The occurrence of animal hypodermosis caused by larvae of insects of *Hypoderma bovis* and *Hypoderma lineatum* species is related to economic losses. They affect not only the breeders, but also the meat and dairy industry. The losses in the meat industry are related to damages caused by movement of L1 larvae in the host tissues. Canals etched with proteolytic en-

zymes of larvae are clearly visible during the post-slaughter examination and the changed tissues are considered non-edible. Digestive enzymes of larvae affect the immunological system, the consequence of which can be secondary bacterial, viral and parasitic infection (Chabaudie and Boulard 1993).

In countries where cattle breeding accounts for a significant share in the national economy losses caused by hypodermosis constitute a serious problem. In many countries, in the past, losses incurred for this reason were estimated (Boulard 2002, Khan et al. 2006, Karatepe and Karatepe 2008, Hassan et al. 2010, Cicek et al. 2011). They were assessed on the basis of the skin changes, post-slaughter changes and a decrease in milk productivity. In Poland, dairy cattle breeding conditions have significantly improved since the country joined the European Union and the performance of dairy cows has increased. In reports prepared by the Institute of Agricultural and Food Economics the average productivity of cows in 2004 was 4140 liters, while in 2010 it increased to 4674 liters (Seremak-Bulge 2011). In the research presented here, it was demonstrated that cows in which positive serological reactions were found reduced their yield by 14%, i.e. by 681.8 kg of milk a year. Losses caused by a reduction in milk capacity in farms resulting from this were highly variable. On farms where the extent of the invasion was the lowest (3.1%) the resulting losses amounted to 3,409 kg of milk a year. On farms where the extent was the highest (58.85%) losses amounted to 13,636 kg of milk. In June 2010, in the province of Warmia and Mazury, in the area where the research was conducted, the dairy cattle stock consisted of 187,372 animals (Safacki 2010). Losses at the provincial level, taking into account the calculated mean extent, amounted to about 1,277,502 kg.

Economic losses of farmers are deepened by a reduced protein content (Fig. 1c) which is one of the factors affecting the price of milk. A reduction in the content of milk protein lowers its technological suitability and the quality of the final products. However, it is difficult to estimate losses resulting from this because of the different levels of extra payment of applied by dairy plants for the content of protein.

Statistical analyses revealed differences in the milk yield of seronegative and doubtful cows (Fig. 1a and 1c). The milk production of animals with doubtful reactions towards hypodermosis was higher than for animals which had no antibodies found in milk samples. A similar relation was observed for the milk protein, although those differences were not statistically significant. Perhaps the reason for the lack of significance of differences lies in the low number of animals demonstrating indeterminate reactions. An

explanation of this phenomenon requires carrying out examinations on a larger group of animals.

To summarize the results of the study, it can be claimed that the extent of *Hypoderma* spp. invasion in dairy cattle herds in north-eastern Poland was diversified and the milk yield of cows revealing seropositive reactions was significantly reduced. A decrease in milk productivity was accompanied by the reduction of milk protein content in seropositive animals. The content of other components, i.e. fat and total solids, did not reveal any statistically significant differences between milk of seropositive and seronegative cows.

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