ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LIX 13 Number 5, 2011

THE EFFECT OF PERORAL COPPER SUPPLEMENTATION ON SELECTED HAEMATOLOGICAL INDICATORS OF HORSES

P. Jančíková, P. Horký, L. Zeman, P. Mareš

Received: April 13, 2011

Abstract

JANČÍKOVÁ, P., HORKÝ, P., ZEMAN, L., MAREŠ, P.: *Effect of peroral copper supplementation on selected haematological indicators of horses.* Acta univ. agric. et silvic. Mendel. Brun., 2011, LIX, No. 5, pp. 119–124

The purpose of our work was to assess the impact of copper on selected haematological indicators of horse. The trial was performed at the Boudky – Velké Němčice farm. The total number of 23 mares was divided into three groups that were subjected to different mineral feeding modes. The experiment duration was 14 days and the blood samples were taken 1st, 7th and 14th day of experiment.
The obtained results show that the enrichment of the horse feeding ration with copper changed the monitored haematological parameters values. Assessment of the changes of haematological indicators between individual trial groups fed with copper-enriched feed with the copper coming from different sources showed the best results in the group with the intake of copper in the inorganic form. This group showed a statistically significant ($P < 0.01$) increase in erythrocyte count (by 0.85 $T.l^{-1}$) and haematocrit value (by 0.04 $l.l^{-1}$) and a statistically significant ($P < 0.05$) change in haemoglobin level (increasing by 8.9 $g.l^{-1}$) between initial and final blood sample. Although the group taking organic form of copper showed a significant ($P < 0.01$) increase in erythrocyte count and haematocrit value in the second blood sample comparing with initial one, the final sample did not corroborate this highly significant correlation. The control group showed no difference between the initial and final blood samples in any of the monitored parameters.

horses, copper, organic and inorganic form, erythrocytes, haemoglobin, haematocrit

Haematological tests are generally used to obtain information about infectious conditions, performance issues and health of horses (Gul *et al.*, 2007). The amount of functional and healthy erythrocytes and the high level of haemoglobin are very important for tissue oxygenation and aerobic performance of horses (Doubek *et al.*, 2003; Jelínek *et al.*, 2003; Hanák, 1996). Copper is an irreplaceable blood-forming element that helps to mobilise iron from liver, absorption from intestine and to bind it in hems (Zelenka and Zeman, 2006). The purpose of our work was to assess the impact of organic and inorganic form of copper on the selected haematological indicators of horse blood.

MATERIAL AND METHODS

The trial was realized at the Boudky – Velké Němčice farm and included 23 trial mares of the Bohemian Warmblood breed. The experiment lasted for 14 days. The horses were stabled in box stalls with feeding pump, with the access to an outdoor pen. The mares of various ages (3.6–19.8 years), similar weight categories and light workload were divided into three groups and subjected to different mineral feeding modes. The mares were fed two times a day by concentrates (at 7 a. m. and 17 p. m.). In the course of the trial, all animals received the same basic feeding ration consisting of *ad libitum* intake to hay that was measured, 1.5 kg of wheat-barley meal and 1.0 kg of oat. Horses in groups No. 1 and 2 received additional copper (Cu) in various forms at level of 120 mg/day with their evening fodder ration. Group No. 1 (n = 8) received Cu in the organic form – proteinate (Bioplex Cu, ALLTECH), while Group No. 2 (n = 8) was fed with Cu in the inorganic form – $CuSO_4.5H_2O$, and Group No. 3

(n = 7) received fodder without the Cu enrichment and was used as a control group. Blood samples for the required analyses were taken on day 1st, 7th and 14th of the trial. The samples were taken at the same time (at 9 a. m.) and the horses were not burdened or stressed before the blood sampling. The blood was taken from the *vena jugularis externa* into plastic samplers with the anticoagulation solution – EDTA. Immediately after the collection, the samples were placed in a transport cooling box in compliance with the relevant regulations governing animal trials. All haematological examinations were performed by using the ABC Vet apparatus (HORIBA ABX, France). All samples of feedstuffs were taken according Act. No. 91/1996 Coll., and Act No. 544/2002 Coll. The obtained results were evaluated statistically by the mean difference test – the Student pair t-test.

RESULTS

The trial horses showed no health problems in the course of the test. The experiment involved a comparison of the baseline values change of the selected haematological parameters between the separate groups and the values obtained from the separate sampling. The initial sampling did not show any significant difference between the groups in any of the monitored parameters. Statistically significant difference ($P < 0.05$) was found only in case of the haemoglobin value change between mares of the control group and mares whose basic feeding ration was supplemented inorganic form of copper. Difference was found between the 1st sampling in the control group and the 2nd sampling in the group receiving with inorganic Cu.

Different changes of the selected haematological indicators occurred between samplings within the separate groups. Mean values of the selected parameters, their standard deviations and statistical correlations are shown in Tab. I.

Blood samples of mares, whose basic feeding ration was supplemented with inorganic source of copper showed a statistically significant ($P < 0.01$) difference in average erythrocytes count that increased by 0.85 $T.l^{-1}$ between initial and final blood sample with the highest value of increasing 2.06 $T.l^{-1}$. Progress of changes of erythrocyte counts is depicted in Fig. 1. The addition of inorganic form of copper during the experiment resulted in a significant increase ($P < 0.01$) of haematocrit level by 0.15 $l.l^{-1}$ comparing with initial blood sampling. This increase was about 0.04 $l.l^{-1}$ higher than in group supplemented with proteinate form of copper.

Both groups whose basic feeding ration was enriched with Cu showed increased haemoglobin levels (Fig. 2). This increase was only statistically significant ($P < 0.05$) in the group receiving Cu in the inorganic form, while in the group receiving organic Cu the difference was not statistically significant. The control group showed no statistically significant difference between the initial and final samples. In the course of the trial, all animals received the same basic feeding ration and had similar workload – low initial values of HGB in mares supplemented with Cu in the inorganic form can be attributed to the individuality of individuals included in this group.

DISCUSSION

Copper is necessary for iron absorption from the gastrointestinal tract (Marycz *et al.*, 2009), represents the essential element for haemoglobin creation and iron transport (Jain, 1993; cit., Nazifi *et al.*, 2005). Physiological trials have shown that the haemoglobin synthesis is only possible at the presence of copper, even though copper is not its direct constituent (Tyleček *et al.*, 1961). Pursuant to Hanák (1996), erythrocytes represent the main mass of cellular blood elements and their count is usually directly proportional to the haematocrit and haemoglobin levels. The observation was also corroborated by our findings (Tab. I).

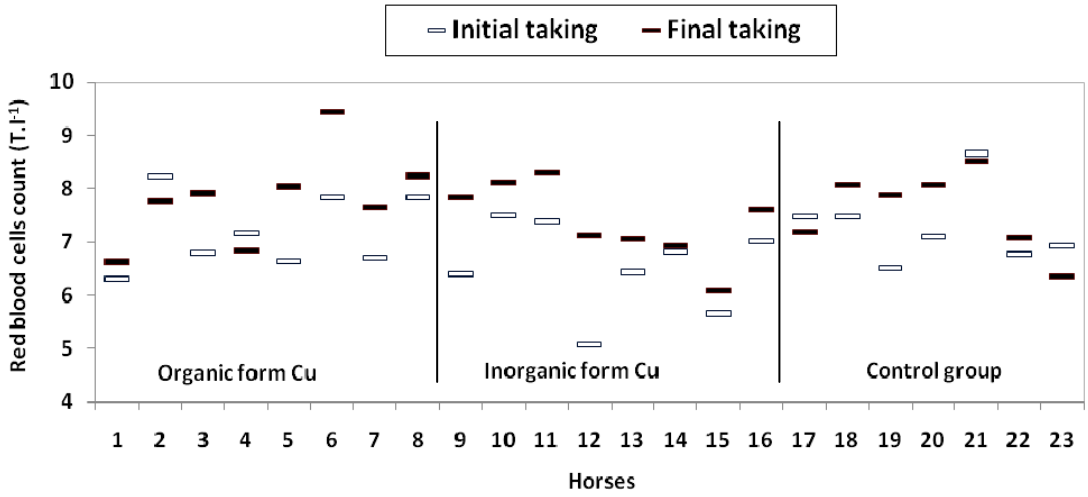
The found values of the basic haematological indicators correspond to reference values for erythrocyte (RBC) 5.50–12.9 $T.l^{-1}$, haemoglobin (HGB) 110–190 $g.l^{-1}$ a haematocrit (HCT) 0.30–0.42 $l.l^{-1}$, published by Doubek *et al.* (2007) and Reece (1998). Values found out in the experimental monitoring of the effect of lactation in mares by Gul *et al.* (2007), the effect of gravidity in mares by Lumsden *et al.* (1980), the effect of gender by Altinsaat (2008), age by Nazifi and Rategh (2005) and workload of horses by Ogoński and Cieśla (2009) on these parameters ranged within these limits too.

Nazifi and Rategh (2005) and Nazifi *et al.* (2003) report to have found no significant correlation between the levels of haemoglobin, copper, iron and ceruloplasmin in adult Turkish horses and Caspian mini-horses. Cupps and Howel (1949) monitored the effect of copper on haematological parameters of foals. The basic feeding ration of foals was enriched with 0, 10 and 100 mg of copper/kg dry matter. The most favourable values were noticed at the foal supplemented with 100 mg of copper/kg dry matter. RBC count of this particular foal was 10.8 $T.l^{-1}$ while the control group reached the level 9.45 $T.l^{-1}$. HGB value of the control group achieved an average level of 132 $g.l^{-1}$ and HCT of 0.35 $l.l^{-1}$. The foal fed with a diet enriched with 100 mg of copper/kg dry matter increased amount of HGB by 14.4% and HCT value by 11.3% compared to the control group, without copper addition. Jančíková *et al.* (2010) in trial on mares observed a statistically significant ($P < 0.05$) increase of the erythrocyte count from 7.46 ± 0.67 $T.l^{-1}$ to 7.89 ± 0.76 $T.l^{-1}$ following the ten-week application of fodder additive containing Cu in the organic form and some vitamins of the B group affecting the blood formation. At the beginning of their experiment, haemoglobin achieved an average level of 114.7 ± 8.3 $g.l^{-1}$. Even though the experimental sampling showed an increase of HGB to 120.1 ± 12.2 $g.l^{-1}$, the difference was not

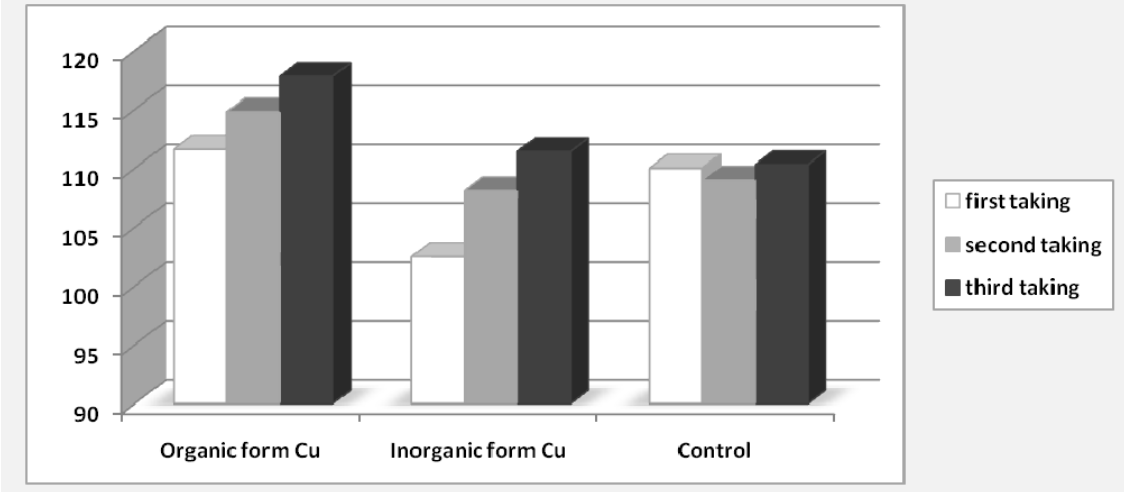
I: *Statistical evaluation (Student pair t-test) of changes in haematological parameters between sampling within experimental groups*

Source	Mares	Monitored parameter								
		RBC			HGB			HCT		
		Day of experiment			Day of experiment			Day of experiment		
		1st	7th	14th	1st	7th	14th	1st	7th	14th
Organic form Cu	1	6.31	7.05	6.63	101	107	102	0.29	0.33	0.31
	2	8.23	8.32	7.77	124	121	114	0.36	0.37	0.35
	3	6.80	7.65	7.91	114	119	125	0.31	0.35	0.37
	4	7.17	7.30	6.84	107	105	101	0.31	0.32	0.30
	5	6.64	7.48	8.04	95	102	113	0.27	0.31	0.34
	6	7.84	8.61	9.45	126	134	147	0.36	0.40	0.45
	7	6.71	7.71	7.65	101	111	110	0.28	0.33	0.33
	8	7.84	7.71	8.24	125	119	130	0.36	0.35	0.38
Average ± SD		**7.19 ± 0.65 a**	**7.73 ± 0.48 B**	**7.82 ± 0.81 ab**	**111.6 ± 11.6 a**	**114.8 ± 9.9 a**	**117.8 ± 14.5 a**	**0.32 ± 0.03 a**	**0.35 ± 0.03 B**	**0.35 ± 0.04 b**
Inorganic form Cu	9	6.40	7.63	7.83	95	108	112	0.27	0.32	0.33
	10	7.50	7.68	8.11	123	122	128	0.36	0.37	0.40
	11	7.38	8.52	8.29	118	127	124	0.34	0.39	0.38
	12	5.07	6.68	7.13	84	104	114	0.24	0.32	0.34
	13	6.43	6.42	7.06	102	98	107	0.30	0.30	0.33
	14	6.82	6.78	6.93	96	92	98	0.28	0.28	0.29
	15	5.66	6.26	6.09	97	101	100	0.28	0.31	0.30
	16	7.01	7.85	7.62	105	113	108	0.30	0.34	0.33
Average ± SD		**6.53 ± 0.78 a**	**7.23 ± 0.75 b**	**7.38 ± 0.68 B**	**102.5 ± 11.9 a**	**108.1 ± 11.2 ab**	**111.4 ± 9.9 b**	**0.29 ± 0.04 a**	**0.33 ± 0.04 B**	**0.34 ± 0.03 B**
Control	17	7.47	7.48	7.19	125	122	117	0.36	0.36	0.35
	18	7.48	8.03	8.07	108	112	110	0.31	0.34	0.34
	19	6.50	7.20	7.87	104	106	120	0.29	0.33	0.36
	20	7.10	7.94	8.07	106	110	115	0.30	0.34	0.35
	21	8.66	8.29	8.51	124	110	120	0.36	0.35	0.36
	22	6.77	6.96	7.09	102	97	100	0.28	0.29	0.30
	23	6.92	7.53	6.36	101	106	90	0.29	0.32	0.27
Average ± SD		**7.27 ± 0.66 a**	**7.63 ± 0.44 a**	**7.59 ± 0.69 a**	**110.0 ± 9.4 a**	**109.0 ± 7.0 a**	**110.3 ± 10.5 a**	**0.31 ± 0.03 a**	**0.33 ± 0.02 b**	**0.33 ± 0.03 ab**

a, b – letters expressing changes within experimental groups in separate parameters between sampling: b – statistically significant ($P < 0.05$), B – highly statistically significant ($P < 0.01$)
RBC – erythrocytes ($T.l^{-1}$), HGB – haemoglobin ($g.l^{-1}$), HCT – haematocrit ($l.l^{-1}$)



1: *Variation of red blood cells (RBC) count from individual mares*



2: *Mean haemoglobin levels (g.l^{-1}) in several sampling experimental groups*

statistically significant. The addition of premix during the experiment also resulted in a significant increase ($P < 0.05$) of haematocrit from 0.33 ± 0.037 l.l^{-1} to 0.35 ± 0.037 l.l^{-1}.

The comparison of the effect of various Cu forms on other animal species and categories resulted in the following findings. Already in 1961 Šimek *et al.*, cit., Zelenka *et al.* (1963) fed pigs with additional high doses of cupric sulphate. The treatment resulted in the highly significant effects on haematological parameters. Angelov *et al.* (2010) found that the Cu in the organic form had positive effects on haematological parameters of growing pigs. Zelenka *et al.* (1963) did not find significant difference in the blood parameters of chickens receiving high doses of cupric sulphate. Aksu *et al.* (2010) observed in their trial on chickens receiving the organic form of Cu a statistically significant ($P < 0.05$) increase in HGB level and insignificant difference in RBC count.

The discussion does not lead to a single evaluation of the used forms of mineral elements and their effects on the monitored haematological parameters. The blood count mirrors the current condition of the horse. Further trial repetitions are needed for a more precise assessment of the effects of various Cu forms on the haematological parameters of horses.

CONCLUSION

The obtained results show that the feeding treatment represented by the enrichment of horse diet with copper led to the change of studied haematological parameters. Best results in the evaluation of changes in haematological parameters within the separate groups fed with the addition of different copper source were achieved in the group with the Cu intake in the inorganic form. This group showed a statistically significant ($P < 0.01$) increase in erythrocyte count and haematocrit value and a statistically significant ($P < 0.05$) change in haemoglobin level between initial and final blood sample. Although the group taking organic

copper showed a significant ($P < 0.01$) increase in erythrocyte count and haematocrit value in the second blood sample comparing with initial one, the final sample did not corroborate this highly significant correlation. The control group showed no difference between the initial and final blood samples. The increased amount of functional erythrocytes and the level of haemoglobin are very important to improve tissue oxygenation and aerobic performance of horses.

SUMMARY

The purpose of our work was to assess the impact of organic and inorganic form of copper on the selected haematological indicators of horse blood.

The 23 healthy mares were divided into three groups and subjected to different mineral feeding modes. Mares in groups No. 1 and 2 received additional copper in various forms at level of 120 mg/day with their evening fodder ration. Group No. 3 received fodder without the Cu enrichment and was used as a control group. Blood samples for the required analyses were taken on day 1st, 7th and 14th of the trial. All haematological examinations were performed by using the ABC Vet apparatus (HORIBA ABX, France).

The obtained results show that the feeding treatment represented by the enrichment of horse diet with copper led to the change of studied haematological parameters. Best results in the evaluation of changes in haematological parameters within the separate groups fed with the addition of different copper source were achieved in the group with the Cu intake in the inorganic form. This group showed a statistically significant ($P < 0.01$) increase in erythrocyte count and haematocrit value and a statistically significant ($P < 0.05$) change in haemoglobin level between initial and final blood sample. Although the group taking organic copper showed a significant ($P < 0.01$) increase in erythrocyte count and haematocrit value in the second blood sample comparing with initial one, the final sample did not corroborate this highly significant correlation. The control group showed no difference between the initial and final blood samples in any of the monitored parameters. Organic forms of trace elements in the animal diet have been recently increased as compared to their inorganic forms in spite of their high cost, problematic specification and less favourable effect on the haematological indicators in our experiment.

Acknowledgements

This project was funded from IGA AF MENDELU Grant No. TP 8/2010.

REFERENCES

AKSU, D. S., AKSU, T., ÖZSOY, B., 2010: The Effects of Lower Supplementation Levels of Organically Complexed Minerals (Zinc, Copper and Manganese) Versus Inorganic Forms on Haematological and Biochemical Parameters in Broilers. *Kafkas Universitesi Veteriner Fakultesi Dergisi,* 16, 4: 553–559.

ALTINSAAT, C., 2008: The Effects of Age and Gender on Blood Parameters in Arabian Horses. *Kafkas Universitesi Veteriner Fakultesi Dergisi,* 14, 2: 173–178.

ANGELOV, L., VRABCHEVA, V., PETRICHEV, M., BORISOVA, L., 2010: The effect of copper complex of methionine compared with copper sulfate in growing pigs. *Turkish Journal of Veterinary and Animal Sciences,* 34, 1: 1–5.

CUPPS, P. T., HOWELL, C. E., 1949: The Effects of Feeding Supplemental Copper to Growing Foals. *Journal of animal science*, 8, 2: 286–289.

DOUBEK, J., BOUDA, J., DOUBEK, M., FÜRLL, M., KNOTKOVÁ, Z., PEJŘILOVÁ, S., PRAVDA, D., SCHEER, P., SVOBODOVÁ, Z., VODIČKA, R., 2003: *Veterinární hematologie*. 1. vyd. Brno: Noviko, a. s., 464 s. ISBN 80-86542-02-5.

DOUBEK, J., ŠLOSÁRKOVÁ, S., ŘEHÁKOVÁ, K., SCHEER, P., BERÁNKOVÁ, J., 2007: *Interpretace základních biochemických a hematologických nálezů u zvířat*. Brno: Noviko, a. s., 77 s. ISBN 80-86542-16-5.

GUL, S. T., AHMAD, M., KHAN, A., HUSSAIN, I., 2007: Haemato-biochemical Observations in Apparently Healthy Equine species. *The Pakistan Veterinary Journal*, 27, 4: 155–158.

HANÁK, J., 1996: *Základy diagnostiky u koní z aspektu sportovní veterinární medicíny*. 1. vyd. Plzeň: Medicus veterinarius, 203 s.

JAIN, N. C., 1993: *Essentials of veterinary haematology.* 1. vyd. USA: Wiley-Blackwell, 417 s. ISBN 0-8121-1437-X.

JANČÍKOVÁ, P., HORKÝ, P., ZEMAN, L., 2010: Vztah krmných aditiv ke krvetvorbě a výkonnosti sportovních koní. *MendelNet 2010: Proceedings of International Ph.D. Students Conference*. Brno: Mendel University in Brno, Faculty of Agronomy, s. 52. ISSN 978-80-7375-453-2.

JELÍNEK, P., KOUDELA, K., DOSKOČIL, J., ILLEK, J., KOTRBÁČEK, V., KOVÁŘŮ, F., KROUPOVÁ, V., KUČERA, M., KUDLÁČ, E., TRÁVNÍČEK, J., VALENT, M., 2003: *Fyziologie hospodářských zvířat*.

1. vyd. Brno: Mendelova zemědělská a lesnická univerzita v Brně, 414 s. ISBN 80-7157-644-1.

LUMSDEN, J. H., ROWE, R., MULLEN, K., 1980: Haematology and Biochemistry Reference Values for the Light Horse. *Canadian Journal of Comparative Medicine*, 44, 1: 32–42.

MARYCZ, K., MOLL, E., ZAWADZKI, W., NICPOŃ, J., 2009: The correlation of elemental composition and morphological properties of the horses hair after 110 days of feeding with high quality commercial food enriched with Zn and Cu organic forms. *Electronic Journal of Polish Agricultural Universities*, 12, 3.

NAZIFI, S., RATEGH, S., 2005: Haemoglobin types and blood concentrations of haemoglobin, copper, ceruloplasmin and iron in adult Caspian miniature horses. *The Revue de Medecine Veterinaire,* 156, 1: 50–52.

NAZIFI, S., ROWGHANI, E., NIKOOSEFAT, Z., 2005: Studies on the relationship between haemoglobin, copper, ceruloplasmin, iron and superoxide dismutase activity in blood of Iranian fat-tailed sheep. *Comparative Clinical Pathology*, 14, 2: 114–117.

NAZIFI, S., SAEB, M., KHOSHAVAGHTI, A., 2003: Studies on the relationship between haemoglobin types of adult Turkoman horses and the concentrations of haemoglobin, copper, ceruloplasmin and iron. *Comparative Clinical Pathology*, 12, 1: 53–56.

OGOŃSKI, T., CIEŚLA, A., 2009: Total antioxidants and antioxidant system microelements in the blood plasma of recreation saddle-horses after a ten-week-long working season. *Medycyna Weterynaryjna*, 65, 6: 394–398.

REECE, W. O., 1998: *Fyziologie domácích zvířat*. 1. vyd. Praha: Grada Publishing, 456 s. ISBN 80-7169-547-5.

TYLEČEK, J., ČECHOVSKÝ, J., ŠPAČEK, F., ZELENKA, J., 1961: Vliv $CuSO_4.5H_2O$ na růst a jatečnou hodnotu vykrmovaných kuřat. *Živočišná výroba*, 6, 10: 747–760.

ZELENKA, J., ZEMAN, L., 2006: *Výživa a krmení drůbeže*. 117 s.

ZELENKA, J., DRESSLER, J., PONÍŽILOVÁ, E., PANEŠOVÁ, J., 1963: Změny periferního krevního obrazu a obsahu mědi v krevním séru a játrech kuřat přikrmovaných vysokými dávkami $CuSO_4.5H_2O$. *Živočišná výroba*, 8, 1: 57–66.

Adresa

Ing. Petra Jančíková, Ústav výživy zvířat a pícninářství, Mendelova univerzita v Brně, Zemědělská 1, 613 00 Brno, Česká republika, e-mail: petra.jancikova@mendelu.cz