

found on the surface of the eggshell: brown (brown pigmentation), black (greyish white eggshell pigmented with various sizes of black pigments), white (non-pigmented eggshell), spotted (small black pin dots on greyish brown eggshell) and blue (slightly blue pigments). This research was conducted to investigate the effects of eggshell colour on the egg weight, egg shape index, eggshell weight, eggshell percentage, eggshell thickness, eggshell strength, albumen weight, albumen percentage, albumen index, Haugh unit, yolk weight, yolk percentage, yolk index and yolk colour. Results revealed that eggshell colour had significant ($P < 0.05$) effect on the egg weight (from 11.66 g in spotted group to 12.98 g in black group). The egg shape index did not express any significant ($P > 0.05$) differences between different eggshell colours. Non-significant ($P > 0.05$) effects were recorded for eggshell weight, eggshell percentage, eggshell thickness and eggshell strength among different eggshell colours. Results also showed that eggshell colour had significant ($P < 0.05$) effect on albumen index (from 9.32 % in brown group to 9.63 % in white group), Haugh unit (from 87.51 in brown group to 89.85 in white group), yolk weight (from 4.11 g in spotted group to 4.36 g in black group) and yolk percentage (from 32.22 % in spotted group to 34.89 % in black group). For albumen weight, albumen percentage, yolk index and yolk colour no significant ($P > 0.05$) differences among eggshell colours were observed.

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

COMPOSITION OF ALVEOLAR AND CISTERNAL MILK OF TSIGAI AND IMPROVED VALACHIAN BREEDS

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The aim of this study was to investigate the composition of the milk in the cisternal and alveolar compartments of the udder in the most bred breeds in Slovakia [Tsigai (TS), $n = 9$ and Improved Valachian (IV), $n = 9$]. Measurements took place at the middle stage of lactation. Cisternal milk was milked out after i.v. administration of atosiban ($10 \mu\text{g}\cdot\text{kg}^{-1}$ body weight) and alveolar milk after i.v. oxytocin administration (4 IU/animal). Milk composition was analysed for percentage of fat, protein, lactose, solids and solids-not-fat with MilkoScan FT120 (Foss, Hillerød, Denmark). The ratios between cisternal and alveolar milk were 55:45 in TS and 65:35 in IV. No significant differences ($P > 0.05$) in milk composition were found in both milk fractions between breeds in protein content of alveolar milk (5.63 ± 0.57 vs. 5.17 ± 0.37 % in TS and IV, resp.) and milk fat concentration in the cisternal as well as in the alveolar fraction (7.94 ± 1.49 vs. 7.55 ± 0.97 % and 9.38 ± 2.02 vs. 9.27 ± 1.39 %, resp.). In conclusion, in both breeds

the large amount of milk is present in the alveolar compartments of the udder, indicating the need of milk ejection reflex occurrence for complete milk removal during milking.

Key words: alveolar milk; cisternal milk; ewes; Tsigai; Improved Valachian

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ASSOCIATION BETWEEN SELECTED TRACE ELEMENTS AND HEPATIC PROFILE IN COMMON CARP (*CYPRINUS CARPIO*)

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Chronic exposure to heavy metals and other trace elements causes several injuries in organism. Biomarkers, such as hepatic enzymes, are good indicators of liver injury. The aim of the present study was to investigate the associations between responses to selected trace elements (aluminium – Al, barium – Ba, lithium – Li, molybdenum – Mo) and hepatic enzymes (aspartate aminotransferase – AST, alanine aminotransferase – ALT, alkaline phosphatase – ALP, bilirubin (Bili) and creatine kinase – CK) in Common Carp blood serum.

Totally, 42 freshwater fishes (*Cyprinus carpio*) were caught by seine net. The blood samples, taken by a cardiac puncture method, were allowed to coagulate and then centrifuged for 20 min at 3000 rpm. Blood serum concentrations of AST, ALT, ALP, Bili and CK were measured using DiaSys (Diagnostic Systems GmbH, Holzheim, Germany) commercial kits and the semi-automated clinical chemistry analyzer Randox RX Monza (Randox Laboratories, Crumlin, UK). The content of selected trace elements (Al, Ba, Li, Mo) in blood serum was determined by inductively-coupled plasma optical emission spectrometry (ICP-OES, Agilent Technologies Australia (M) Pty Ltd.). Statistical analyses were performed using STATGRAPHICS Centurion software (©StatPoint Technologies, Inc., USA). The following scheme of descending concentrations of trace elements in blood serum was used: Al ($0.61 \text{ mg}\cdot\text{L}^{-1}$) > Ba ($0.17 \text{ mg}\cdot\text{L}^{-1}$) > Mo ($7.13 \mu\text{g}\cdot\text{L}^{-1}$) > Li ($5.24 \mu\text{g}\cdot\text{L}^{-1}$). Levels of serum markers were comparable with other authors, except higher ALP ($6.36 \mu\text{kat}\cdot\text{L}^{-1}$). Correlation analysis showed significant positive relationship between ALT and

Mo ($r = 0.4032$; $P < 0.01$) and significant negative association between CK and Ba ($r = -0.3780$; $P < 0.05$) or Li ($r = -0.3925$; $P < 0.05$). Insignificant Pearson correlations were detected between other trace elements and hepatic profile markers. Al and Li were in positive association with AST and Bili (n.s.). Mo and Ba insignificantly positively correlated with ALP and negatively with cholesterol and bilirubin. On the other hand, the analysis showed insignificant positive correlation between Al and cholesterol or bilirubin. In conclusion, obtained data indicate that trace elements affect hepatic profile markers of Common Carp. However, there were no serious damages observed in the health status except for ALP, which may indicate the bile duct epithelial damage. The correlation analysis confirmed statistically significant interactions.

Key words: trace elements; hepatic profile; Common Carp

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ADDITIVES IN JAPANESE QUAIL NUTRITION

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The aim of this study was to analyse the effect of humic substances (HS) on the quality traits (colour, water-holding capacity and pH), serum minerals (calcium, phosphorus, sodium, potassium and chlorides) and the serum metabolites (glucose, total protein, triglycerides and cholesterol) in breast and thigh meats of Japanese quails. A total of 60 animals (30 males and 30 females) were involved in the experiment. The birds, fed by the standard basal diet, were divided into four experimental groups as follows: probiotic females (PF, $n = 10$) and males (PM, $n = 10$) received probiotic preparation at the single dose of $1 \text{ g} \cdot \text{kg}^{-1}$ of feed mixture, humic acids females (HF, $n = 10$) and males (HM, $n = 10$) received humic acids at the single dose $3 \text{ g} \cdot \text{kg}^{-1}$ of feed mixture. The groups fed basal diet without any additive served as the control groups (CF; $n = 10$, CM, $n = 10$). After 210 days the quails were slaughtered and the blood samples and samples of muscles (breast and thigh) were collected. The treatments by probiotic and humic acids caused significant increase in serum calcium levels in the female groups when compared to the male groups. Serum phosphorus was significantly increased in the PF group

in comparison to the PM group. Both treatments significantly decreased amount of HDL cholesterol in the female groups in comparison to the female control. 24 hours after slaughter, the meat pH in different muscles showed significant differences. In conclusion, the effect of the treatment with probiotics and humic acids was dose dependent. This suggests that the estimation of an effective dose of additives used in poultry feeding plays an important role.

Key words: Japanese quails; probiotics; humic acids; blood biochemical parameters; meat quality

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CYTOTOXIC EVALUATION OF NONYLPHENOL IN TM3 LEYDIG CELL LINE

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Current widespread research of health effects potentially associated with endocrine disruptors has a high priority nowadays. Alkylphenol ethoxylates, a class of non-ionic surfactants, are degraded into alkylphenol diethoxylates and alkylphenol monoethoxylates. These are subsequently degraded into other sub-products and persist in the environment for a long time. Alkylphenols are common environmental contaminants originating from industrial processes, which are widely used as a component of paints, pesticides and herbicides. The most studied alkylphenols are nonylphenols and octylphenols. They have the ability to mimic effects of reproductive hormones and can interfere with the endocrine system leading to reproductive disorders at different levels of cellular system. A significant body of evidence, based upon laboratory experiments and meta-analysis, indicates that exposure to alkylphenols is associated with male reproductive malfunctions and impairment of spermatogenesis followed by irreversible changes in steroidogenesis. The primary objective of our *in vitro* study is to provide a knowledge about the cytotoxic effect of nonylphenols on TM3 cell line. In our study, the effect of 4-nonylphenol on the Leydig cell functions at lower doses ($0.04\text{--}5.0 \mu\text{g} \cdot \text{mL}^{-1}$) was evaluated. Cytotoxicity was assessed by measuring inhibition of metabolic activity (AlamarBlue™) and loss of membrane integrity (CFDA-AM) in order to identify the mode of toxic action after 24 h of culture. Significant ($P < 0.001$) increase in metabolic inhibition at 2.5 and $5.0 \mu\text{g} \cdot \text{mL}^{-1}$ was revealed, whereas significant ($P < 0.001$) loss of membrane integrity was occurred at the highest dose ($5.0 \mu\text{g} \cdot \text{mL}^{-1}$) of 4-nonylphenol. Additional *in vivo* and *in vitro* studies are required to better understand the nature of the effects of alkylphenols and their mechanisms of action in altering male reproductive functions.

Key words: nonylphenol; cytotoxicity; Leydig cells

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