ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS
SBORNÍK MENDELOVY ZEMĚDĚLSKÉ A LESNICKÉ UNIVERZITY V BRNĚ

Ročník LIV 15 Číslo 1, 2006

ACTIVITY AND ACTION SCREENING OF SELECTED DISINFECTANTS

K. Balharová, K. Šustová

Received: June 6, 2005

Abstract

BALHAROVÁ, K., ŠUSTOVÁ, K.: *Activity and action screening of selected disinfectants.* Acta univ. agric. et silvic. Mendel. Brun., 2006, LIV, No. 1, pp. 157–164

This research work is aimed to monitoring of selected disinfectants´activity in operational conditions. Hereby there have been monitored two acidic disinfectants Despon K and Mikasan D, which have had--by their producer-stated different recommended concentration. These solutions were monitored in viewpoint of their activity at different temperature, time of circulation, pH and water hardness. In this work there were measured pH of solutions in unloaded medium to be compared with pH of solutions in loaded medium and this measuring was carried out regularly each week within a one month period. During this period there was also monitored total plate count (TPC), which was stated in the dairy, where samples were taken two-times monthly. It has been found, that the disinfectants Mikasan D and Mikal 94D are effective even by high water hardness.

disinfection, sanitation, cleaning

Very important precondition for securing of economic results in milk-cattle breeding is production of high quality milk, delivered to further dairy procession. To acquire milk with a high microbiological quality it is necessary to disinfect milking equipment. Disinfecting is a part of complete file of measures which liquidates undesirable micro-organisms and impurities. Moreover there is need to observe strict hygienic principles to avoid contamination of milk. Importance of disinfecting should be accepted in its whole range and should become the main interest of a breeder.

Cleaning is a process during heterogeneous material (such as salts, organic material, micro-organisms) are being removed from the equipment which. During disinfecting number of pathogenic micro-organisms (ANONYM 2, 2003) is being reduced. Especially in large herds of milk-cows milking machines should be cleaned after each milking in order to achive the lowest possible pollution of milking machine and consequently also micro-biological loading of milk including transfer of micro-organisms (DOLEŽAL, 2003).

Sanitation of milking equipment aims to achieve micro-biological purity. If a milking equipment is not cleaned up or if it is cleaned insufficiently this will be reflected in an enormous growth of bacteria and contamination of milk streaming through the milking equipment. Chemical cleaners used in sanitation of milking equipment can be divided into detergents and disinfectants (KIS, 2001). Detergents not only help in removing of substances´ impurities and sediments of organic and inorganic origin, but they are able to keep these impurities in washing solution circulating in the equipment. Detergents can be divided into the acidic and the alkaline ones. Alkaline group removes milk fat, proteins and fatty residues. Acidic detergents remove milk scale, minerals and sediments caused by hard water. To keep the milking equipment clean it is unavoidable to apply regularly both of the detergent groups. They must not be mixed when applied

(BELL, GALLAGHER; 1999 a). Oftentimes there is used alternating method of washing, when primary alkaline detergent for cleaning of milking equipment is used in the morning and secondarily – in the evening - acidic detergent is used. Disinfectants serve for suppressing of undesired micro-organisms´ occurrence and they are most frequently based on chlorine (SEYDLOVÁ, SEYDL; 1997). Alkaline cleaning detergents are composed of several chemicals. Each component has its specific result.

The most frequent substances contained in these detergents are nitric acid or phosphoric acid. Nitric acid is more effectual but it indeces the higher corrosion in milking equipment (TYBOR, GILSON; 1989). Hydrogen peroxide is active against lipophile virus and bacteria, it does not effect however against hydrophilic virus and spores, if it is not combined with per-acetic acid. It is a strong oxidant and it reacts very fast. It can cause corrosion with some metals e.g. with aluminium, brass, cooper and zinc. During cleaning there are most important temperature, concentration, time of circulation and effectiveness of detergents (ANONYM 3, 2003). In the opinion of SCHWARZ (1993) alternating of alkaline and acidic disinfectants is also a very important ecological point of view and it brings energy saving and can reduce the waste water.

Temperature of disinfectants is a very important factor during sanitation of milking plant and it influences their effectiveness. With increasing of solution temperature from 40 °C to 80 °C also impacts of their action are markedly higher. Cleaning detergents applied during lower temperature than 40 °C are not acting so effectively as during higher values of temperature (OLKONEN, HENNO; 1998).

In his experiments ORDOLFF (1992) find out accordance with the relationship between the cleaning time and the bacterial contamination that the removing of the milk residues (by the rinsing or by the total cleaning and disingecting) can be postponed within 60 mintes. On the other side EDMONDSON (2002) claims, that a milking system should be cleaned up immediately after stopping of milking, when the milking equipment remains still warm and the milk residues are not still starting their sedimentation. Rinsing cycle should remove 90–95% of resting milk and all galactose from the system. Resting residues are removed during washing with a chemical agent. Temperature of rinsing water should be in the range of 38–43 °C.

Accordingly to HERRERO et al. (2002) water is a key factor in dairy industry and it should be regularly checked. Therefore its microbiological and chemical purity and hardness must be watched and also eventual contamination, that could decrease quality of milk, must be prevented. Sediments in water could facilitate growth of bacteria and sedimentation on milking equipment surfaces.

Minerals and salts as e.g. calcium and magnesium create high hardness of water. Hard water reacts with detergents and it can bring along insufficient cleaning of system. This causes increasing of bacteria quantity in milking equipment (BELL, GALLAGHER; 1999 b). Too hard water can cause damaging of heating plant and milking tubing. Therefore regions with hard water should use disinfectants and cleaning agents specially developed for these cases (ANONYM 1, 2000).

Accordingly to MILLER (1996) each breeds should choose cleaning methods in accordance with the type of milking equipment, energy costs and their medium requirements. Suitable using of cleaning and disinfecting solutions does not bring any risks of milk contamination (LINDERER, GUTHY; 1994). The quality of all workong surfaces of the milking equipment which come in the contact with the milk during the milking is the conclusive factor. There is posed the claim for the quality of the teatcups, which – as the only ones – come in the contact with the living organisms and milk. The work medium (milk, animal fats, disinfectants and cleaning agents, mechanical loading during pulsation etc.) causes the deterioration of the rubber mechanical properties and the quality of the teatcup internal surface. Moreover the teatcups are exposed during the milking process to the complicated mechanical, physical and chemical stress which causes the ageing. Nowadays there aren´t any suitable testing aparature determining the teatcups long-life. There exist just facilities, by means of which only concrete functional property can be watched (MAŠKOVÁ, 2002 a, b). Extraordinarily harmful influence has a long-time treatment of disinfectants, which very negatively influence rubber material of teatcups. (JANDÍK, KLEPAL, BENEŠ; 1995).

This work aims to verify effectivity and functioning of selected disinfectants in region with a very high hardness of water.

MATERIAL AND METHODS

The measuring was caried out there in the bredd with the dimensioned milking house 2×8 (dairy cows). Washing of milking equipment is secured by washing cleaning solutions. Automatic machine enables setting up of water doses and setting up of milking equipment sanitation time. Water and disinfectants were mixed together in an opened disinfection tank, from the samples (*1 Litre*) for the pH value determination were taken which.

In this breed main cleaning and sanitation were carried out after each milking. First of all rinsing by cold water at the temperature ±30 °C taking 5 minutes is carried out, then follows c/a 10 minutes sanitation and then five-minutes rinsing by water with the temperature ±20 °C. Total time of cleaning is 20 minutes.

For sanitation of milking equipment in this milking house were used 0.5% alkaline solution Despon A (based on sodium hypochlorite and sodium hydroxide), acid 0.25% solution Despon K (*based on orthophosphoric acid*) for the first measuring during October and November and for the second measuring 0.5% alkaline solution Mikal 94 D (*based on sodium hypochlorite and sodium hydroxide*) and acid solution 0.5% Mikasan D (*based on hydrogen peroxide, sulfuric acid and phosphoric acid*) during February. Time of sanitation was 10 minutes at temperatures on input ±40 °C. Cleaning agents and disinfectants were alternated, in the morning acid agents were applied and after the evening milking alkaline solutions were used. Before starting of regular monitoring of sanitation effectiveness there was carried out general sanitation by disinfectants 5% Mika General A (*based on potassium hydroxide and sodium hydroxide*) and 5% Mika General K (*based on sulfuric acid and phosphoric acid*). At the first use of disinfectants there was always probed pH of acid solution in unloaded medium, that means immediately after general cleaning and they were re-used after evening milking. So in the first day Despon K and Mikasan D were used twice consequently. Then there was measured pH of acid disinfecting solutions in loaded medium – always after morning milking. pH of solutions was measured by means of a portable pH meter WTW pH 320, which was calibrated before each measuring.

Total hardness of water was stated by means of a complete-metric method of titrimetry with Titriplex III towards mixed indicator. This concerns titrimetry where free ions of calcium and magnesium are bound onto indicator, by means of Titriplex addition indicator is released and appears change of colouring from the red into the grey-green. Into a vessel there was taken out 5 ml of water, which is used for sanitation and cleaning of milking equipment, then there were added three drops of indicating solution and they both were carefully mixed together. By constant mixing there was added tittering agent till the solution became green. 1 drop of tittering agent = 1 °d. This methology is according to company MERCK KgaA, Darmstadt, Germany. Total water hardness was stated at 31 °d.

Values of total plate count (TPC) were stated– acc. to the standard CSN ISO 4833 – General rules for stating of TPC, in the diary where the samples were taken twice a month.

DISCUSSION AND RESULTS

The first sample for determination of pH was taken before the very cleaning process. The second value of pH was influenced by circulation, when the solution had been diluted by water which remained in tubing and in this way the temperature was lowered and pH was increased, in some cases even by one whole number. In the unloaded medium 0.5 % Mikasan D showed values of pH ranged from 2.54 to 2.71, which differed just by hundredths, as you can see from the Fig. 1, that can not be considered to be a reasonable change. In other cases pH did not drop down under the limit 2.52, this was noted just in only one case and namely it was the value measured up as the last one (Tab. I). From the Table I it is legible that during sanitation pH of solutions is increasing, namely during falling temperature. That´s why last measured values are higher than the values noted at the start of measuring. This is however invalid in case when the temperature moves between the values 25 °C and 26 °C, when pH is lower than initial values – this is well evident from the Table I.

The second used agent was 0.25% Despon K, where there were measured less values, because stabilising on one concrete value took a long time. This solution in unloaded medium showed values of pH from 2.88 to 2.98, and the highest value was not measured by the highest temperature. In this measuring no changes in whole numbers were discovered, but only in the decimal positions, which is evident from the Fig. 2. In remaining measuring of Despon K solutions were changed even in whole numbers, ─ see the Table II. and here it is evident, that the pH values were lowered with dropping-down temperature. This effect can be caused by a worse quality of water as this problem with water was actual at the breed at that time. Values shown in the Table I. and in the Table II. Are measured in loaded medium

Water could contain more quantity of agents with those the substance in solution reacted. Probably in this way there were created salts which caused larger acidulation. This is also confirmed by BELL and GALLAGHER (1999 b), that hard water reacts with detergents and this results in insufficient cleaning of system. Therefore water should be regularly checked--up. This fact has been, like it has been referred by HERRERO et al. (2002). Despon K is used in concentration of 0.25%, as it contains more components than Mikasan D.

Deteriorated quality of water was projected into total number of microorganisms (TPC) which in November exceeded permissible hygienic limit 100 × 103 CFU × ml^{-1}, as is evident in the Fig. 3. In other months this value was exceeded. According to the values TPS it can be concluded the used agents acted effectively.

In breed there there is used alternating method of washing, in the morning they use acid agent and in the evening the alkaline one, as has been recommended by SEYDLOVÁ and SEYDL (1997). SCHWARZ (1993) emphasises, that this alternating is also an im-

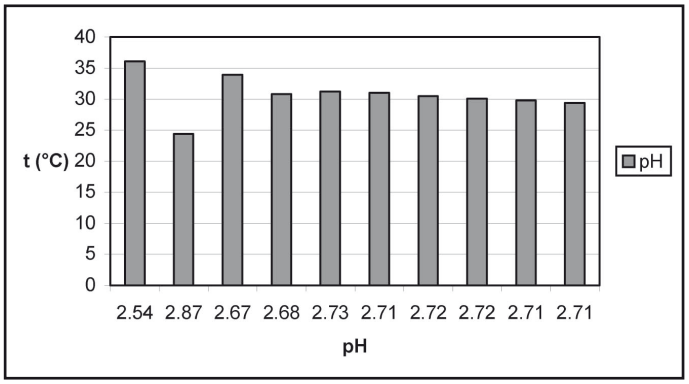
portant ecological aspect, as it brings saving of energy and reduction of waste water.

Temperatures during cleaning are moving between 30–40 °C, in some cases they are even lower. Accordingly to OLKONEN and HENNO (1998) with increasing temperature there are substantially increased impacts of solution and detergents´ effects while detergents applied by the temperature lower than 40 °C are not acting so effectively like by higher temperatures.

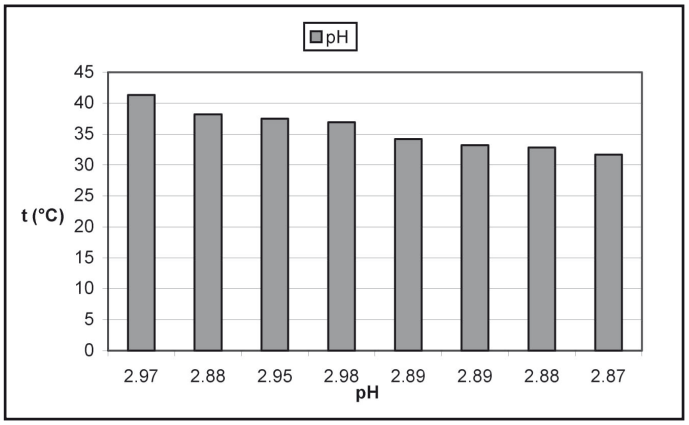
During watching of selected agents their values were moving within the range stated by the producer. These agents are not purposed for regions with very hard water, they were however deliberately tested just in this region. Measured total water hardness was 31 °d. Producer indicates to use detergents for moderately hard water (*10–20 °d*). During checking of milking units when there were used Mikasan D and Mikal 94D no sediments of scale were found in them, while already after a few use of Despon A, K these sediments started to appear.

From these results it ensues, that for the region with very hard water acid Mikasan D and alkaline Mikal 94D are better disinfectants, with regard to the fact, that during their application no sediments were appearing in milking system. From the point of disinfecting however both the agents were acting in a very good quality. Breed should regularly watch microbiological quality of water and in this way eventually prevent contamination of milk. During sanitation there should also be respected temperatures of solutions stated by a producer to enable them correct functioning and disinfecting of milking equipment.

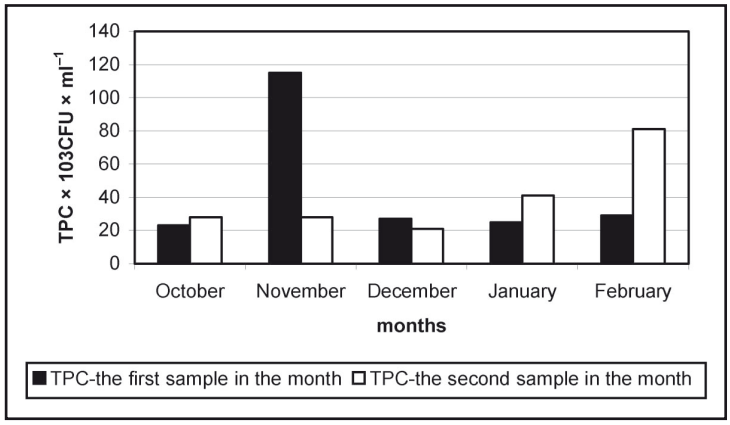
pH values of the cleaning and disinfecting solutions were tested on onechoice T-test account pH values recommended by the producer. No statistic significant differences were recorded.



1: *pH 0.5% solution of Mikasan D in unloaded medium*



2: *pH 0.25% solution of Despon K in unloaded medium*



3: *Dynamics TPC × 10³ CFU × ml⁻¹ in pool samples of milk*

I: *Changes of pH and temperature of the tested solution Mikasan 0.5% during the cleaning cycle (in loaded medium)*

	First week of using									
pH (1)	2.55	3.41	2.69	2.77	2.75	2.74	2.83	2.83	2.83	2.75
t (°C)	31.9	24.4	29.5	28.6	29.3	28.7	28.2	27.9	27.6	27.3
	Second week of using									
pH (2)	2.54	3.22	2.68	2.73	2.75	2.72	2.73	2.75	3.01	2.95
t (°C)	53	26.8	39.7	35.1	36.6	35.2	36	35.1	34.3	31.7
	Third week of using									
pH (3)	2.78	2.86	2.88	2.88	2.88	2.87	2.86	2.85	2.84	2.82
t (°C)	42.7	39.7	34.8	36.6	35.2	35.3	34.5	33.9	33.2	32.3
	Fourth week of using									
pH (4)	2.54	2.75	2.67	2.66	2.67	2.62	2.61	2.59	2.55	2.52
t (°C)	30.1	21.6	30.1	27.1	28.3	26.9	26.2	26.1	25.8	25

– one value of pH corresponds to an average value of 15 measured out partial values

II: *Changes of pH and temperature of the tested solution Despon K 0.25% during the cleaning cycle (in loaded medium)*

	First week of using							
pH (1)	2.93	2.89	2.91	2.94	2.89	2.9	2.95	2.94
t (°C)	38.8	34.8	35.8	33.7	34.1	33.6	32.4	30.9
	Second week of using							
pH (2)	2.94	3.13	3.26	3.1	3.12	3.21	3.21	3.15
t (°C)	38.8	36.3	32.8	34.1	33.5	32.5	31.9	31.6
	Third week of using							
pH (3)	2.92	2.85	2.85	2.83	2.85	2.84	2.85	2.85
t (°C)	40.2	37.7	35.2	34.1	34.3	33.7	32.8	32
	Fourth week of using							
pH (4)	2.92	2.99	3.12	3	3.1	3.12	3.15	3.18
t (°C)	40.5	38.7	36.5	34.3	33.8	32.7	31.6	29.8

– one value of pH corresponds to an average value of 15 measured out partial values

SOUHRN

Ověření účinnosti a působení vybraných dezinfekčních prostředků

Cílem práce bylo ověření účinnosti a působení dezinfekčních prostředků v provozních podmínkách. Sledovalo se pH roztoků, jejich teplota a doba čištění. Byly sledovány dva kyselé dezinfekční přípravky, Mikasan D a Despon K, které měly odlišnou doporučovanou koncentraci od výrobce. V dané oblasti se používala k čištění a dezinfekci velmi tvrdá voda (31 °d). Vzorky roztoků se odebíraly z otevřené dezinfekční vany, kde se míchaly dezinfekční přípravky s vodou. Nejprve se pH roztoků měřilo v nezatíženém prostředí, což znamená, že se dojicí zařízení pročistilo silnějšími přípravky pro generální čištění a potom se použily Mikasan D a Despon K. Následně se měřilo pH v zatíženém prostředí ihned po dojení a následně v týdenních intervalech. Při měření pH roztoků se zároveň sledovala teplota cirkulujícího roztoku. Účinnost přípravků se sledovala podle zjištění celkového počtu mikroorganismů (CPM), které byly stanovovány v laboratoři v mlékárně dvakrát měsíčně.

Z výsledků vyplývá, že pH dezinfekčních prostředků se pohybovalo v rozmezí, které je doporučováno výrobcem (*výrobce udává pH 2.15 pro 0,25% roztok Desponu K a pH 2.1 pro 0,5% roztok Mikasanu D*) a jejich dezinfekční schopnosti i podle výsledků CPM zůstaly zachovány (*viz graf 3*). Pouze v listopadu u prvního vzorku mléka CPM překročil hygienický limit 100×10^3 CFU × ml^{-1}. Jak bylo dodatečně zjištěno, toto překročení způsobila nedostatečná kvalita vody, se kterou měl v té době chov problémy. Další výsledky již byly dobré. Ve většině případech teploty dezinfekčních roztoků byly nižší než doporučuje literatura a jejich zvýšení by mohlo znamenat ještě větší působení a účinnost dezinfekčních roztoků. S vysokou tvrdostí si lépe poradil přípravek Mikasan D, protože při jeho používání se při kontrole dojicích jednotek v nich nenacházely žádné usazeniny. Při používání Desponu K se po několika jeho aplikacích tyto usazeniny objevily. Lze tedy říct, že lepší přípravek pro oblast s velmi tvrdou vodou se projevil Mikasan D. Jednovýběrovým T-testem byly testovány hodnoty pH monitorovaných čisticích a dezifekčních roztoků vzhledem k hodnotám pH, které doporučuje výrobce. Nebyly zjištěny statisticky významné rozdíly.

dezinfekce, sanitace, čištění

REFERENCES

ANONYM 1: National Milk National Harvesting Centre, Australia. Milk Good water quality is essential for good quality milk. [online].c2000 [cit. 20-1-2003] <www.milkharvesting.au.com/links/good.pdf>

ANONYM 2: Iternational Federation of Infection Control.Cleaning, disinfecting and sterilisation, Infection control. [online].c2003 [cit. 14-2- 2003] < www.ific.narod.ru/Manual/Clean.htm>

ANONYM 3: Getting organised for better milking. *Chevre*, 1996, 214, 34 - 35

BELL, J. R. M., GALLAGHER, S. J.: Cleaning and sanitising milking equipment. *Agriculture Western Australia, Farmnote* 42/99, 1999 a

BELL, J. R. M., GALLAGHER, S. J.: Water quality for dairying. AgricultureWestern Australia, Farmnote 41/99, 1999 b

ČSN ISO 4833 – General rules for stating of total number of micro-organisms

DOLEŽAL, O.: Čištění dojícího stroje jako další faktor.[online]. c2003, last revision 12. 1. 2005 [cit. 8-2-2003] <www.vuzv.cz/technol/otazkaO2.htm>

EDMONDSON, P. W.: Shepton Veterinary Group, Caring for Animals in Somerset. Cleaning the Milking Mashine. [online].c2002 [cit. 20-1-2003] <http://web.ukonline.co.uk./sotirios.karvountzis/referral/cleaning.htm>

HERRERO, M. A., IRAMAIN, M. S., KOROL, S., BUFFONI, H., FLORES, M., POL, M., MALDONADO, M. V., SARDI, G., FORTUNATO, M. S.: *Water quality and contamination in dairy farms in the Basin of Abasto Sur*. Revista Argentina de Produccion Animal, 2002, 22: 1, 61–702

JANDÍK, M., KLEPAL, V., BENEŠ, J.: *Vývoj pro dojící zařízení, která neobsahují sazová plnidla*. Institut gumárenské technologie a testování, 1995, 28

KIS, E.: Účinná sanitace (čištění a dezinfekce) dojícího zařízení. *Náš chov*, 61, 2001, 2, 50

MAŠKOVÁ, A.: *Influence of disinfectants on dug socket*. Final report, 2002, Mendel´s agriculture and forestry university in Brno, Institute of technique and processing of agricultural products, Faculty of Agronomy, 2002 a, 5

MAŠKOVÁ, A.: *Rubber materials in milking technique* Final report, 2002, Mendel´s agriculture and forestry university in Brno, Institute of technique and processing of agricultural products, Faculty of Agronomy, 2002 b, 40

LINDERER, M., GUTHY, K.: Investigations on problems with residues in milk after cleaning and disinfecting procedures. *Deutsche Milchwirtschaft*, 1994, 45: 17, 780–782

MILLER, J.: Comparing different cleaning processes for milking machines. *Milchpraxis*. 1996, 34: 4, 166–168

OLKONEN, A., HENNO, M.: On the mistakes in milking equipment cleaning and results of estimating the efficiency of washing detergents. *Proceedings of the Institute of Animal Husbandry of the Estinian Agricultural University*, 1998, 68,18–24

ORDOLFF, D.: Randprobleme des Robotmelkens. *Landtechnik*, 47, 1992, 12, 631–633

SEYDLOVÁ, R., SEYDL, M.: Vztah cen sanitačních prostředků k nákladům na výrobu mléka. *Farmář*, 3, 1997, 6, 49

SCHWARZ, P.: Cleaning in dairies with regard to environmental aspects. *Deutsche Milchwirtschaft*, 1993, 44: 22, 1097–1101

TYBOR, P. T., GILSON, W. D.: Cleaning and Sanitising in the Milking System. The University of Georgia College of Agricultural Bulletin 1025. [online]. c1989 [cit. 14-2-2003] <www.ces.uga.edu./pubcd/B1025-W.HTML>

Address

Ing. Kateřina Balharová, Ing. Květoslava Šustová, Ph.D., Ústav technologie potravin, Mendelova zemědělská a lesnická univerzita v Brně, Zemědělská 1, 613 00 Brno, Česká republika, e-mail: kbalharova@seznam.cz