THE EFFECT OF HERBAL PLANTS *YUCCA SHIDIGERA* AND *CURCUMA LONGA* ON RABBIT FEMALE REPRODUCTION

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The aim of the study was to evaluate the effect of Yucca shidigera and Curcuma longa herbal additive on rabbit female reproduction. White New Zealand line rabbit females (two months old) were used in the experiment. The animals were divided into the control (C: without Yucca shidigera and Curcuma longa addition, n = 29) and experimental groups (E1: Yucca shidigera, n = 17; E2: Yucca shidigera, n = 12; E1: Curcuma longa, n = 15; E2: Curcuma longa, n = 12). All the studied animals were housed in the wired-floor cages at the air temperature of $22 \pm 3^{\circ}$ C, humidity 75 ± 5 %, ad libitum access to water and feed. The rabbits in the control group (C) were fed with a commercially available feed; in the first experimental groups (E1) 5 g of Yucca shidigera and Curcuma longa plant powder was added to 100 kg normal feed. In the second experimental groups (E2) 20 g of Yucca shidigera and Curcuma longa plant powder was added to 100 kg normal feed. The animals were fed for 50 days and weighed weekly. The rabbit females were hormonally treated and inseminated using heterospermic insemination dose (0.5 ml I.D. per female) after reaching the sufficient weight (at least 3.5 kg). Conception rate, kindling rate, the number of liveborn and number of stillborn young rabbits were evaluated in this experiment.

The highest conception and kindling rate (p < 0.05) were found out in the *Yucca shidigera* E2 group (100 %-conception rate; 100 %-kindling rate) and *Curcuma longa* E2 group (83.3 %-conception rate; 80.0 %-kindling rate), when compared to control group (65.5 % -conception rate; 58.6 % -kindling rate). Both herbal additives improved reproduction parameters in rabbit females; furthermore, *Yucca shidigera* addition was more efficient than *Curcuma longa* addition in our experiment.

Key words: rabbit does; *Yucca shidigera*; *Curcuma longa*; conceptional rate; kindling rate

Acknowledgement: This experimental work was supported from the grant APVV-0854-11: Phytochemicals as natural regulators of ovarian functions of livestock.

COMPARISON OF GENETIC VARIABILITY BETWEEN SLOVAK AND CZECH VALACHIAN SHEEP POPULATIONS

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The aim of this study was to compare the genetic variability in small populations of Valachian sheep maintained as genetic resources in Slovakia and the Czech Republic. Ninety eight animals from different regions of Slovakia (SR) and forty eight animals from the Czech Republic (CZ) were individually genotyped using 18 microsatellite markers in two multiplex PCR. The number of alleles, frequency of alleles, heterozygosity, polymorphism information content, Hardy-Weinberg equilibrium and null allele frequency estimate were calculated for all microsatellites using Cervus 3.0.3. All loci were polymorphic in both populations showing between 4 alleles (D5S2, McM527, SPS113, TCRVB6 in CZ) and 22 alleles (OarFCB304 in SK), PIC between 0.377 (TCRVB6 in CZ) and 0.887 (HSC in SK). The Slovak population showed apparently higher average number of alleles per locus (15.05 in SR vs 7.27 in CZ), higher mean expected heterozygosity $(0.83 \pm 0.06 \text{ in SR vs } 0.67 \pm 0.11 \text{ in CZ})$ and mean observed heterozygosity (0.75 \pm 0.08 in SK vs 0.61 \pm 0.13 in CZ) than the Czech population. The deviation from Hardy-Weinberg equilibrium ($P \le 0.05$) was detected only in two loci common for both populations, D5S2 and MAF 214, probably due to the presence of null-alleles. Despite the dramatic reduction in the population size of Valachian sheep breed in both countries in the past these data suggest higher genetic variability in the Slovak population.

Key words: Valachian sheep; genetic resources; genetic variability; microsatellites

ANTIOXIDANT STATUS OF PORCINE OVARIAN GRANULOSA CELLS EXPOSED TO IRON *IN VITRO*

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The objective of present study was to determine the dosedependent effect of iron (Fe) in vitro on total antioxidant status (TAS) of porcine ovarian granulosa cells. The cells were cultured with iron sulphate (FeSO4.7H2O) at various doses of Fe in the experimental groups 0.17, 0.33, 0.5 and 1.0 mg.ml⁻¹ (E1, E2, E3, and E4) for 18 hrs. The cells without Fe exposure served as the control. TAS was analysed by spectrophotometer Genesys 10. Antioxidant status was dependent on Fe doses and decreased in all experimental groups, when compared to the control. Statistical analysis showed significantly lower value (P < 0.05) in the E4 group with the highest dose of Fe in comparison with the control group. Trace elements may adversely affect reproductive system of animals through oxidative stress induction. The research in the area of antioxidant system and iron effects will be worthy of further investigation.

Key words: porcine ovarian granulosa cells; iron; antioxidant status

Acknowledgment: This work was financially supported by VEGA scientific grant 1/0084/12.

CRYOPROTECTANTS USED FOR SEMEN FREEZING AFFECT RABBIT REPRODUCTIVE PERFORMANCES

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The aim of this study was to compare the effect of two different cryoprotectants (CPAs), dimethylacetamide (DMA) and dimethylsulfoxide (DMSO) on the fertility and prolificacy of rabbit does artificially inseminated with cryopreserved semen. Semen was collected using an artificial vagina from 32 mature hybrid rabbit bucks. Ejaculates were pooled (four ejaculates/pool) in order to avoid individual differences and transported to the laboratory in a water bath at 30°C and cooled at 5°C for 90 minutes. Each pool was divided into 2 semen samples, that were diluted 1:1 (v:v) with the freezing extender composed of Tris-citric acid-glucose, 2 % sucrose (as a nonpermeable CPA) and 12 % DMA or 16 % DMSO (as permeable CPAs) to give a final concentration of 1 % sucrose and 6 % DMA or 8 % DMSO. After dilution, the semen was packed into 0.25 ml plastic straws, equilibrated at 5°C for 45 min and frozen in liquid nitrogen vapour for 10 min before plunging into liquid nitrogen for storage (-196°C). Three groups of rabbit does (n = 114) were inseminated with a fresh semen and with semen frozen using the DMA and DMSO protocols. All the does were i.m. treated with cyclogonine. (20 IU/doe) for estrous synchronization 48 h before insemination. At the time of insemination, each female was treated by intramuscular injection of buserelin acetate to induce ovulation (1 µg/doe). Fertility for each doe was checked by abdominal palpation 12 days after the insemination. Kindling rate, total number of kids born and the number of young born alive were determined at parturition. Similar fertility rates and litter size (total number of born) were recorded in the DMSO group (79.8 % and 7.7 ± 0.3 young per kindling) and fresh semen group (81.6 % and 8.6 \pm 0.3), what was higher ($P \le 0.05$) in comparison with the DMA (47.4 % and 6.7 ± 0.4) group. Moreover, the number of kids born alive in DMSO group (7.2 ± 0.3) was lower $(P \le 0.05)$ in comparison with fresh semen (8.3 ± 0.3) but higher $(P \le 0.05)$ compared to the DMA (5.9 ± 0.4) group.

In conclusion, our results show that DMSO is more effective cryoprotectant than DMA for rabbit sperm freezing, because the semen frozen with DMSO showed a fertility potential similar to that of fresh semen.

Key words: rabbit; spermatozoa; DMA; DMSO; cryopreservation; fertility

PATULIN – INDUCED CHANGES IN HAEMATOLOGICAL PARAMETERS OF RABBITS FED BY STRAWBERRY LEAVES

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Key words: haematological parameters; strawberry leaves; patulin; rabbit

Acknowledgments: This work was financially supported by VEGA scientific grant 1/0084/12, 1/0022/13, and KEGA grant 030SPU-4/2012.

CARNIOLAN BEE IN SLOVAKIA

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In Slovakia, the original local bee belongs to the Carniolan bee (*Apis mellifera carnica*). Kepena (1975), an employee of the Institute of Apiculture in Liptovský Hrádok, confirmed this fact by detailed morphometric measurements. The origin of Carniolan bees is located in the area of the Carpathians. Carpathians formed a natural boundary of its original distribution to the north and east. Carniolan race is the best race for the climatic conditions of the Central Europe, but it can also survive outside this zone. These bees are peaceful and quiet. In the spring they have a rapid development. They are adapted to mild and severe

winter very well. To overcome the winter, they are grouped in medium weight clusters, 1 to 1.5 kg of winter bees. They have very economical consumption of stocks during the winter as well as during a whole year. They collect nectar and honeydew very well from all insectophile / insect-pollinated plants and they can quickly shift to new food sources. They lid stocks airy. Carniolan honeybee race derives several ecotypes. We classify all accepted Slovak lines (Tatranka, Vojničanka, Sitňanka, etc.) to the Carpathian ecotype.

Bees, kept in our area since ancient times, have been attacked by bees imported from other countries. We can say that besides Carniolan bee, Italian bee also participated in the development of Slovak bees, but not in such a large extent. Slovak Carniolan bee may also be negatively affected by uncontrolled individual import of mothers from other breeds. Bastardization of Slovak Carniolan bee population may lead to reduction of bee activity and raise of their aggression. Affiliation to Carniolan race is assessed numerically by evaluating of the typical morphological features. For the conservation of the gene pool of the original Carniolan bees it is necessary to involve also modern molecular methods for the verification of genetic markers, besides the traditional ones.

Due to these characteristics Carniolan bees are the most widespread in central Europe because they have adapted to the climate and environmental conditions. The fact that the current Slovak Carniolan bees are racially profiled and best adapted to the conditions of Slovakia is proved by the fact that Slovakia has not been affected by large colony losses (so-called CCD) to so high extent as it is happening abroad.

In Slovakia, the breeding of Carniolan bees is covered by queen breeders organized into the Association of Slovak Carniolan bee breeders. Its members have to prove that they kept Carniolan bee, what is currently determined by morphometric measurement of wings and according to colour of tergits. The DNA mapping of bees reared in Slovakia is performed using molecular biology.

After Slovenia, we are probably the second country in Europe, which aims using pure-bred breeding to keep only one breed, autochthonic one, which lives and has been developed here for long period. It is unethical and unnecessary to import other breeds and try to introduce them here. In Slovakia, from the breeders' point of view, the only Carniolan race could be kept. For recovery of the gene pool, pure-bred Carniolan lines may be imported from abroad with permission from authorized breeders' organization - Institute of Apiculture Liptovský Hrádok, Animal Production Research Centre Nitra.

Key words: Carniolan bee; bee breeding; morphometric measurement of wings; importation of queen bees

THE RELEASE OF ESTRADIOL BY PORCINE OVARIAN GRANULOSA CELLS AFTER AMYGDALIN ADDITION *IN VITRO*

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Amygdalin, sometimes termed as vitamin B_{17} , is a cyanogenic glycoside abundant in the seeds of bitter almond, apricots of

the Prunus genus and other rosaceous plants. It has been used as a traditional drug because of its wide range of medicinal benefits, including curing or preventing cancer, relieving fever, suppressing cough and quenching thirst. The aim of this in vitro study was to examine the secretion activity (steroid hormone estradiol) of porcine ovarian granulosa cells after amygdalin addition. Granulosa cells from non-cyclic and cyclic porcine ovaries were incubated with amygdalin at the doses of 1, 10, 100, 1000 and 10000 µg/mL for 24 hours and compared to the control without amygdalin addition. The release of estradiol by granulosa cells from non-cycling and cycling porcine ovaries was assessed by ELISA. Our results showed that amygdalin at the highest dose (10 000 μ g/mL) significantly ($P \le 0.05$) affected releasing of estradiol by granulosa cells from non-cyclic and cyclic porcine ovaries. In the experimental groups with lower doses of amygdalin no statistically significant differences in the estradiol release were found. In conclusion, our results indicate possible dosedependent effect of amygdalin on secretion activity of porcine ovarian granulosa cells.

Key words: amygdalin; secretion activity; estradiol; ovarian granulosa cells

Acknowledgments: This work was financially supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic; projects no. 1/0790/11 and 1/0022/13.

CHARACTERIZATION OF ORAVKA BREED: CHICKEN GENETIC RESOURCE

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Growth and some reproduction traits (fertilization, hatchability and egg weight) of Oravka breed were measured in two chicken lines (OR2 and OR3). The in situ conservation flock kept in Animal Production Research Centre Nitra (APRC Nitra) during the period between 2009 and 2012 was included in the experiment. During each season the breeding males outside the APRC Nitra flock were used. The breeding females were originated from the mating between females raised in the APRC Nitra flock and those new males. The weight at age of 5, 12 and 20 weeks was monitored. The average weights of OR2 were ranged from 371.3 ± 68.9 g to 538.1 ± 79.4 g at 5 weeks, from 1246.1 ± 254.0 g to 1464.5 ± 242.2 g at 12 weeks and from 2076.3 ± 381.8 g to 2286.1 ± 535.4 g at 20 weeks of age. The fertilization rate for each line and season was higher than 84 % except for OR2 line in the season 2011/2012. The hatchability from fertilized eggs was higher than 80 % except for OR2 line in the season 2011/2012. The average weights of eggs in the middle of laying period (from March to May) were ranged from 53.9 ± 3.5 g to 56.6 ± 4.3 g for OR2 and from 52.9 ± 4.5 g to 56.4 ± 3.6 g for OR3 during the whole experiment.

Key words: Oravka; growth; fertilization; hatchability; egg weight; genetic resources

EFFECT OF GENOTYPE ON SOME EXTERNAL AND INTERNAL EGG QUALITY PARAMETERS OF JAPANESE QUAIL (*COTURNIX JAPONICA*) IN GENETIC RESOURCE

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A research was carried out to determine effect of different genotypes of Japanese quail (Coturnix japonica) in genetic resource on some external and internal egg quality parameters. The birds were housed as 1 male and 3 females per cage of 0.12 m² area at Animal Production Research Centre Nitra and fed with a mixture of 9.0 MJ ME and 145.0 g of crude protein during the experiment. Feed and water were given ad libitum. Analysis of external and internal parameters of Japanese quail eggs was performed in the laboratory of the Department of Poultry Science and Small Animal Husbandry at the Faculty of Agrobiology and Food Resources of the Slovak University of Agriculture in Nitra. This research was conducted to investigate the effects of genotype on egg weight, egg length, egg width, egg shape index, shell weight, percentage of shell, shell thickness, shell strength, albumen weight, percentage of albumen height, albumen width, albumen length, albumen index, Haugh unit, volk weight, percentage of yolk, yolk height, yolk width, yolk index and yolk colour. We have found significantly higher values for meat type in terms of all egg parameters ($P \le 0.05$). In case of shell parameters, we observed significant ($P \le 0.05$) difference between genotypes only in shell weight in benefit of the meat type. There were significant ($P \le 0.05$) differences found between the genotypes in points of albumen height and albumen index for laying Japanese quail. The significant (P < 0.05) difference in benefit of the meat type was found in yolk weight, yolk percentage, yolk height and yolk index. For all other characteristics no significant differences in egg quality between the laying and the meat type of Japanese quail were observed.

Key words: Japanese quail; egg; external quality; internal quality

VITRIFIED RABBIT EMBRYOS AND SPERM AS POTENTIAL GENETIC RESOURCES

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The aim of our study was to assess the effect of Ficoll 70 on the post-thaw quality of New Zealand White rabbit embryos and spermatozoa. Rabbit embryos at morula stage were cryopreserved using OPS (open pulled straw) vitrification method (CIM - CO_2 independent medium + 40 % EG + 18 % ficoll + 0.3 M sucrose). Rabbit ejaculates of good initial quality (> 75 % motility) were diluted in a freezing medium composed

of commercial diluent, 8 % dimethylsulphoxide and 1 % sucrose enriched with 0.5, 1, and 2 % Ficoll 70, respectively. The semen samples were packed in straws and frozen in liquid nitrogen vapour according to the protocol.

The cleavage rate of vitrified/warmed early morula stage embryos up to the hatched blastocyst stage *in vitro* was 64.70 % after 2 hours of storage in liquid nitrogen. Computer Assisted Sperm Analyser showed that 2 % Ficoll 70 had a positive effect (p < 0.05) on the total motility and progressive motility (E4; 42.235 ± 5.695 and 30.385 ± 5.488) of rabbit spermatozoa when compared to the medium without Ficoll 70 (E1; 35.684 ± 7.468 and 24.968 ± 6.030), respectively.

Our preliminary results indicate that vitrification medium enriched with Ficoll 70 had a positive effect on the quality of cryopreserved rabbit embryos and spermatozoa compared to medium without Ficoll 70. However, other experiments are required to confirm our suggestion.

Key words: rabbit; embryo; spermatozoa; cryopreservation

Acknowledgments: This work was supported from the grant APVV-0556-011 coordinated by the Slovak Research and Development Agency. The research leading to these results has received funding from the European Community under project no 26220220180: Building Research Centre "AgroBioTech".

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

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The target of rapamycin (mTOR)-dependent intracellular mechanisms can play an important role in regulation of ovarian cell functions, but its effects and mechanisms of action are studied insufficiently. The aim of this in vitro study was to examine the effect of rapamycin, a plant mTOR inhibitor, (at the additions of 0; 1; 10; 100 µg/ml) on proliferation, apoptosis and hormone release of porcine granulosa cells. The presence of the proliferation-associated peptide (PCNA) and apoptotic peptide (BAX) in the ovarian granulosa cells was determined by immunocytochemistry. The secretion of hormones (progesterone, testosterone) was analysed by RIA. It was observed that the addition of rapamycin decreased the PCNA and increased the BAX accumulation in the ovarian cells at all doses added. The rapamycin treatment also suppressed progesterone release (at dose of $1 \mu g/ml$), whereas the release of testosterone was unaltered. These observations demonstrate the involvement of the mTOR signalling system in control of female reproductive functions via changes in ovarian cell proliferation, apoptosis and steroidogenesis. Rapamycin, an inhibitor of mTOR system, can directly affect these ovarian cell functions and, therefore, it could be potentially useful for the suppression of ovarian functions.

Key words: Rapamycin; mTOR; proliferation; apoptosis; steroidogenesis; granulosa cells

Acknowledgments: We thank Mrs. Katarína Tóthová and Ing. Žofia Kuklová (Animal Production Research Centre Nitra) and Mrs. Iris Stelter (Institute of Animal Science, Neustadt, Germany) for technical assistance. This work was financially supported by the Ministry of Education of the Slovak Republic (project no. 1/0790/11), Slovak Agency for Promotion of Research and Development (APVV, projects no. 0137-10 and 0854-11), Slovak Academic Information Agency (SAIA, project no. 740531-OPVaV-2011/2.2/07-SORO) and Operational Programme Research and Development funded from the European Regional Development Fund (project no. 26220220176).

THE EFFECT OF CHOSEN BEE PRODUCTS ON BIOCHEMICAL CONSTITUENTS OF CHICKEN BLOOD

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The aim of this work was to investigate the effect of chosen bee product (propolis and bee pollen) inclusion to the diet on some serum biochemical parameters (glucose, total cholesterol, triglycerides) of chickens. Broiler chickens (n = 99) were divided into 3 groups. First group without any addition served as a control (C, n = 33). Experimental groups received propolis extract (E1, n = 33) in feed mixture at the dose of 200 mg.kg⁻¹, bee pollen extract (E2, n = 33) at the dose of 800 mg.kg⁻¹. After 42 days of feeding chickens were slaughtered and blood samples (n = 10 in each group) were collected. The blood serum was separated from whole blood by centrifugation at 3000 g for 30 min. The concentrations of serum components in blood serum of broiler chickens were analysed using automatic analyser Microlab 300 (Merck, Germany) using Ecoline kits according to the manufacturer guidelines. Significant decrease in serum glucose in the experimental group E2 (11.76 mmol.1-1), when compared to the control (14.70 mmol.1-1) and the E1 (14.73 mmol.l⁻¹) group, was measured. The addition of propolis extract and bee pollen extract to the diet of broiler chickens had slight or no effect on values of triglycerides, cholesterol, as differences among the groups were insignificant (P > 0.05).

Key words: propolis; bee pollen; broiler chickens; blood biochemistry

Acknowledgments: This work was financially supported by VEGA scientific grants 1/0022/13, 1/0084/12, and KEGA 030 SPU-4/2012.

SPERMIOPHAGES IN RABBIT EJACULATES AFFECT FUNCTIONAL PARAMETERS OF SPERMATOZOA

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The objective of our study was to access the influence of spermiophages on the membrane stability and motility of the rabbit spermatozoa. Semen samples from rabbit males were collected using an artificial vagina and spermiophages were analysed using an Alexa-AcLDL fluorescent dye (Acetylated Low Density Lipoprotein; Molecular Probes, USA). The sperm samples were divided into two groups basing on the number of spermiophages. Spermatozoa were stained for plasma membrane integrity (peanut agglutinin, PNA-FITC) and occurrence of dead/necrotic sperm (propidium iodide, PI). The quality of semen samples was evaluated by CASA system (Sperm Vision[™], Minitube, Germany). Our results show that the percentage of the spermatozoa with disturbed plasma membrane was lower in the experimental group S1 with less than 20 % of spermiophages in sperm samples, than in the experimental group S2 with 40 % of spermiophages (S1 vs. S2; 16.83 ± 2.33 vs. 20.03 ± 2.21). The negative impact of spermiophages was expressed also by the higher number of dead/necrotic spermatozoa (S1 vs. S2; 7.57 ± 1.66 vs. 10.89 ± 1.46) and the lower motility (M) and progressive movement (PM) of the spermatozoa (S1 vs. S2; M: 84.05 ± 7.22 vs. 79.33 ± 6.89 ; PM: 70.35 ± 7.04 vs. 68.29 ± 6.84). These preliminary results indicate that the higher occurrence of spermiophages may have negative influence on the quality of rabbit spermatozoa.

Key words: rabbit; spermatozoa; spermiophages; plasma membrane; motility

Acknowledgement: This work was supported from the grant of Slovak Research and Development Agency - APVV-0556-011.

PRODUCTION AND EXTERIOR TRAITS OF ORIGINAL VALACHIAN BREED

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The objective of this study was to analyse the population of original Valachian breed in Slovakia in concern to the animal genetic resource. Breed promotion and breeding operations are managed under the guidance of the Original Valachian Breeders' Club, which works under the umbrella of the Sheep and Goat Breeders' Association of the Slovak Republic. The breed standard and breeding programme are in place. Breeding is aimed at maintaining of genetic variability and of typical wool and exterior characteristics (colour variability and both horned and polled individuals). At the end of 2012, there were 31 living adult males and 225 living adult females registered in the flock book. The animals are kept in two nucleus and five multiplier flocks. The number of ewes per flock ranges

from 6 to 93. In the period between 2010 and 2012 the average fertility was 90.3 ± 12.0 %, the average fecundity was 123.4 ± 23.1 % and the average prolificacy was 136.4 ± 17.1 %. In milk recorded ewes, the average milk yield per 150-day lactation length was 96.3 ± 6.1 l, the average fat percentage was 7.660 ± 0.462 %, the average protein percentage was 5.920 ± 0.221 % and the average lactose percentage was 4.580 ± 0.066 %. The average daily gain in male and female lambs till weaning at age of 45 to 55 days was 246.70 ± 81.51 g and 242.90 ± 48.80 g, respectively. According to the analysis of breed phenotype, which was done in four original Valachian flocks in 2010 (in total 102 heads), 48.0 % were polled animals and 36.3 % were animals with lyre horns (so called "širaňa" animals). The majority (30.4%) were the animals of "bekaša", "murga" or "mucha" colour pattern (white coat with black spots on head and legs), 28.4 % were the animals of "lajka" colour pattern (wool with black and white hairs, head and legs are mostly black) and 18.6 % were the animals of "belica" colour pattern (white coat and wool without any markings). About 52 % were the animals with white wool and 15.7 % were the animals with black wool. About 42 % were the animals with hair diameter between 37.1 and 40.0 µm (grade D) and 39 % were the animals with hair diameter between 40.1 and 43 µm (grade DE). None animals with reduced auricle (so called "čulka" animals) were found. According to PrP genotyping (there were 55 tested individuals in the period between 2010 and 2012), 36.4 % were the animals of the ARR/ARR genotype and 38.2 % were the animals of the ARR/ARQ genotype. The ARR allele frequency was 0.582 and the ARQ allele frequency was 0.327. The monitoring based on genetic and phenotypic evaluation of the original Valachian sheep population, aimed at the maintaining typical adaptability, production and exterior traits, is recommended to be done regularly.

Key words: sheep; original Valachian breed; animal genetic resource; production traits; exterior; wool

EFFECT OF GREEN TEA POLYPHENOLS (GTPP) ALONE OR IN COMBINATION WITH T-2 TOXIN ON STEROID HORMONE PRODUCTION BY THE OVARIAN GRANULOSA CELLS

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Green tea extracts have beneficial effects on live organisms. It is well known that green tea has strong antioxidant, anticarcinogenic, anti-inflammatory and anti-radiation effect. T-2 toxin is a mycotoxin, which occurs predominantly in cereals and cereal-based products. T-2 toxin is considered to be a strong cytotoxic mycotoxin. The aim of present study was to examine the ability of ovarian granulosa cells (GCs) to produce progesterone and estradiol after addition of green tea polyphenols (GTpp) either alone or in combination with T-2 toxin. Ovarian granulosa cells were incubated without (control) or with green tea polyphenols $(1, 10, 100 \ \mu g.ml^{-1})$ and in combination of green tea polyphenols (50 µg.ml⁻¹) with T-2 toxin (1, 5, 50 ng.ml⁻¹) (experimental groups) for 48h. After the addition of GTpp (1, 10, 100 µg.ml⁻¹) no significant changes in secretion of progesterone were observed. Secretion of estradiol by ovarian GCs was significantly stimulated after the addition of GTpp (1 µg.ml⁻¹). No significant changes in estradiol secretion were observed after the addition of higher doses (10, 100 µg. ml⁻¹). After addition of green tea polyphenols with T-2 toxin no significant changes in progesterone secretion were observed. Estradiol secretion was significantly stimulated after the addition of GTpp (50 µg.ml⁻¹) with T-2 toxin (5 ng.ml⁻¹). No significant changes in estradiol secretion by ovarian GCs were observed after the addition of GTpp (50 µg.ml⁻¹) with T-2 toxin (1, 50 ng.ml⁻¹). In conclusion, because of the lack of information about the effect of GTpp and its combination with T-2 toxin on ovarian cells, it is necessary to continue in this investigation. Green tea extracts with their high antioxidant status could have protective effect against cytotoxicity of mycotoxins.

Key words: Green tea polyphenols (GTpp); T-2 toxin; progesterone; estradiol; granulosa cells

Acknowledgments: The authors are thankful to Romer Labs Division Holding GmbH, Tulln, Austria for providing mycotoxins and to the colleagues from the Institute of Genetics and Reproduction of Farm Animals, Animal Production Research Centre Nitra, Mrs. Katarína Tóthová and Ing. Žofia Kuklová for their skillful technical assistance. This work was financially supported by the Ministry of Education of the Slovak Republic - project no. 1/0790/11, 1/0084/12, 1/0022/13.

LH-INDUCED PROGESTERONE SECRETION BY PORCINE OVARIAN GRANULOSA CELLS AFTER FUSARIOTOXINS EXPOSURE

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Normal ovarian steroid production in most mammals occurs via the "two cell/two gonadotropin" model. Granulosa cells (GCs) become "luteinized" after aspiration from follicles because they have limited oxygen and cholesterol supply, thereby are able of progesterone synthesis in large amounts. Luteinizing hormone (LH)-induced cultivation of GCs reveals a large and rapid rise in progesterone production in porcine ovarian granulosa cells from non-cyclic gilts. The aim of the study was to examine the LH-induced progesterone secretion by porcine ovarian GCs after addition of Fusarium toxin deoxynivalenol (DON), zearalenon (ZEA) and T-2 toxin each at dose of 100 ng/ml for 24h. Direct measurements of ovarian progesterone content in culture medium were accomplished by ELISA. The doses of 0.1, 1 and 10 µg/ml of LH in culture medium significantly ($P \le 0.05$) increased progesterone secretion by GCs. The combination of the lowest LH dose (0.1 µg/ml) and 100 ng/ml of T-2 toxin (but not ZEA and DON) significantly ($P \le 0.05$) decreased GCs progesterone release. Progesterone secretion was significantly ($P \le 0.05$) stimulated by 1 µg/ml of LH in combination with DON, ZEA and T-2 toxin (each at dose of 100 ng/ml). DON and ZEA (but not T-2 toxin) each at dose of 100ng/ml in combination with 10 µg/ml of LH significantly ($P \le 0.05$) increased progesterone secretion. Our findings reveal that *Fusarium* toxins can act as a possible endocrine disruptor in the steroidogenesis.

Key words: DON; ZEA; T-2 toxin; LH; progesterone; granulosa cells

Acknowledgments: The authors are thankful to Romer Labs Division Holding GmbH, Tulln, Austria for providing mycotoxins and colleagues from the Institute of Genetics and Reproduction of Farm Animals, Animal Production Research Centre Nitra. This work was financially supported by the Ministry of Education of the Slovak Republic - project no. 1/0790/11, 1/0084/12, 1/0022/13.

SCREENING FOR TOLL-LIKE RECEPTOR GENE POLYMORPHISM IN THE TRADITIONAL CATTLE BREEDS

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The traditional breeds are supposed to be a valuable source of genes in the breeding programmes for sustainable agriculture. Notably, the genes responsible for the health traits might be of interest due to the presumed adaptation of the traditional breeds to the local environmental and breeding conditions and thanks to a higher intravarietal diversity. Additional variants of the microbe resistance genes are demanded by the changing spectrum of pathogens, legal and practical limits on the antibiotic prevention and therapy, and by the eroding genetic diversity in modern breeds. Therefore, a screening has been carried out for the structural polymorphism in the innate immunity receptors of the TLR family in two historical Czech cattle breeds that are conserved in frame of the national program of Conservation and Use of Farm Animal Genetic Resources. The survey in the Czech Red and Czech Pied cattle will include 50 individuals of each breed and is aimed at the genes coding for receptors participating in the interactions with the bacterial pathogens in frame of the innate immunity. The sequencing of the PCR-fragments of the TLR4 gene discovered four haplotypes. To date, no new gene variants specific for the local breeds have been revealed. The comparatively low diversity in the completed locus TLR4 can be ascribed to the population bottleneck in the history of both breeds and to the outcrossing with modern breeds. Nevertheless, the polymorphism in the highly conserved TIR domain of the TLR4 receptor resulting in the exchange Ile674Thr has been shown to occur in the nucleus herds of both breeds. The difference in the amino acid character has been predicted to associate with a functional change of the molecule. Accordingly, the variant of the receptor with threonine has been reported to lead to an increased infection resistance in the Canadian and Chinese Holstein populations. The validation of its effect in both historical breeds and in the production herds of the Czech Pied cattle is a logical next step.

Key words: cattle; disease resistance; diversity; innate immunity; Toll-like receptor

THE EFFECTS OF BENZOIC ACID AND PROTEIN LEVEL ON URINE PH AND AMMONIA EMISSION IN PIGS

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Twelve hybrid gilts (initial body weight 29.9 ± 1.7 kg) were used for the evaluation to identify the effect of benzoic acid and dietary protein in the diet on urine pH and ammonia of the slurry in growing piglets. We used two protein concentrations (high protein 18.8 %, HP and low protein 14.0 %, LP) and two benzoic acid levels (0.0 % and 1.0 %). The same energy level (13.3 MJ. kg) in the diets was achieved by means of the supplementation with rapeseed oil. The piglets were housed in metabolic cages and fed with two equal doses at 7 a.m. and 5 p.m. at a daily rate of 90 g.kg^{0.75}. Water was supplied ad libitum. Each experimental period consisted of a 6-day adaptation, which was followed by a 4-day collection phase, when faeces and urine were collected using bladder catheters. Experimental data were subjected to ANOVA and when significant value was observed for treatment effect, the differences between means were assessed using Fisher's LSD procedure.

Nitrogen (N) and dry matter intake were not significantly affected in any diets. The amount of retained nitrogen from intake was significantly increased only in pigs fed the diet with benzoic acid and HP. We found a significant decrease in urine pH (-0.7 and -0.9) in both experimental groups fed with benzoic acid diets, regardless of the nitrogen content in the diet. The coefficients of excretion determination between hippuric acid and urine pH were R2 = 0.57 both for the HP and LP diets. The higher but not significant decrease in ammonia nitrogen was observed in the experimental LP groups.

Key words: ammonium excretion; benzoic acid; urine pH; piglets

Acknowledgments: This article was written during realization of the project "BELNUZ No. 26220120052" supported by the Operational Programme Research and Development funded from the European Regional Development Fund.

EFFECT OF QUERCETIN AND T-2 TOXIN ON SERUM ALBUMIN AND BILIRUBIN CONTENT IN RABBITS

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The aim of the present paper was to determine the effect of long-term application of quercetin at various doses and T-2 toxin at acute dose on the content of bilirubin (BR) and albumins in rabbit's blood.

The animals were divided into two control groups (C1 and C2) and six experimental groups (E1 - E6). Experimental groups received quercetin (Sigma Aldrich, Saint Louis, USA)

in injectable form (intramuscularly) at 10 µg.kg⁻¹ in E1 and E2 group, 100 µg.kg⁻¹ in E3 and E4 group and 1000 µg.kg⁻¹ in E5 and E6 group without T-2 toxin for 90 days. T-2 toxin (Romer Labs Division Holding GmbH, Tulln, Austria) was applied to C2, E2, E4 and E6 groups at dose 0.08 mg per kg of body weight 72 hours before slaughter. Control group (C1) was injected with water (Imuna Pharm a.s. Šarišské Michal'any, Slovak Republic).

After T-2 toxin treatment statistical analysis showed insignificantly (P > 0.05) lower levels of bilirubin in the experimental groups (E4, E6) in comparison with the control group with T-2 toxin (C2). When differences between groups with/without T-2 toxin were compared, insignificant increase in the groups treated with T-2 toxin (C2, E2 and E6) and decrease in the E4 group was found. Our results are consisted with results of other authors. In case of albumins slight, but not significant, decrease in the content of albumins in all experimental groups (with or without T-2 toxin) vs. the control group was observed. Based on the literature natural substances could slightly modify the serum content of bilirubin and albumins and consequently influence antioxidant balance in the organism. To prove this assumption further research using quercetin and T-2 toxin is required.

Key words: quercetin; superoxide dismutase; rabbits

Acknowledgments: This work was financially supported by VEGA scientific grant VEGA 1/0084/12 and 1/0022/13 and KEGA 030 SPU-4/2012.

EXPERIMENTAL TESTING OF LAPAROSCOPIC INTRAUTERINE INSEMINATION OF MOUFFLON FEMALES WITH FROZEN SEMEN

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The aim of the research was to evaluate the use of laparoscopic intrauterine insemination (LII) of moufflon females using deep-frozen moufflon semen. Fourteen moufflon females were synchronized during the anestric period using CIDR and i.m. administration of FSH. The LII was performed by deposition of 0.03 ml of thawed moufflon semen $(20x10^6 \text{ motile spermatozoa})$ into the middle of each uterine horn using laparoscope and inseminating pipette, fitted with a 25-gauge needle either 40 or 48 h after the CIDR removal. Ovulation was monitored by ultrasonography with an abdominal linear probe as well as by laparoscopic examination. Embryos at blastocyst stage were recovered retrogradually using a Folley catheter. The highest ovulation rates were detected between 56 and 60 h after the CIDR removal. Recovery rate of embryos was 30 % and 38 %, fertilization rate 43 % and 58 %, percentage of unfertilized oocytes - 56 % and 41 %, of intact-transferable embryos - 28.2 % and 33.8 % and percentage of damaged embryos - 64.6 % and 66.7 % after insemination following 42 and 50 h after the CIDR removal, respectively. The results showed that the middle of the uterine horn is an acceptable deposition site for the insemination dose

(ID). Insemination at 48 h after the CIDR removal seems to be better than at 40 h when using LII with frozen-thawed semen. The volume of ID and number of progressively motile sperm cells per ID were sufficient. Therefore, LII can be used successfully in moufflon and may be implemented to use in controlled reproduction.

Key words: moufflon; frozen semen; laparoscopic insemination; embryo recovery rate

Acknowledgement: This research was supported by VEGA 1/0498/12

IMPACT OF SELECTED METABOLIC PARAMETERS IN PROFITS AND QUALITY OF EMBRYOS IN FALLOW DEER DONORS

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The aim of this work was to study the observation of prevalence serum cholesterol value, urea and total protein before and after superovulation on the yield and quality of embryos in fallow deer donors. Positive correlation was found between the level of cholesterol and superovulatory response (r = 0.64), production of embryos (r = 0.55) and transferable embryos (r = 0.51). Levels of urea were in negative correlation in the superovulatory response (r = 0.34), to flushed embryos (r = 0.58) and transferable embryos (r = 0.61). The influence of total proteins in blood serum of donor fallow deer on effectiveness of embryo transfer was not proved in our experiments.

Key words: biochemical parameters; blood serum; fallow deer; heat

Acknowledgement: This research was supported by the grant KEGA 016PU - 4/2012.

TYPICAL ALLELES IN THE GENOTYPES OF NATIONAL RABBIT BREEDS

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The present Pattern Book of Rabbit Breeds by the Slovak Association of Rabbit Breeders contains more than 50 registered breeds and dozens of varieties. Their classification is based on live weight (giants, medium, small and tiny – dwarf) and fur structure (fur-bearing, Angoras, short-haired coat – rex). A group of national rabbit breeds is part of the

national heritage; they are the result of breeding activities of breeders associated in the national breeders' organization. These breeds represent in some cases unique genotypes with typical configuration of alleles, which do not occur in other zootechnical units. These breeds are a source of genetic variability, which is used to improve them further or in new breeding. In spite of the fact that the frequency of populations of national breeds is limited by the breeders' basis, these animals are regularly exhibited during exhibitions at home and abroad and they often award prizes. At present there are following national breeds in Slovak herds (with the year of origin):

Slovak grey-blue rex (1965)

Blue of Holic (1975)

Rabbit of Nitra (1977)

Zemplin pastel rabbit (1987)

Zobor rabbit (2005)

Liptov bold-spotted rabbit (2005)

Dwarf Slovak pastel rex (2005)

Slovak pastel rex (2007)

At the same time, these zootechnical units are bearers of unique nucleotide sequences, which can be used for a number of practical and experimental applications in the future.

Key words: rabbit; national breeds; alleles; coat colour

EXPRESSION OF THE CELL MEMBRANE ANTIGENS CD9 AND CD41/61 IN TRANSGENIC RABBITS WITH THE RECOMBINANT HUMAN FACTOR VIII (HFVIII)

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The blood coagulation is a complex cascade process in which a set of blood factors is comprised. Some cell surface molecules (CD antigens) mediate the interaction of thrombocytes and coagulation factors.

In current study, we investigated whether the insertion of the hFVIII gene construct into the rabbit genome could modify the expression of cell surface molecules CD9 and CD41/61 taking part in the process of blood coagulation. The presence of both molecules in blood and milk have been analysed by indirect immunofluorescence and ELISA during two lactations of transgenic rabbits and in the udder tissue after the killing of animals, in comparison with the expression of the same molecules in non-transgenic rabbits. The study showed that the insertion of the WAP-hFVIII does not influence significantly the expression of CD9 and CD41/61 on the blood cells, milk somatic cells and mammary gland tissues.

Key words: transgenic animals; CD molecules; factor VIII; rabbits

Acknowledgement: This work was supported by grants VEGA 2/0006/12 and APVV/0137/10

EXPLORING GENE DIVERSITY IN CZECH TRADITIONAL BREEDS INCLUDED INTO THE CONSERVATION PROGRAMME

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Biotechnology based on the genetic markers provides a potential for effective management of genetic resources of animals, including the control of their health and development of new products with specific (desirable) properties.Genetic markers are used alongside to improve the efficiency of selection programs. For the assessment and prevention of inbreeding the polymorphic variant testing of selected candidate genes is used. In practice, the genes with high association to one of the indicators of these traits have been used.

The most common techniques to determine the genetic diversity of livestock breeds are the screening for microsatellite profiles, mtDNA variability, AFLP technique and variation present in the coding region and noncoding parts of particular genes. To assess inter- and intra-varietal diversity in the conserved traditional breeds, we have used various methods of molecular genetics.

The aim of this study was to evaluate the occurrence of gene diversity in goat, sheep, hen and horse breeds included into the National Programme of Conservation and Exploitation of Genetic resource. For analysis in different species, we have used different number of polymorphic microsatellite markers recommended by ISAG or FAO.

Goat: 7 recommended microsatellite markers were used. Results indicate that high level of genetic variations has been maintained in two Czech dairy goat breeds (White Short-Haired (WSH) and Brown Short-Haired (BSH). The low value of calculated genetic distance between them (D = 0.0517; SD = 0.0075) was expected since both breeds are related and have been bred for the same natural conditions. On the other hand, both breeds exhibited relatively high level of heterozygosity.

Sheep: 6 microsatellite loci divided into 2 multiplex were used to detect genetic variation in Sumava sheep, Valachian sheep and Improved Valachian sheep breeds native to the territory of the Czech Republic, engaged into the Animal Genetic Resource. The results showed high level of polymorphism in the second multiplex. Heterozygosity of loci in the first multiplex was lower than in the second in the followed sheep breeds.

Horse: 16 recommended microsatellite markers in Old Kladruber horse were used. The results showed that the average number of alleles per microsatellite locus was 8.25 with a range of 4 to 14. The estimated average value for the observed heterozygosity across microsatellite loci was 0.637, while the estimated mean value of genetic diversity was 0.678. Based on the result, the genetic diversity (heterozygosity) in monitored Old Kladruber horse is consistent with other populations of horses.

Rabbit: For molecular analysis we used 7 breeds of rabbit and 17 microsatellite markers, divided into 5 multiplex. The analysis revealed better than expected large differences between breeds (due to their relatively recent common origin). This is underlined by the finding of unique alleles that are unique to each breed. With their help they can be reliably identified. From this we can also conclude that breeds carry the unique genes within a population of rabbits. Further results show that despite the limited number of individuals thrive maintain desirable genetic variability. The Fis coefficient (can be seen as a measure of inbreeding) acquires medium to high values at least in numerous breeds.

Chicken: To date, we have used 16 microsatellite loci to characterize Czech Gold Speckled Hen. Average values of heterozygosity (0.37), polymorphic information content (0.38) and total number of alleles (4.19), as well as a high frequency of the most common allele (0.7) show a high level of inbreeding, high intensity of selection and other factors negatively affecting genetic variability. The above-mentioned parameters obtained in the control group correspond to approximately half of the normal value for this type of markers in other livestock populations or to one third of the value of wild populations.

In conclusion, microsatellite analysis is one of the powerful tools for evaluating gene diversity, genetic variation within species and their populations. The obtained data are (or will be) a guide for breeding measures for the management of conserved breeds.

Key words: gene diversity; microsatellite DNA; polymorphism

QUALITY OF BOVINE EMBRYOS FOLLOWING CRYOPRESERVATION AND STORAGE

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Cryopreservation procedure still causes significant morphological and biochemical alterations, which may lead to cell death and loss of embryo viability. The loss of viability can be attributed among other factors also to the length of storage in liquid nitrogen. One potential way to improve post-thaw survival and post-transfer efficiency is to modify post-thaw culture conditions to more closely simulate embryo physiology in vivo. One of macromolecules, which can regulate embryonic development, may be insulin-like growth factor I (IGF-I), which promotes development to the blastocyst stage, increases blastocyst cell number and decreases proportion of apoptotic blastomeres in in vitro-produced embryos. The goal of this study was to examine the effect of IGF-I added during the postthaw culture (48h) of bovine embryos stored for twenty years in liquid nitrogen on their post-thaw survival and quality.

The embryos recovered from the Czech Fleckvieh dairy cows within the years 1989-1990 were frozen using slowfreezing procedure. Following thawing the embryos were either processed immediately (the control group) or cultured alone (Exp 0) or in the presence of rhIGF-I (10 or 100 ng/ml; Exp10 and Exp100, resp.). Following 48h post-thaw culture 41 % of the embryos developed up to advanced blastocyst stage. Thawed embryos were represented mostly by the grade III actin quality and less of them (12 %) were of the grade II actin, whilst none grade I actin embryos were noted. In the cultured embryos IGF-I at both doses significantly elevated the cell number compared to non-cultured embryos. However, in comparison to embryos cultured without IGF-I, only higher IGF-I dose (100 ng/ml) elevated the total cell number. Apoptotic (TUNEL) index was significantly lowered by both IGF-I doses. These observations indicate that addition of IGF-I during post-thaw culture, can improve the quality of bovine embryos following long-term cryostorage.

Key words: embryo; post-thaw viability; apoptosis; actin; IGF-I

Acknowledgment: Supported from the APVV grant (APVV-0137-10).

KNOCK-OUT STEM CELLS IN PHYSIOLOGICAL RESEARCH

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Knockout mice have been widely used in human and veterinary medicine, biology, pharmacology and other related fields to the study of gene function, drug and therapeutic development and disease research. Mice remain the most efficient and easily genetically manipulated animal research model. Their genetic similarity to humans allows valuable advancements in human health research. Generation of knockout mice is based on introduction of specific mutation(s) into the genome of embryonic stem cell, subsequent generation of chimeric animals and transmission of the mutant allele to the offspring. In conventional knockout, the targeted gene is functionally eliminated. Conditional knockout enables a post-natal or tissuespecific gene inactivation. Knockout mice with a reporter gene are used to monitor target gene expression. In my presentation I will focus on several models of knockout mice previously or currently used in our laboratory. I will discuss our results in studies related to male infertility, developmental separation of blood and lymphatic vessels, restenosis and introduce our current projects in the area of cancer research. Finally, I will give a brief overview of current requirements of the Ethic commission of Medical Faculty in Vienna and Austrian Federal Ministry for Science and Research to breed knockout mice and to accomplish the experiments.

Key words: knockout mice; stem cells; gene function; gene expression; disease research

DIRECT IMMUNOMAGNETIC SEPARATION OF MESENCHYMAL STEM CELLS FROM THE RABBIT BONE MARROW

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The objective of this study was to assess the effectiveness of MACS technique used for the isolation of mesenchymal stem cells (MSCs) from the rabbit bone marrow. Rabbit bone marrow was harvested from humanely sacrificed New Zealand White rabbits (n = 4) under sterile conditions and mononuclear cells

(BMMCs) were isolated using ficoll centrifugation. BMMCs were divided and placed into prepared tubes, incubated with Anti-SSEA-4 or Anti-MSCA-1 microbeads (Miltenyi Biotec, Germany) and sorted using AutoMACS Pro Separator (Miltenyi Biotec, Germany) according to the producer's manual. Inactivated rabbit serum was also used in order to exclude nonspecific labelling to Fc receptors. The control samples were not sorted. After MACS sorting APC-conjugated Labelling Check Reagent (LCR; Miltenvi Biotec, Germany) was used to control the sorting efficiency. Labelled cells before and after sorting were evaluated using the flow cytometer FACSCalibur (BD Biosciences, USA). At least 100,000 events (cells) were analysed in the control samples and negative fractions. In the positive fractions at least 10,000 cells were analysed. Propidium iodide was used to exclude the dead cells from the evaluation. The sorting efficiency, proved by the LCR positivity in both positive fractions (SSEA-4⁺ and MSCA-1⁺), was significantly higher (P < 0.05) in comparison to the sample before sorting or negative fractions. We observed higher percentage of MSCs using SSEA-4 Microbeads (34.70 ± 2.29) in comparison to the MSCA-1 Microbeads (7.00 \pm 0.87). Our observations indicate that MACS technique could be potentially used for the positive selection of MSCs from rabbit bone marrow. However, further experiments are required in order to substantiate this assumption.

Key words: rabbit; MACS; MSCs; SSEA-4; MSCA-1

Acknowledgement: This work was supported from the grants of Slovak Research and Development Agency: APVV LPP-0119-09 and APVV-556-011.

THE HEALTH-PROMOTING FATTY ACIDS OF MILK FAT AND MILK QUALITY IN RELATION TO THE BREED OF DAIRY COWS REARED IN SLOVAKIA

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The aim of this study was to investigate the effect of gene pool on milk production and fat nutritive value of raw cow's milk from different upland farming areas in Slovakia. We examined Braunvieh (B, n = 8), Pinzgau (P, n = 74), Holstein (H, n = 105), Slovak Spotted (S, n = 61) and Red Holstein (R, n = 120) cows. We have found that P had significantly lower values of the total amount of produced milk, fat and protein compared to the other breeds. The B and H breeds had the lowest content of the fat-free dry matter (FFDM) and dry matter (DM) in the milk. The R and S breeds reached the highest content of fat and protein, and also the highest FFDM and DM. The S and P breeds showed significantly lower content of saturated fatty acids (SFAs 69.34 % and 70.89 %, respectively). This parameter was also affected at the value of the atherogenic index (AI), which is the lowest in both breeds. The highest values of unsaturated fatty acids (UFAs 30.66 % and 29.11 %, respectively) and essential fatty acids (EFAs 3.33 % and 2.98 %, respectively) were observed. From those the most distinguished FA, the conjugated linoleic acid (CLA) had significant proportion in milk of the P (0.67 %) and S (0.56 %) breeds. We conclude that among the observed breeds of dairy cows the lowest values of the health-undesirable

components of milk fat in relation to cardiovascular diseases of people were observed in the P and S breeds. **Key words:** cow's milk; milk fat; fatty acids; breed; gene pool

EFFECT OF EPICATECHIN ON HAEMATOLOGICAL PARAMETERS OF RABBITS: TWO-WEEK EXPOSURE

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In this study haematological parameters (total white blood cell count, lymphocyte count, medium size cell count, granulocyte count, red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, platelet count, platelet percentage, mean platelet volume and platelet distribution width) in blood of rabbits after epicatechin administration during two weeks were analysed. The animals were divided into four groups: control group (C) and experimental groups (E1 - E3). Experimental groups received epicatechin in the injectable form at 10 µg.kg⁻¹ in E1, 100 µg.kg-1 in E2 and 1000 µg.kg-1 in E3 for 14 days three times a week. Significant decrease (P < 0.05) of mean corpuscular haemoglobin concentration in E2 and E3 groups in comparison with the C group was observed. Higher platelet and medium size cell count in the experimental groups and slight decrease in haemoglobin in the experimental groups was found in comparison with the control group but without significant differences (P > 0.05). Other haematological parameters were not affected by this natural antioxidant. Epicatechin has a number of positive effects on organism including the improvement of vasodilation, reduction of blood pressure, insulin resistance and glucose tolerance, the attenuation of platelet reactivity and the improvement of immune responses and antioxidant defence systems.

Key words: epicatechin; haematology; rabbit

Acknowledgments: This work was financially supported by VEGA scientific grants 1/0084/12 and 1/0022/13.