Potravinarstvo, **vol. 8, 2014, no. 1, p. 87-91**
doi:10.5219/357
Received: 28 February 2014. Accepted: 3 March 2014.
Available online: 7 May 2014 at www.potravinarstvo.com
© 2014 *Potravinarstvo*. All rights reserved.
ISSN 1337-0960 (online)

THE EFFECT OF YUCCA ON PROLIFERATION, APOPTOSIS AND STEROIDOGENESIS OF PORCINE OVARIAN GRANULOSA CELLS

Aneta Štochmaľová, Attila Kádasi, Richard Alexa, Roland Grossmann, Alexander Sirotkin

ABSTRACT

Yucca shidigera is a medicinal plant native to Mexico. Is a plant widely used in folk medicine to treat a variety of ailmentary disorders, but its action on reproductive processes and possible mechanisms of such action remains unknown. *Yucca schidigera* extract contains a number of steroidal saponins that, because of their biological activity, have attracted attention from the food industry for many years. Yucca extract is used as a natural feed additive with positive effect to microflora, digestion, metabolism and to improve animal muscle growth. Its extract has been used as a foodstuff and folk medicine to treat a wide variety of diseases for many years. Nevertheless, it remaines unknown, whether consumption of yucca can affect reproductive system. The aim of this study was to examine the effects of yucca on basic ovarian cell functions – proliferation, apoptosis and steroidogenesis. Porcine ovarian granulosa cells were cultured with and without yucca extract (added at doses 0; 1; 10 and 100 $\mu g.mL^{-1}$ of medium). Markers of proliferation (% of PCNA-positive cells) and apoptosis (% cells containing bax) were analysed by immunocytochemistry. Release of steroid hormones (progesterone and testosterone) was measured by EIA. It was observed, that addition of yucca inhibited proliferation (expression of PCNA), increased apoptosis (expression of bax), stimulated progesterone and inhibited testosterone release. The ability of yucca to reduce ovarian cell proliferation, to promote ovarian cell apoptosis and affect steroidogenesis demonstrates the direct influence of yucca on female gonads. Furthermore, our observations suggest the multiple sites of action (proliferation, apoptosis, steroidogenesis) of yucca on porcine ovarian cell functions. It is not to be excluded, that consumption of yucca can suppress female reproductive functions.

Keywords: yucca; proliferation; apoptosis; steroidogenesis; porcine granulosa cell

INTRODUCTION

Medicinal plants contain a variety of molecules with potent biological activities **(Coran et al., 2001)**. Changes in nutrition can affect human and animal reproductive processes **(Sirotkin, 2010)**. Herbal medicine and functional food provides plentiful alternatives to synthetic compounds in the treatment of almost any condition, as it is believed to be less toxic. *Yucca schidigera*, know as yucca, is a plant belonging to the *Agavaceae* family, native to the South-Western United State and Mexico. Indians recognized yucca as one of the nicest desert plants, "a tree of life" with health promoting activity. Its extracts have been used for centuries in folk medicine to treat a wide variety of inflammatory disorders, especially headaches, gonorhea, arthritis, and rheumatism **(Cheeke, 1998)**. Yucca powder has powerful anti-inflammatory activity, mediated via inhibition of NFkB activation. Yucca polyphenolics inhibit NFkB, a transcription factor which stimulate iNOS, an inducible enzyme which produced the inflammatory agent nitric oxide **(Cheeke et al., 2006)**. In recent time, this plant and its extract have been used to produce drugs that are applied to treat various human diseases. Furthermore, yucca is used as a food additive increasing the performance in sportsmen. It also improves performance and health of the livestock in addition to feed in various concentrations **(Duffy et al., 2007)**. The ability of yucca to affect food digestion and metabolism are well known. It has been showed that *Yucca schidigera* and yucca-supplemented diets had lower rumen ammonia N concentrations compared to the control diet. *Yucca schidigera* can be used to modify rumen fermentation in order to decrease ruminal ammonia concentrations and reduce urinary N excretion thereby reducing the environmental impact of ruminant production systems **(Santoso et al., 2006)**. The mode of action of *Yucca schidigera* is believed to be related to their steroidal saponins (sarsapogenin, smilagenin, markogenin, samogenin, gitogenin and neogitogenin) and phenolic compounds (resveratrol and yuccaols) were found in the yucca plant bark **(Abaza and Said, 2005)**. Yucca polyphenols are potent antioxidants **(Oleszek et al., 2001)**. Yucca phenolics also are antioxidants and free-radical scavengers, which may aid in suppressing reactive oxygen species (ROS) that stimulate inflammatory responses **(Cheeke et al., 2006)**. Yuccaols inhibit the generation of free radicals in blood platelets may be beneficial in protecting against cardiovascular diseases **(Olas et al., 2003)**. According to **Jaques (1989)** saponins contained in the yucca plant it just one of the many natural biosecurity substances which increase the efficiency of farm animals. Steroidal saponins in yucca were reported to exhibit antiyeast or antifungal activities **(Tanaka et al., 1996)**. Saponin-containing yucca extracts are currently used in the feed industry for control of ammonia and odour **(Cheek,**

1999). Yucca saponins are known to reduce iron absorption **(Southon et al., 1988)** and may reduce fatty acid absorption by sequestering bile acids necessary for micelle formation and fat absorption **(Oakenfull and Sidhu, 1989)**. The evidence demonstrates that saponins enhance feed efficiency and weight gain in pigs, as well as feed efficiency, weight gain, and increased egg production in chickens, increase production of milk in dairy cows. Livestock clearly benefits when the extract from this plant is included in the feed. Because it is 100% natural, the *Yucca schidigera* dry extract is environmentally safe and is the best answer to naturally lowering toxic ammonia levels in housing and improving the quality and potential output of animals **(Cheeke et al., 2006)**. **Balazi, et al., (2013)** suggests that the addition of Yucca shidigera plant into the normal feed had positive effects on male's spermatozoa parameters. Effect of yucca on ovarian functions and steroidogenesis has been unknown. Progesterone (P) is the ovarian steroid hormone that is needed for ovarian functions, embryonic development and in mammary gland development **(Hagan et al., 2009)**. It is produced by porcine **(Sirotkin et al., 2008; Kolesarova et al., 2010 a, b)**, rabbit **(Sirotkin et al., 2009)**, sheep **(Al-Dabbas et al., 2008)** and goat **(Blaszczyk et al., 2009)** and other animal ovarian cells. Progesterone governs ovarian functions of pigs **(Sirotkin et al., 2008, Kolesarova et al., 2010a,b)** and rabbits **(Sirotkin et al., 2009)**. Testosterone (T) is a steroid hormone that is produced in the testes, ovaries and a small amount in adrenal gland **(Cox et al., 2005; Reed et al., 2006)**. It is important for healthy development of the individual and the establishment of secondary sexual characteristics **(Swaab et al., 2009)**. Progesterone and testosterone can affect ovarian functions via influence on ovarian cell proliferation and apoptosis, whose in turn define ovarian follicullogenesis, ovulation and fecundity **(Sirotkin, 2014)**. The influence of yucca on ovarian cell proliferation, apoptosis, steroidogenesis, the interrelationships between these processes and their mechanisms remain unknown yet.

MATERIAL AND METHODOLOGY

Isolation and culture of granulosa cells

Ovaries of non-cycling pubertal gilts, about 180 days of age, were obtained after slaughter at a local abattoir. They were washed several times in sterile 0.9% NaCl and 95% alcohol. Granulosa cells were aspirated by syringe and sterile needle from follicles 3-5 mm in diameter and granulosa cells isolated by centrifugation for 10 min at 200 g. Cells were then washed in sterile DMEM/F12 1:1 medium (BioWhittakerTM, Verviers, Belgium), resuspended in the same medium supplemented with 10% fetal calf serum (BioWhittakerTM) and 1% antibiotic-antimycotic solution (Sigma, St. Louis, MO, USA) at a final concentration 106 cells/ml medium. Portions of the cell suspension were dispensed to 16-well chamber slides (Nunc Inc., International, Naperville, USA, 200 µL/well) and incubated at 37.5 °C in 5% CO_2 in humidified air until 60-75% confluent monolayer was formed (3-5 days), at which point the medium was renewed. Further culture was performed in 200 µL/medium in the same chamber slides, as described previously.

After medium replacement experimental cells were cultured in the presence of yucca (KONFIRM, Brno, Czech Republic) alone at concentrations of 0; 1; 10 and 100 µg/mL^{-1}. Yucca was dissolved in culture medium immediately before its addition to the cells. Control cells were cultured without yucca. After two days in culture, the medium from the 24-well plates was gently aspirated and frozen at -24 °C to await EIA. After removing the medium from chamber slides, cell were washed in ice-cold PBS (pH 7.5), fixed in paraformaldehyde (4% in PBS, pH 7.2-7.4; 60 min) and held at 4 °C to await immunocytochemi. cal analysis.

Immunocytochemical analysis

Following washing and fixation, the cells were incubated in the blocking solution (1% of goat serum in phosphate-buffered saline - PBS) at room temperature for 1 h to block nonspecific binding of antiserum. Afterwards, the cells were incubated in the presence of monoclonal antibodies against either PCNA (marker of proliferation) and bax (marker of apoptosis) (all from Santa Cruz Biotechnology, Inc., Santa Cruz, USA; dilution 1:500 in PBS) for 2 h at room temperature at overnight at 4 °C. For the detection of binding sites of primary antibody, the cells were incubated in secondary swine antibody against mouse IgG labeled with horse-radish peroxidase (Servac, Prague, Czech Republic, dilution 1:1000) for 1 h. Positive signals were visualized by staining with DAB-substrate (Roche Diagnostics GmbH, Manheim, Germany).

Following DAB-staining, the cells on chamber-slides were washed in PBS, covered with a drop of Glycergel mounting medium (DAKO, Glostrup, Denmark); then coverslip was attached to a microslide. Cellular presence and localization of PCNA and bax positivity in cells was proved on the basis of DAB-peroxidase brown staining. A ratio of DAB-HRP-stained cells to the total cell number was calculated.

Immunoassay

Concentrations of P4 and T were determined in 25-100 µL samples of incubation medium by EIA. Previously validated for use in culture medium by using antisera against steroids produced in the Institute of Animal Science, Neustadt, Germany. P4 concentrations were measured by using EIA as described previously **(Prakash et al., 1987)**. Rabbit antiserum against P was obtained from Research Institute for Animal Production, Schoonoord, Netherlands. It cross-reacted <0.1% with 17 β-estradiol, dihydrotestosterone, testosterone and 17 β-hydroxyprogesterone. Sensitivity was 12.5 pg/mL. Inter and intra-assay coefficients of variation did not exceed 3.3% and 3.0% respectively. T was assayed by using EIA according to **Münster (1989)**. Sensitivity was 10 pg/mL. The antiserum cross-reacted <96% with dihydrotestosterone, <3% with androstenedione, <0.01% with progesterone and estradiol, <0.02% with cortisol and <0.001% with corticosterone. Inter and intra-assay coefficients of variation were 12.3% and 6.8% respectively.

Statistical analysis

Each experimental group was represented by three Chamber-slide wells. The proportions of cells containing specific immunoactivity were calculated from at least 1000 cells per chamber. The percentage of cells containing antigen in different groups of cells was calculated. Each series of experiments was performed twice. The data shown are the means of values obtained in these two separate experiments performed on separate days with separate groups of granulosa cells, each obtained from 9 animals.

Significant differences between the experiments were evaluated using Student´s T-test and one/two-way ANOVA folowed by paired Wilcoxon-Mann Whitney test, by using Sigma Plot 11.0 software (Systat Software, GmbH, Erkhart, Germany). Differences from control at P <0.05 were considered as significant.

RESULTS AND DISCUSSION

These data constitute first reports concerning the influence of yucca on reproduction. Moreover, this is the demnstration of direct influence of this medical plant on ovarian cells. We have shown, that yucca significantly increased the percentage of cells containing apoptosis marker (bax) at doses 10 and 100 $\mu g.mL^{-1}$. Dose 1 $\mu g.mL^{-1}$ did not affect the apoptosis. The percentage of cells containing proliferation marker (PCNA) was significantly increased at all doses added. The effect of yucca on both proliferation and apoptosis has a dose-dependent manner (Table 1).

We have been observed yucca has effect on steroidogenesis. In our experiment yucca stimulated release of P and inhibited T release by a dose-dependent manner (Table 2).

This study demonstrated effect of yucca addition on porcine granulosa cells. It was find dose-dependent effect on hormone release of yucca. Yucca at the highest dose (100 $\mu g.mL^{-1}$) significantly stimulated the release of P4, while doses 1 and 10 $\mu g.mL^{-1}$ did not effect on secretion. In our experiment, T release was inhibited by yucca at doses 1 and 10 $\mu g.mL^{-1}$ and the highest dose (100 $\mu g.mL^{-1}$) did not affect secretion. This is the first finding, that yucca can influence not only P4, but also androgen output. P4 and T have antiproliferative and proapoptotic properties. They can suppress growth of ovarian follicles. Therefore, it might be hypothesized, that yucca through promotion of P4 and T can inhibit porcine ovarian development. This hypothesis was supported by the ability of yucca to affect markers of ovarian cell proliferation and apoptosis. We have been observed that yucca can directly suppress the accumulation of proliferative peptide PCNA and promote the expression of apoptotic peptide bax. Physiological influence of yucca on ovarian granulosa cells could be important practical viewpoint. It is not to be excluded the yucca may used in the regulation of functions including fertility in pigs, other animals and humans, although this hypothesis should be verified by further, *in vivo* experiments. An inhibitory effect of yucca on porcine ovarian functions observed in our experiments could indicate that this plant substance could be potentially useful for synchronisation of porcine ovarian cycles. If the negative effect of yucca on ovarian functions occurs not only in pigs, but also in humans, and not only in-vitro, but *in vivo* too, it could be hypothesised, that yucca could jeopardize human reproduction and fertility.

Table 1 The percentage of granulosa cells containing markers of proliferation (PCNA) and apoptosis (Bax) in the porcine granulosa cells exposed by yucca

Supplement	PCNA	bax
Control (no adition)	49.94 ±1.92 (1700)	35.06 ±1.48 (1600)
Yucca 1 µg.mL^{-1}	33.29 ±2.17* (850)	40.47 ±3.15 (850)
Yucca 10 µg.mL^{-1}	26.00 ±1.92* (800)	45.00 ±2.84* (800)
Yucca 100 µg.mL^{-1}	22.71 ±1.60* (900)	54.38 ±2.53* (800)

All the values represent P or T release, means ± SEM, * - significant (P <0.05) differences with control (cells not treated with yucca).

Table 2 The secretion of steroid hormones in the porcine granulosa cells treated and not treated with yucca (EIA)

Supplement	P4 secretion ng/106 cells/day	T secretion pg/106 cells/day
Control (no adition)	19.98 ±1.22	392.80 ±33.23
Yucca 1 µg.mL^{-1}	17.28 ±2.31	108.18 ±48.81*
Yucca 10 µg.mL^{-1}	20.42 ±3.22	171.71 ±11.47*
Yucca 100 µg.mL^{-1}	31.53 ±1.71*	375.58 ±31.23

All the values represent P or T release, means ± SEM, * - significant (P <0.05) differences with control (cells not treated with yucca).

CONCLUSION

This study is the first evidence possible stimulatory effect of yucca on the release of progesterone, inhibitory effect on the release of testosterone, inhibitory impact on proliferation (accumulation of PCNA) and stimulatory influence on apoptosis (accumulation of bax) on granulosa cells of porcine ovary. Our results suggest a direct effect of yucca on steroidogenesis, proliferation and apoptosis in porcine ovaries. It is possible, that consumption of yucca by animal and human females may suppress their reproductive functions.

REFERENCES

Abaza, I. M., El-Said, H. 2005. Effect of using *Yucca Schidigera* as feed additive on performance of growing rabbits. The 4th Inter. Con. on Rabbit Prod. in Hot Clim., Sharm El-Sheikh, Egypt, p. 259-266. Retrieved from the web: http://www.abazacenter.8m.net/rich_text.html

Al-Dabbas, F. M., Hamra, A. H., Awawdeh, F. T. 2008. The effect of arginine supplementation on some blood parameters, ovulation rate and concentrations of estrogen and progesterone in female Awassi sheep. *Pakistan Journal of Biological Science*, vol. 11, p. 2389-2394.

http://dx.doi.org/10.3923/pjbs.2008.2389.2394 PMid:19137847

Balazi, A., Foldesiova, M., Chrastinova, L., Sirotkin, A. V., Chrenek, P. 2013. Effect of the herbal additive "Yucca" on rabbit spermatozoa characteristics. 2013. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 2, p. 1829-1837.

Blaszczyk, B., Stankiewicz, T., Udala, J., Gaczarzewicz, D. 2009. Plasma progesterone analysis by time-resolved fluorescent antibody test to monitor estrous cycles in goats. *Journal of Veterinary Diagnostic Investigations*, vol. 21, p. 80-87. http://dx.doi.org./10.1177/104063870902100112 PMid:19139505

Cheeke, P. R. 1998. Saponins: Surprising benefits of desert plants. *The linus Pauling Institute Newsletter*; Oregon state university, p. 4-5.

Cheeke, P., R., Piacente, S., Oleszek, W. 2006. Anti-inflammatory and anti-arthritic effects of *Yucca shidigera*: A review. *Journal of Inflammation*, vol. 3, p. 6. http://dx.doi.org./10.1186/1476-9255-3-6 PMid:16571135

Cox, R. M., John-Alder, H. B. 2005. Testosterone has opposite effects on male growth in lizards (*Sceloporus spp.*) with opposite patterns of sexual size dimorphism. *Journal of Experimental Biology*, vol. 208, no. 24, p. 4679-4687. http://dx.doi.org./10.1242/jeb.01948 PMid:16326949

Duffy, C., Brooks, P. 2007. Using Yucca shidigera in Pig Diets: Effects on Nitrogen Metabolism. [cit.2014-02-24] Available at: http://en.engormix.com/MA-pig-industry/management/articles/using-yucca-schidigera-pig-t717/124-p0.htm

Hagan, C. R., Faivre, E. J., Lange, C. A. 2009. Scaffolding actions of membraneassociated progestrerone receptors. *Steroids*, vol. 74, no. 7, p. 568-572. http://dx.doi.org/10.1016/j.steroids.2008.12.004 PMid:19135465

Department of Animal science, Oregon State University, Corvallis, OR 97333, USA.

Jaques, K. A. 1989. Yucca sarsaponin mode of action: Effects independent of air quality problems. *Animal feed, Biological additives: Proceeding,* Veterinary science University of Sydney, no. 119, p. 39-46.

Kolesárová, A., Roychoudhury, S., Slivková, J., Sirotkin, A., Capcarová, M., Massanyi, P. 2010a. *In vitro* study on the effect of lead and mercury on porcine ovarian granulosa cells. *Journal of Environmental Science and Health, Part A Toxic/ Hazard Substances Environmental Engeneering*, vol. 45, p. 320-331. http://dx.doi.org/10.1080/10934520903467907 PMid:20390873

Kolesárová, A., Capcarová, M., Sirotkin, A., Medveďová, M., Kováčik, J. 2010b. Cobalt-induced changes in the IGF-I and progesteron release, expression of proliferation- and apoptosis-related peptides in porcine ovarian granulosa cells *in vitro*. *Journal of Environmental Science and Health, Part A Toxic/ Hazard Substances Environmental Engeneering*, vol. 45, no. 3, p. 810-817. http://dx.doi.org./10.1080/10934521003708968 PMid:20397087

Münster, E., 1989. *Entwicklung von enzymimmunologischen Messverfahren auf Mikrotitrationsplatten zur Bestimmung von Testosteron und Progesteron im Blutplasma.* Doctoral Thesis. Institut for Animal Production and Breeding of the University of Hohemheim, p. 154.

Oakenfull, D., Sidhu, G.S., Cheeke, P., R. 1989. Saponins. *Toxicants of Plant Origin,* vol. 2, p. 97-141.

Olas, B., Wachowicz, B., Stochmal, A., Olezsek, W. 2003. Inhibition of oxidative stress in blood platelets by different phenolics from *Yucca schidigera* roezl. bark. *Nutrition,* vol. 19, p. 633-640. PMid:12831950

Oleszek, W., Sitek, M., Stochmal, A., Piacente, S., Pizza, C., Cheeke, P. 2001. Resveratrol and other phenolics from the bark of *Yucca schidigera* roezl. *Journal if Agricultural and Food Chemistry,* vol. 49, p. 747-752. PMid:11262023

Prakash, B. S., Meyer, H. H., Schallenberger, E., van de Wiel, D. F. 1987. Development of a sensitive enzyme immunoassay (EIA) for progesterone determination in unextracted bovine plasma using the second antibody technique. *Journal of Steroid Biochemistry*, vol. 28, no. 6, p. 623-627. http://dx.doi.org/10.1016/0022-4731(87)90389-X PMid:3320573

Reed, W. L., Clark, M. E., Parker, P. G., Raouf, S. A., Arguedas, N., Monk, D. S., Snajdr, E., Nolan, V., Ketterson, E. D. 2006. Physiological effects on demography: a long-term experimental study of testosterone's effects on fitness. *The American Naturalist*, vol. 167, no. 5, p. 667-83. http://dx.doi.org/10.1086/503054 PMid:16671011

Santoso, B., Mwenya, C., Sar, C., Takahashi, J. 2006. Ruminal fermentation and nitrogen metabolism in sheep fed a silage-based diet supplemented with *Yucca Schidigera* or *Yucca schidigera* and nisin. *Animal Feed Science and Technology*, vol. 129, no. 3-4, p. 187-195. http://dx.doi.org/10.1016/j.anifeedsci.2006.01.001

Sirotkin, A. V., Chrenek, P., Darlak, K., Valenzuela, F., Kuklova, Z. 2008. Some endocrine traits of transgenic rabbits. II. Changes in hormone secretion and response of isolated ovarian tissue to FSH and ghrelin. *Physiological Research*, vol. 57, p. 745-751. PMid:17949242

Sirotkin, A. V., Rafay, J., Kotwica, J. 2009. Leptin controls rabbit ovarian function *in vivo* and *in vitro*: possible interrelationships with ghrelin. *Theriogenology*, vol. 72, no. 6, p. 765-72. http://dx.doi.org/10.1016/j.theriogenology.2009.05.011 PMid:19616295

Sirotkin, A. V. 2010. Effect of two types of stress (heat shock/high temperature and malnutrition/serum deprivation) on porcine ovarian cell functions and their response to hormones. *The Journal of Experimental Biology*, vol. 213, p. 2125-2130. http://dx.doi.org/10.1242/jeb.040626 PMid:20511527

Sirotkin, A. V. 2014. *Regulators of Ovarian Functions*. Nova Publishers, New York, p. 193. ISBN 978-1-61324-468-5

Southon, S., Wright, A. J. A., Price, K. R., Fairweather-Tait, S. J., Fenwick, G. R. 1988. The effect of three types of saponin on iron and zinc absorption from a single meal in the rat. *British Journal of Nutrition*, vol. 59, p. 389-396. http://dx.doi.org/10.1079/BJN19880048 PMid:3395602

Swaab, D. F., Garcia-Falgueras, A. 2009. Sexual differentiation of the human brain in relation to gender identity and sexual orientation. *Functional Neurology*, vol. 24, no. 1, p. 17-28. PMid:19403051

Tanaka, O., Tamura, Y., Masuda, H., Mizutani, K. 1996. Application of saponins in foods and cosmetics: saponins of Mohave yucca and *Sapindus mukurossi*. *Advances in Experimental Medicine and Biology*, vol. 405, p. 1-11. http://dx.doi.org/10.1007/978-1-4613-0413-5_1 PMid:8910691

Watanabe, C. M. H., Wolfram, S., Ader, P., Rimbach, G., Packer, L., Maguire, J. J. 2001. The *in vivo* neuromodulatory effects of the herbal medicine ginkgo biloba. *Proceedings of*

the National Academy of Sciences, vol. 98, p. 6577-6580. http://dx.doi.org/10.1073/pnas.111126298 PMid:11381109

Acknowledgments:
The authors thank to Ing. Ž. Kuklová and Mrs. K. Tóthová (Animal Production Research Centre in Nitra-Lužianky) for technical assistance. This publication was written during realization of the project "ZDRAVIE" no. 26220220176 supported by the Operational Programme Research and Development funded from the European Regional Development Fund.

Contact address:
Aneta Stochmalova, Constantine the Philosopher University, Tr. A. Hlinku 1, Department of Zoology and Anthropology, 949 74 Nitra, Slovakia; E-mail: Anetka@centrum.sk.

Attila Kadasi, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and and Food Science, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia; E-mail: attila.kadasi@gmail.com

Richard Alexa, Constantine the Philosopher University, Department of Zoology and Anthropology, Tr. A. Hlinku 1, 949 74 Nitra, Slovakia; E-mail: rikialexa@gmail.com.

Roland Grossmann, Department of Functional Genomics and Bioregulation, Institute of Animal Science, Mariensee, Neustadt, Germany, E-mail: grossman@tzv.fal.de

Alexander Sirotkin, Institute for Genetics and Reproduction of Farm Animals, Animal Production Research Centre Nitra, Hlohovecka 2, 951 41 Lužianky, Slovak Republic and Department of Zoology and Anthropology, Constantine the Philosopher University, Tr. A. Hlinku 1, 949 74 Nitra, Slovakia; E-mail: sirotkin@cvzv.sk