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CHARACTERIZATION OF TWO EGYPTIAN NATIVE CHICKEN BREEDS USING GENETIC AND IMMUNOLOGICAL PARAMETERS

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Abstract: In order to identify and characterize our native chicken breeds we used two approaches, one of them is genetic and the other concerns with the immunological status of the chickens. In this study, the first 539 bases of the mtDNA D-loop region of two Egyptian native breeds (Fayoumi and Dandarawi, from El-Fayoum research station) were amplified and sequenced. The alignment results showed an approximate tandem repeat of 60-base units with the first 34 nucleotides being exact. These 34-base units were completely identical and conserved in both Egyptian and GenBank database samples. The multiple alignment results showed also that there are three transversions specific for the Egyptian breeds only. Two of them are not specific for certain breed since both of the two breeds showed the normal and mutated nucleotide. The third transversions seems to be specific to Egyptian Dandarawi breed only. The first mutation site is at the position (457) where Adenine nucleotide transversed to Cytosine nucleotide. The second mutation site is at the position (464) where Guanine nucleotide transversed to Thymine nucleotide. The third mutation site is at the position (483) where Adenine nucleotide transversed to Thymine nucleotide. With respect to immunological parameters, it could be speculated that the Fayoumi strain had hyper responders to phytohemagglutinin-P (PHA-P) injection compared to Dandarawi ones. Opposite trend was noticed for anti-SRBCs antibody response. The results may give an insight into the genetic differentiation and immunological status of the Egyptian domestic fowl. The results did not show direct relation between the two approaches used. However, the results of the two approaches can complete the identification and characterization of the chickens. Also, they could be used in future as bases for more studies.

Key words: mitochondrial D-loop, immunological parameters, Egyptian chickens
Introduction

Fayoumi and Dandarawi breeds are pure Egyptian native chickens. These native breeds have the carcass characteristics and flavor desired by Egyptian consumers. They demonstrate better general disease resistance than imported breeds because they have evolved through natural selection for a long period in the prevailing environment. They showed a strong inherent scavenging and nested habit, less prone to predator attacks and can survive under harsh nutritional and environmental conditions. The Egyptian Fayoumi breed seemed to have more resistance to viral disease than other breeds of chicken (Hoffmann, 2005). Fayoumi has been introduced in countries such as Tanzania (Katule, 1989), Ethiopia (Swan, 1996) and Bangladesh (Jensen, 1996). It was reported that the Sonali birds (male Rhode Island Red x female Fayoumi) reared under the semi-scavenging system in Bangladesh had a higher infection rate with Newcastle disease virus compared with indigenous and Fayoumi birds (Biswas et al., 2005). So it was important to study the immunological status of our chickens in conjunction with other parameter, the genetic ones. Genetic diversity in a population is expected to enhance the chance for survival of the species. Mitochondrial DNA (mtDNA) is an available molecular tool for investigating evolutionary relationships and genetic variations within and between species because of its more rapid variability than nuclear DNA (Avise et al., 1987; Zhang and Shi, 1992). The mitochondrial DNA (mtDNA) control region (CR), the major noncoding region of the animal mtDNA molecule, has a role in the replication and transcription of mtDNA molecules (Clayton 1984, 1992). The central domain of the CR, containing the heavy strand’s origin of replication, is relatively conserved (Saccone et al., 1991). In contrast, the two domains that flank the central domain (domain I and domain III) are typically hyper-variable in base substitutions (Saccone et al., 1987). Due to the fast rate of evolution of domain I and domain III, the CR has been typically deemed to be most appropriate for intra-specific studies (Wenink et al., 1994). For genetic diversity studies, it is also important to identify the positions of nucleotide polymorphism where individuals have differences in their sequences. In this study, the first 539 bases of the mtDNA D-loop region (containing the first variable domain I) of two Egyptian native breeds (Fayoumi and Dandarawi, from El-Fayoum research station) and were amplified and sequenced and the polymorphic bases were recorded.

Materials and Methods

Birds and management. This experiment was carried out at poultry breeding farm, poultry production department, Faculty of Agriculture, Ain Shams University, Egypt. A total of 230 chicks (150 Fayoumi and 80 Dandarawi) were used. Chicks were wing-banded and brooded in electrical brooding batteries from
hatching up to 4 weeks of age, at when they were transferred to rearing batteries. All chicks were brooded and reared under similar environmental, managerial and hygienic conditions. The feed and water were provided ad libitum. They were fed a diet containing 18% crude protein and 2850 kcal ME/kg.

**Amplification of the D-loop fragments and sequencing.** Wing vein blood samples were obtained from eight live birds (four chickens per breed) without harming them; DNA was extracted using PURE Gene™ DNA Purification Kit. As recommended by the manufacturer.

The conserved primer pair, L16750 (forward; 5’-AGG ACT ACG GCT TGA AAA GC-3’) and H 547 (reverse; 5’- ATG TGC CTG ACC GAG GAA CAA G-3’) were used to amplify the first 539 base fragment of the D-loop region of the birds. The primer number refers to the positions of the 3’ end of the primer in the reference sequence *(Desjardins and Morais, 1990).* The amplification reaction was carried out in a 25 µl reaction mixture consisting of 1.25 unit Taq polymerase (DyNAzyme), 1X enzyme buffer (1X is 10 mM Tris-HCl, pH 8.8 at 25 °C, 1.5 mM MgCl2, 50 mM KCl and 0.1% Triton X-100) supplied by the manufacturer, 1 µM of each forward and reverse primer, 0.2 mM dNTPs and 50 ng of DNA. The reaction mixture was overlaid with sterile mineral oil and was run in an MJ research PTC-100 Thermocycler. The PCR cycle profile was 94 °C for 2 min before the first cycle, then 94 °C for 1 min, 63 °C for 1 min and 72 °C for 1 min for 35 cycles. After the last cycle, the PCR mixture was incubated for a further 5 min at 72 °C. The reaction products (5 µl each) were used for electrophoresis with an appropriate size marker on 1.5% agarose in 1X-Tris acetate buffer (TAE). After electrophoresis the gels were stained with ethidium bromide and were examined with UV lamp at a wavelength 312 nm to verify amplification of the D-loop fragment. The PCR products were purified using QIAquick PCR purification kit (Qiagen, Inc.) and the resulting purified products were used in the subsequent sequencing reactions. Sequencing was performed on an Applied Biosystems 310 genetic analyzer (Applied Biosystem) using BigDye terminator cycle sequencing ready reaction mixture according to manufacturer’s instructions (Applied Biosystems). Direct submissions were made to GenBank database using BankIt. Sequence analysis and alignment were carried out using NCBI-BLASTN 2.2.5 version *(Altschul et al., 1997)* and Clustalw (1.82) multiple sequence alignment programs *(Thomson et al., 1994).* The Clustalw program was also used for computing the alignment and the phylogenetic tree. The default parameters program was used. The phylogenetic tree was asserted by the bootstrapping technique.

**Immunological parameters. Phytohemagglutinin injection (In vivo cell-mediated immunity assay).** Response induced *in vivo* by mitogen was evaluated by injection of phytohemagglutinin-P (PHA-P) into the two-web between the second and the third digits of male chicks. Ten male chickens from each genetic line at 6 weeks of age were used. Each male was intradermally injected in the toe-
web of the left foot with 100 μg phytohemagglutin-P (Sigma Chemical Co., St. Louis, MO 63178) in 0.1 ml of sterile saline. The swelling response was measured with a constant tension caliper before injection and at 24, 48 and 72 hr after PHA-P injection. The toe-web swelling was calculated as the difference between the thickness of the toe-web before and after injection.

**Sheep red blood cells (SRBCs).** At 2 weeks of age, 30 chicks per strain were randomly assigned for assessing humoral immunity response. The sheep red blood cells (SRBCs) were collected and washed 3 times in phosphate–buffer saline (PBS). After that, the packed cells were brought to a 7% vol/vol solution in the PBS. At 2 wks of age, chicks were injected into thigh muscle with SRBC (3% suspension in PBS, 1 ml/chick) followed by a booster injection of SRBC suspension at 4 wks (after 14 days of the first injection). Blood samples were drawn at 7, 14 days from first and second injection. Plasma was stored at −20°C until tested. The antibody levels against SRBC were measured by hemagglutination test using 2% SRBCs suspension. Plasma was heat inactivated at 56°C for 30 min and then analyzed for total, mercaptoethanol-sensitive (Presumably IgM) and mercaptoethanol-resistant (IgG) anti-SRBC antibodies as previously described (Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994). Briefly, 50 μL of plasma was added in an equal amount of PBS in the first column of a 96-well V-shaped bottom plate, and the solution was incubated for 30 min at 37°C. A serial dilution was then made and 50 μl of 2% SRBC suspension was added to each well. Total antibody titers were then read after 30 min of incubation at 37°C. The well immediately preceding a well with a distinct SRBC button was considered as the endpoint titer for agglutination. For MER (IgG) response, 50 μl of 0.01 M mercaptoethanol in PBS was used instead of PBS alone, followed by the previous mentioned procedure. The difference between the total and IgG response was considered to be equal to the IgM antibody level.

**Relative weight of lymphoid organs.** After completion of PHA-P assay, the same males (10 per genetic line) were weighed and slaughtered. The bursa of Fabricius, spleen and thymus (all lobes from left side of the neck) were removed and weighed to the nearest milligram.

**Statistical analysis**

Data were subjected to a one-way analysis of variance with genetic group effect using the General Linear Model (GLM) procedure of SAS User’s Guide, 2001.

**Results and Discussion**

**Genetic diversity.** In this study, the first 539 bases of the mtDNA D-loop region of two Egyptian native breeds (Fayoumi and Dandarawi, from El-Fayoum research station) were amplified using polymerase chain reaction. The reaction
products were run on 1% agarose gel and each of them gave only one sharp band in the correct size (Figure 1). Samples from the two breeds were sequenced in both directions (forward and reverse). The sequence of the fragments was corrected using Blast software. The final sequence results were corrected manually and submitted directly to GenBank database under the following accession numbers: (Fayoumi 1: EF 586879, Fayoumi 2: EF 586880, Dandarawi 1: EF 586881, Dandarawi 2: EF 586882, Dandarawi 3: EU352856). The data is available over the network data servers. The multiple sequence alignment results between these two breeds and the published results in GenBank database (Figure 2) showed an approximate tandem repeat of 60-base units with the first 34 nucleotides being exact (caagtcaacctaatgatgtacaggacata). These 34-base units were completely identical and conserved in both Egyptian and GenBank database samples. It also contains in its center the published invariant tetradecamer (aactatgaatggtt) sequence. So we can conclude that the invariant sequence is composed of 34-base unit at the beginning of the approximate tandem repeat of 60-base unit. This agrees with the published tandem duplication of 60-base units which contains in its center the invariant tetradecamer (aactatgaatggtt) sequence in the member of the genus Gallus (Akishinonomiya et al., 1994).

The PCR reactions were run on 1% agarose gel, stained with ethidium bromide and examined with UV. Samples from 1 to 4 for Dandarawi breed and samples from 5 to 8 for Fayoumi breed. The Hae III digest of Ф X174 DNA was used as ladder (1353, 1078, 872, and 603 base pairs).

Figure1. PCR amplification of chicken mitochondrial D loop fragment.

First copy

EgyFay1EF586879
AGCTCCAAACCACCTACTACCAATCTCCTCAGGCTATGTTACAGGCATATACTATGATGACAT
AY704707
AGCTCCAAACCACCTACTACCAATCTCCTCAGGCTATGTTACAGGCATATACTATGATGACAT

AGCCTTCTACCTACCAATCTCCTCAGGCTATGTTACAGGCATATACTATGATGACAT
H. A. I. Ramadan et al.

**Second copy**

EgyFay1EF586879  
TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AY704707  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AP003318  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyDand3EU352856  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AY644968  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyFay2EF586880  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyDand2EF586882  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AP003320  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AB007750  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyFay1EF586879  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AP003318  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyDand3EU352856  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AY644968  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyFay2EF586880  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyDand2EF586882  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AP003320  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AB007750  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyFay1EF586879  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AP003318  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyDand3EU352856  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AY704707  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
ACCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyDand1EF586881  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AY644968  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyFay2EF586880  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyDand2EF586882  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
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TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AB007750  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyFay1EF586879  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
ACCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AY704707  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
ACCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
AP003318  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyDand3EU352856  

TCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
ACCATGTTCTTCCCCCAACAGTCACCTAACCTAATGGAATGTTACAGGACATA CATTTAACT  
EgyFay1EF586879
The first 34-base units in the approximate tandem repeat of 60-base units were underlined. The arrows show the positions of mutation sites in the Egyptian samples. The left column shows the breeds with their accession numbers and the asterisks indicate nucleotide identity between all samples.

The results showed also that there are three SNP sites (transversions) revealed by the Egyptian chicken breeds. Two of them are specific for the Egyptian...
breeds however they are not specific for certain breed since both of the two breeds showed the normal and mutated nucleotide. The third SNP site shows that it may be specific to Dandarawi breed only. The nucleotide numbers in Egyptian GenBank database samples were used to identify the mutation sites in all chicken samples. The first mutation site is at the position (457) where Adenine nucleotide transversed to Cytosine nucleotide. The second mutation site is at the position (464) where Guanine nucleotide transversed to Thymine nucleotide. The third mutation site is at the position (483) where Adenine nucleotide transversed to Thymine nucleotide. Other expected mutation site was noticed in only one bird (Fayoumi 1) where (C-A, at position 276). Mitochondria produce most of our cells’ ATP, and thus their function is critical for our wellbeing. Over 100 proteins are involved in oxidative phosphorylation. Most of these proteins are encoded by nuclear genes, but 13 are encoded by mitochondrial genes. Furthermore, 22 tRNA genes and 2 rRNA genes are also encoded in the mitochondrial genome. In addition, more than 50 different disease-causing mitochondrial SNPs (single nucleotide polymorphism) have been identified in mammals, and this number is expected to increase as we become more proficient at detecting SNPs. Genetic diversity in the population enhances the chance for survival of the species. In conclusion, the sequence results Of the D-loop fragments identified the positions of nucleotide polymorphism where individuals have differences in their sequences. The results showed that there are three expected mutation sites (transversions) revealed by some of the Egyptian breeds only. Two of them are not specific for Dandarawi or Fayoumi since both of the two breeds showed the normal and mutated nucleotide while the third one may be specific for Dandarawi breed only.

**Phylogenetic analysis.** From the multiple alignment, the sequence differences can be summarized as indicated in Table 1. It can be easily noticed that the sequence of Egyptian Dandarawi No.3 is identical to the sequences in the Database. The most diverged pairs are DQ629875 (we consider it as out-group) and Egyptian Dandarawi No.2, where the number of differences is five. The phylogenetic tree based on the alignment is shown in Figure 3. It can be seen that the Egyptian groups are differentiated from the other ones, except for Egyptian Dandarawi No.3, which is identical to the other Database sequences. The Egyptian clade was also asserted when we ran the bootstrapping option of the ClustalW using the default bootstrapping parameters. The results gave an insight into the genetic diversity and divergence of the Egyptian domestic chickens; which might benefited through their evolution.
Table 1. The Polymorphic sites and their positions

<table>
<thead>
<tr>
<th>Breed &amp; Accession number</th>
<th>Variable sites and their positions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23</td>
</tr>
<tr>
<td>EgyDand1    EF586881</td>
<td>T</td>
</tr>
<tr>
<td>EgyDand2    EF586882</td>
<td>T</td>
</tr>
<tr>
<td>EgyDand3    EU352856</td>
<td>T</td>
</tr>
<tr>
<td>EgyFay1     EF586879</td>
<td>T</td>
</tr>
<tr>
<td>EgyFay2     EF586880</td>
<td>T</td>
</tr>
<tr>
<td>DQ629875 A</td>
<td>*</td>
</tr>
<tr>
<td>Database public sequence</td>
<td>T</td>
</tr>
</tbody>
</table>

The nucleotide positions were given with respect to the Egyptian nucleotide numbers in GenBank database. The left column shows the breeds with their accession numbers.

**Lymphoproliferative response to PHA-P.** Phytohemagglutinin-P, a T-cell mitogen, induces proliferation in T-lymphocytes. Injection of PHA-P at a selected site in chickens can be considered as an inducer of localized in vivo T-lymphoproliferative response (Cheema et al., 2003). The results of swelling response measured at 24, 48 and 72h post-injection of PHA-P are presented in Table (2). With respect to caustic basophilic hypersensitivity (CBH) it could be speculated that the Fayoumi strain exhibited significantly higher swelling response than that of Dandarawi ones at 24, 48 and 72h after PHA-P injection. The difference between strains for response to PHA-P injection could be attributed to the lymphoblastogenic response to PHA-P is presumed to be polygenic (Morrow and Abplanalp, 1981). Also, T-cell mediated immune response of chicken has significant variation among birds of different genetic lineage (Lamont and Smyth, 1984; Cheng and Lamont, 1988). Successful divergent selection of chickens for various T-cell functions suggests that many of these functions are highly heritable, and are often negatively correlated with body weight (Yamamoto and Okada, 1990; Afraz et al., 1994).

Table 2. Means (±SE) of Phytohemagglutinin-P mediated (PHA-P) swelling in the toe-webs for Fayoumi and Dandarawi strains at 6 weeks of age

<table>
<thead>
<tr>
<th>Time</th>
<th>Fayoumi</th>
<th>Dandarawi</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>0.203±0.02</td>
<td>0.188±0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>48 h</td>
<td>0.111±0.02</td>
<td>0.103±0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>72 h</td>
<td>0.083±0.02</td>
<td>0.073±0.03</td>
<td>0.05</td>
</tr>
</tbody>
</table>

24 h = toe-web swelling at 24 hr post PHA-P injection.
48 h = toe-web swelling at 48 hr post PHA-P injection.
72 h = toe-web swelling at 72 hr post PHA-P injection.
Body weight and lymphoid organs weight. Six weeks live body weight and relative lymphoid organs weight of Fayoumi and Dandarawi strains are presented in Table (3). There was no significant difference between strains for body weight. However, the Fayoumi strain had significantly higher relative bursa weight compared to Dandarawi ones. The bursa of Fabricius is a key lymphoid organ that is responsible for the development and maturation of B-lymphocytes, and the humoral antibody response is dependent on this central organ (Zhang et al., 2006 and Cheema et al., 2007). For example, a high antibody response to SRBC has been associated with a larger bursa size in White Leghorn chicken strains (Ubosi et al., 1985). Furthermore, Zhang et al. (2006) showed a clear association between non-MHC genes and changes in the size of lymphoid organs by using highly inbred parental and recombinant congenic chicken lines.

The spleen provides microenvironment, which is needed for antigens presentation and concentrating them in the white pulps where T and B cell interactions, leads to the formation of antibodies (White et al., 1975; Williams et al., 1991). Results indicated that the Fayoumi strain significantly has increased relative weight of spleen compared to Dandarawi ones. The immunological function of thymus is to provide a specific environment essential for T-cells differentiation, which is essential for cell-mediated immunity and modulation of immune response (Owen, 1977). The differentiation is through subpopulation of thymus cells including T-helper, T-cytotoxic and T-suppressor cells. The present result indicated that the Fayoumi strain had significantly higher relative thymus weight compared to Dandarawi ones.

Table 3. Means (±SE) of live body weight and percentages of lymphoid organs relative to live body weight for Fayoumi and Dandarawi breeds at 6 weeks of age

<table>
<thead>
<tr>
<th>Trait</th>
<th>Fayoumi</th>
<th>Dandarawi</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>457.50±13.99</td>
<td>429.13±15.71</td>
<td>NS</td>
</tr>
<tr>
<td>Bursa weight, %</td>
<td>0.292±0.03</td>
<td>0.302±0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Spleen weight, %</td>
<td>0.305±0.03</td>
<td>0.245±0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Thymus weight, %</td>
<td>0.371±0.02</td>
<td>0.334±0.03</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Table 4. Means (±SE) of total antibody, IgG and IgM levels for Fayoumi and Dandarawi strains at 6 weeks of age

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time</th>
<th>Total antibody level</th>
<th>Immunoglobulin-G (IgG)</th>
<th>Immunoglobulin-M (IgM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7PPI</td>
<td>Day 14PPI</td>
<td>Day 7PSI</td>
<td>Day 14PSI</td>
</tr>
<tr>
<td>Fayoumi</td>
<td>3.80±0.03</td>
<td>3.40±0.02</td>
<td>5.33±0.02</td>
<td>3.60±0.03</td>
</tr>
<tr>
<td>Dandarawi</td>
<td>4.20±0.02</td>
<td>4.00±0.01</td>
<td>4.80±0.02</td>
<td>4.10±0.01</td>
</tr>
<tr>
<td>Probability</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Fayoumi</td>
<td>2.20±0.02</td>
<td>1.80±0.03</td>
<td>2.11±0.01</td>
<td>2.10±0.01</td>
</tr>
<tr>
<td>Dandarawi</td>
<td>2.60±0.01</td>
<td>2.20±0.01</td>
<td>2.30±0.02</td>
<td>2.00±0.01</td>
</tr>
<tr>
<td>Probability</td>
<td>0.01</td>
<td>0.01</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Fayoumi</td>
<td>1.60±0.03</td>
<td>1.60±0.01</td>
<td>3.22±0.02</td>
<td>1.50±0.03</td>
</tr>
<tr>
<td>Dandarawi</td>
<td>1.60±0.01</td>
<td>1.80±0.02</td>
<td>2.50±0.02</td>
<td>2.10±0.01</td>
</tr>
<tr>
<td>Probability</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

7PPI = 7 days post primary SRBCs injection
14PPI = 14 days post primary SRBCs injection
7PSI = 7 days post secondary SRBCs injection
14PSI = 14 days post secondary SRBCs injection

Anti-SRBCs antibody titer. Sheep red blood cells (SRBCs) have been chosen in this study as antigen because they are natural multi–determinant, non-pathogenic antigen and chicken phagocytosis of SRBCs opsoned with FC receptor for lysis and stimulate T-cell dependant response (Saxena et al., 1997). Data illustrated in Table 4 showed that the Dandarawi breed had a significantly higher total anti-SRBC antibody titer at 7 and 14 days post primary SRBC-injection compared to Fayoumi ones. Conversely, the Fayoumi breed had significantly higher total anti-SRBCs antibody titer at 7 days post secondary SRBCs injection. Similar trend have not been observed at 14 days post secondary SRBCs injection.
Sample DQ629875 was used as out-group for its high diversity.

Figure 3. Phylogenetic tree constructed between the Egyptian and GenBank database chicken samples

The IgG anti-SRBC antibody titer measured at 7 and 14 days post primary injection of Dandarawi strain was significantly higher than that of Fayoumi ones. Inversely, there was no significant difference between breeds for IgG anti-SRBC antibody measured at 7 and 14 days post secondary SRBCs injection. Okada and Yamamoto (1987) demonstrated that the high IgG level was associated with high antibody response to SRBC and lipopolysaccharides. Also,
Martin et al. (1989) reported that IgG level was higher for high antibody level than low antibody level. With respect to IgM anti-SRBC antibody titer, it could be noticed that there was no significant difference between breeds for primary immune response. However, the Fayoumi breed had significantly higher IgM concentration measured at 7 days post secondary SRBC-injection compared to Dandarawi ones. Opposite trend was noticed 14 days post secondary SRBC-injection.

Conclusion

These results may give an insight into the genetic differentiation and immunological status of the Egyptian domestic fowl. These results did not show direct relation between the two approaches used. However, the results of the two approaches can complete the identification and characterization of the Egyptian native chicken breeds. Also, they could be used in future as bases for more studies.

Acknowledgment

The authors thank Mohamed Abouelhoda for his help in constructing the phylogenetic tree and helpful comments concerning the bioinformatics part.

Karakterizacija dve egipatske autohtone rase pilića korišćenjem genetskih i imunoloških parametara


Rezime

U cilju identifikovanja i karakterizacije naših autohtonih rasa pilića, koristili smo dva pristupa, genetski i pristup koji se odnosi na imunološki status pilića. U ovom ispitivanju, prvih 539 baza mtDNK regiona D-petlje kod dve egipatske autohtone rase (Fayoumi i Dandarawi, iz El-Fayoum istraživačke stanice) je amplifikovano i sekvencirano. Rezultati poravnjanja pokazuju približno ponavljanje tandema od 60 baznih jedinica sa prvih 34 nukleotida koji su tačni. Ovih 34 baznih jedinica su bile u potpunosti identične i konzervirane u Egipatskoj banci gena i banci gena gde se čuvaju uzorci. Višestruki rezultati poravnjanja pokazuju takođe da postoje tri transverzije koje su specifične samo za egipatske rase. Dve od njih nisu specifične za određenu rasu.
Obe rase su pokazale normalne i mutirane nukleotide. Treća transverzija se čini da je specifična samo za egipatsku Dandarawi rasu.

Prva lokacija mutacije je na poziciji (457) gde se adenin nukleotid transverzuje u cistin nukleotid. Drugo mesto mutacije je na poziciji (464) gde se guanin nukleotid transverzuje u timin nukleotid. Treće mesto mutacije je na poziciji (483) gde se adenin nukleotid transverzuje u timin nukleotid. U vezi sa imunološkim parametrima, može se pretpostaviti da Fayoumi soj ima hiper odgovor/reakciju na fitoheamglutinin-P (PHA-P) injekcije u poređenju sa Dandarawi sojem. Suprotni trend je registrovan kod anti-SRBCs reakcije antitela. Rezultati mogu dati uvid u genetsku diferencijaciju i imunološki status dve egipatske autohtone rase. Rezultati nisu pokazali direktnu vezu između dva pristupa koji su korišćeni. Međutim, rezultati dobijeni korišćenjem dva pristupa mogu kompletirati identifikaciju i karakterizaciju pilića. Takođe, mogu se koristiti u budućnosti kao osnova za dalja istraživanja.

References


Characterization of two Egyptian ...

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THE EFFECT OF STOCKING DENSITY ON INDIVIDUAL BROILER WELFARE PARAMETERS
2. DIFFERENT BROILER STOCKING DENSITIES

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Abstract: Stocking density is considered one of the most important factors for the welfare of broilers. This paper is continuation of the study in order to obtain full evaluation of the impact of different broiler stocking densities on production performance, condition of the broiler legs and body feathering, as welfare indicators but also indicators of the productivity and quality of produced chickens. The effect of three stocking densities (20, 15 and 10 birds/m²) was investigated in 4 repetitions on broilers of genotype Hubbard at the age of 3 and 6 weeks. At the age of 6 weeks stocking density of 20 birds/m² resulted in significantly lower growth of broilers, higher mortality and higher incidence of leg lesions and problems with body feathering, compared to stocking densities of 15 and 10 birds/m².

Key words: broilers, stocking density, welfare

Introduction

The dependance of the product quality from the state of animal welfare (Sundrum, 2001), as well as legislation at European (Council Directive 2007/43/EC) and national level (Law on animal welfare), cause the introduction of certain changes/corrections into process of broiler production.

Welfare of farm animals is estimated through combination of physical and social indicators. In general, minimal mortality, low level of diseases and lesions, good body conditions, possibility for exhibition of main forms of behaviour and social connections, absence of physiological signs of stress, indicate absence of problems associated with welfare (Broom, 1991). Also, production results, in addition to their importance from the aspect of economical efficiency of production, are also important in estimation of welfare.

Broiler welfare is under the influence of numerous environmental factors which are mainly determined by the production management (Hristov et al., 2006).
Quality of air (temperature, relative humidity, gases, contamination), quality of litter/bedding (humidity, temperature, structure), light (photoperiod, intensity) and stocking density are the most important factors of the environment.

Stocking density exhibits direct and indirect influence on broiler welfare. High stocking density has direct influence of broiler welfare by physical restriction of their movement which reflects on development of locomotive apparatus and exhibition of main forms of broiler behaviour. Indirect effects are associated with the quality of air and litter/bedding and effect on incidence of diseases, condition of legs and body feathering (lesions, blister, dermatitis).

This paper is continuation of the study in order to obtain full evaluation of the impact of different broiler stocking densities on production performance, condition of the broiler legs and body feathering, as welfare indicators but also indicators of the productivity and quality of produced chickens. Obtained results (Škrbić et al., 2009) indicated presence of certain tendencies of deterioration of investigated welfare indicators, presented through higher frequency of lower scores with the increase of stocking density of housed broilers (10, 13, 16 birds/m²). Considering that methods used for evaluation of welfare indicators are subjective, we find that in order to objectively evaluate in general the effect of stocking density on broiler welfare it is necessary to acquire more scientifically based results.

**Materials and Methods**

Trial was carried out during spring season, in production facility with floor system of rearing and controlled environment conditions. Total of 1380 one day old chickens of Hubbard genotype were placed into boxes in 3 stocking densities (A: 20 birds/m²; B: 15 birds/m²; C: 10 birds/m²) and 4 repetitions. Chopped straw was used as litter/bedding. Continuous light regime 23L:1D was implemented. All chickens had the same adequate space where they received food and water. Nutrition was *ad libitum* with 4 mixtures, formulated based on corn/soy bean.

Control of the body weight of broilers and evaluation of welfare parameters were done at the age of 3 and 6 weeks on a sample of 50 chickens per box, i.e. 200 chickens per treatment. Mortality and feed conversion were calculated for whole fattening period. The locomotion ability was evaluated according to the method of Kestin et al. (1992). Gait score marks six categories relating to locomotion ability ranked from 0 (completely normal) to 5 (immobile), described in the paper by Thomas et al. (2004). In the same paper also the method is described based on which hock burns in chickens were determined: score 1 = no burns; score 2 = mild burns; score 3 = severe burns. Feathering of chickens was evaluated according to method of Gyles et al. (1962) modified to changes in body weight and breast width of modern broiler hybrids (Perić et al., 2007). Foot pad
lesions were scored using the three point scale (Thomas et al., 2004): score 1 = no lesions; score 2 = mild lesions; score 3 = severe lesions.

In order to determine the quality of litter, on 21st and 42nd day temperature of litter was measured on 5 locations/points in the box, and in this way average litter temperature was obtained per box as trial unit. At the end of trial, in the same way litter samples were taken to determine the moisture content by method of drying of samples at the temperature of 105°C until they reach constant weight.

Data was analyzed by method of variance analysis and Tukey test (Stat.Soft, Inc. STATISTICA, version 6). In addition to average values, analysis also included frequency of the welfare indicator scores in trial groups. Percentage data were converted to arcsine prior to analyses.

**Results and Discussion**

General tendency of decrease of body weight with the increase of stocking density was confirmed by average body weightes obtained at the end of the sixth week of age of broilers (table 1). In the third week, the positive effect of higher stocking density on growth rate was registered, through increased generating of metabolic energy in groups with more chickens in the box, and the ability of chickens, in this stage, to utilize it for more intensive growth (Dozier et al., 2005).

In the group with the highest stocking density the mortality of chickens was the highest (6.67%). In conditions of equal and constant feeding space, as a response to increase of stocking density, the consumption of food is reduced (Cravener et al., 1992). Depression in food consumption was greater in relation to reduction of body weight increase, so that ultimately we have better feed conversion in higher stocking densities (Škrbić, 2007).

**Table 1. Production parameters of chickens housed in studied stocking densities at the age of 3 and 6 weeks**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T R E A T M E N T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td><strong>3 weeks</strong></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>200</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>733.9 ± 90.4</td>
</tr>
<tr>
<td><strong>6 weeks</strong></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>200</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>2307.5 ± 304.5B</td>
</tr>
<tr>
<td>Mortality, %</td>
<td>6.67</td>
</tr>
<tr>
<td>Feed conversion, kg</td>
<td>1.92</td>
</tr>
</tbody>
</table>

A-B p<0.01
Average scores of welfare indicators, presented in table 2, are very high, with small differences between groups but certain deterioration observed in the group with the highest stocking density and in the function of time, i.e. age of chickens. It is difficult to give adequate assessment of the welfare condition in studied stocking densities on that basis alone. Therefore, in subsequent tables (3,5,6) frequencies of welfare indicator scores according to groups and age of broilers are presented.

Table 2. Average scores of welfare indicators at the age of chickens of 3 and 6 weeks

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Gait score</th>
<th>Foot pad</th>
<th>Hock burns</th>
<th>Feathering</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>200</td>
<td>0.04</td>
<td>1.08</td>
<td>1.01</td>
<td>2.34</td>
</tr>
<tr>
<td>B</td>
<td>200</td>
<td>0.04</td>
<td>1.04</td>
<td>1.00</td>
<td>2.18</td>
</tr>
<tr>
<td>C</td>
<td>200</td>
<td>0.04</td>
<td>1.04</td>
<td>1.00</td>
<td>2.14</td>
</tr>
<tr>
<td>6 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>200</td>
<td>0.25</td>
<td>1.21</td>
<td>1.37</td>
<td>1.21</td>
</tr>
<tr>
<td>B</td>
<td>200</td>
<td>0.08</td>
<td>1.12</td>
<td>1.27</td>
<td>1.13</td>
</tr>
<tr>
<td>C</td>
<td>200</td>
<td>0.16</td>
<td>1.00</td>
<td>1.18</td>
<td>1.07</td>
</tr>
</tbody>
</table>

If we accept the criteria of Kestin et al. (1992) that chicken welfare is severely compromised when gait score is 3 and above, we can conclude that welfare situation in all three groups of chickens at the age of 3 weeks was not disturbed. At the age of chickens of 6 weeks, 0.61% of evaluated chickens had score 3 and 0.61% score 5, which indicates severely disturbed welfare, considering that chickens in such conditions experience pain and discomfort and/or are immobile which compromises the fulfillment of their basic needs for food and water. However, also in group C, 1.44% chickens were scored 3. Cause for this is probably significantly higher body weight of chickens which leads to physical inactivity regardless of the available space for movement.

Table 3. Impact of stocking density on the gait score of broilers at 3 and 6 weeks of age

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stocking density</th>
<th>3 weeks</th>
<th>6 weeks</th>
<th>3 weeks</th>
<th>6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>A</td>
<td>NS</td>
<td>96.0</td>
<td>4.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>NS</td>
<td>97.0</td>
<td>2.5</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>NS</td>
<td>96.0</td>
<td>4.0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* p<0.05
In order to better understand the frequency of incidence of foot pad lesions and hock burns, it is necessary to analyze the changes in the quality of litter with the increase of stocking density (table 4). Stocking density affects the humidity and temperature of the litter/bedding, in a way that they increase with the increase of stocking density which is in accordance with the results of Mendes et al. (2004), Dozier et al. (2006), Škrbić et al. (2009). Quality of litter/bedding directly affects the condition and health of skin of chickens, moist and coarse litter is high risk for incidence of lesions and contact dermatitis.

Table 4. Average temperature and humidity of litter in studied broiler stocking densities

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average temperature, °C</th>
<th>Average contain humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 weeks</td>
<td>6 weeks</td>
</tr>
<tr>
<td>A</td>
<td>26.69</td>
<td>32.96</td>
</tr>
<tr>
<td>B</td>
<td>25.19</td>
<td>29.37</td>
</tr>
<tr>
<td>C</td>
<td>23.75</td>
<td>27.52</td>
</tr>
</tbody>
</table>

In stocking density of 20 birds/m² determined frequency of incidence of medium severe foot pad lesions was highly significantly higher compared to stocking density of 10 birds/m². Accordingly, frequency of scores for hock burns were similar, but without statistical significance between established differences (table 5). This effect was not present in previous study (Škrbić et al., 2009) which indicates the significance of commitment to a certain stocking density, but also significance of factors within the facility, primarily air humidity which, in conditions of high stocking density, inadequate air circulation and high outside humidity, also increases and has adverse effect on quality of litter and at the same time on incidence of foot pad dermatitis and hock burns. Elwinger (1995) defines them as factors of the farm and points out the importance of the season in determination of the optimal broiler stocking density.

Good feathering provides for broilers protection from injuries and negative influences from the environment, including low temperatures. Proces of forming of featheringis determined by genetic, but also hormonal status of the organism which is under significant influence of the environment and nutrition (Leeson and Walsh, 2004). With the increase of stocking density the feathering of broilers at the age of 6 weeks was of poorer quality (table 6). Differences in frequency of scores 1 and 2 were more expressed between groups A and C (p=0.068). Also, in group A, 2.45% of evaluated broilers had relatively great areas of skin not covered with feathers, whereas in groups B and C, there were no broilers with such scores.
Table 5. Impact stocking density on the incidence of foot pad lesions and hock burns of broilers at 3 and 6 weeks of age

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 weeks</th>
<th></th>
<th>6 weeks</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Foot pad lesions</td>
<td>%</td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Stocking density</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
</tr>
<tr>
<td>A</td>
<td>91.50</td>
<td>8.50</td>
<td>0</td>
<td>79.45&lt;sup&gt;bbl&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>96.50</td>
<td>3.50</td>
<td>0</td>
<td>87.50&lt;sup&gt;bAB&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>96.00</td>
<td>4.00</td>
<td>0</td>
<td>100&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hock burns</td>
<td>%</td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Stocking density</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>A</td>
<td>98.50</td>
<td>1.50</td>
<td>0</td>
<td>64.42</td>
</tr>
<tr>
<td>B</td>
<td>99.50</td>
<td>0.50</td>
<td>0</td>
<td>73.21</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>81.65</td>
</tr>
</tbody>
</table>

** p<0.01; a-b p<0.05; A-B p<0.01

Table 6. Effect of stocking density on feathering in broilers at 3 and 6 weeks of age

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 weeks</th>
<th></th>
<th>6 weeks</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Stocking density</td>
<td>%</td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>12.50</td>
<td>42.50</td>
<td>45.00</td>
<td>80.99</td>
</tr>
<tr>
<td>B</td>
<td>16.00</td>
<td>46.50</td>
<td>37.50</td>
<td>87.15</td>
</tr>
<tr>
<td>C</td>
<td>19.00</td>
<td>46.00</td>
<td>35.00</td>
<td>93.17</td>
</tr>
</tbody>
</table>

**Conclusion**

Stocking density, based on investigated indicators, cannot be considered a factor which influences broiler welfare at the age of 3 weeks. The assessment of locomotion ability based on gait score indicated that the best results can be achieved in conditions of “moderate” stocking densities (B group), considering that in this way the inactivity of broilers caused by lack of space, i.e. high body weights, is avoided.

Stocking density of 20 birds/m² leads to significantly lower growth of broilers, higher mortality and higher frequency of incidence of leg lesions and
problems with body feathering, compared to stocking densities of 15 and 10 birds/m².

Acknowledgment

This research is part of the Project EVB: TP-31033 financial supported by Ministry of Science and Technological Development of the Republic of Serbia.

Uticaj gustine naseljenosti na pojedine parametre dobrobiti brojlera 2. različite gustine naseljenosti brojlera


Rezime

Gustina naseljenosti se smatra jednim od važnijih faktora za dobrobit brojlera. Rad predstavlja nastavak istraživanja u cilju potpunijeg sagledavanja efekata različitih gustina naseljenosti brojlera na proizvodne performanse, stanje nogu i telesnog pokrivača, kao indikatore dobrobiti ali i proizvodnosti i kvaliteta proizvedenih pila. Ispitan je uticaj tri gustine naseljenosti (20, 15 i 10 grla/m²) u 4 ponavljanja na brojlerima genotipa Hubbard u uzrastu 3 i 6 nedelja. Gustina naseljenosti se, na osnovu ispitanih indikatora, ne može smatrati faktorom koji utiče na dobrobit pila u uzrastu od 3 nedelje. U uzrastu od 6 nedelja gustina naseljenosti od 20 grla/m² je rezultirala značajno manjim porastom brojlera, većim mortalitetom i većom frekvencijom pojavljivanja problema sa nogama i telesnim pokrivačem u odnosu na gustine od 15 i 10 grla/m².

References


Zakon o dobrobiti životinja. Službeni glasnik RS 41/09

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EFFECTS OF PHYTOGENIC ADDITIVE ON PRODUCTION AND QUALITY OF TABLE EGGS IN EARLY STAGE OF LAYING CYCLE

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Original scientific paper

Abstract: The aim of this paper is to present results achieved by adding dietary phytogenic additive (Biomin® P.E.P. 125 poultry) on production and quality of table eggs in the early stages of laying period in commercial Hy Line Brown hens. The experiment was conducted from 16 to 28 weeks of hens age. During the experimental period the following parameters were determined every week: egg production, percentage of second grade eggs and egg weight. The examination of egg quality was conducted at 21, 24, 26 and 28 weeks of hens age. Based on the obtained results we can conclude that the addition of dietary phytogenic additive induced an increase in egg production and egg weight and reduced the percentage of second grade eggs. Significant effects of phytogenic additive on some egg quality parameters were not established.

Key words: phytogenics, laying hens, performance, egg quality

Introduction

Intensive egg production, after a complete ban or restricted use of antibiotic growth promotors (Castanon, 2007), imposes a comprehensive researches about their replacement with additives that on acceptable way increase production performance, maintain animal health and not create harmful residues in food. One of the possible alternatives are phytogenic additives, which are specially prepared parts of plants or their extracts containing active ingredients (Lee et al., 2004, Windisch et al., 2009). Most research is focused on the evaluation of the effects of aromatic herbs and spices added in feed or water, because they contain a large number of substances with antimicrobial, antiviral and antioxidant activities (Bölükbaşı and Erhan, 2007, Bölükbaşı et al., 2009). Digestive stimulation by phytogenic additives is achieved through stimulation of saliva secretion, liver
function, pancreas and intestine enzymes activities (Platel and Srinivasan, 2004), intestine function and morphohistology (Incharoen and Yamauchi, 2009, Perić et al., 2010) and metabolism (Zhou et al., 2009, Chowdhury et al., 2002).

The aim of this study was to investigate the effects of a commercial dietary phytogenic additive in laying hens nutrition on production parameters and table eggs quality.

**Materials and Methods**

The study was conducted in the experimental farm "Pustara", at Faculty of Agriculture in Novi Sad. A total of 360 commercial Hy Line Brown hens divided in two equal groups were used in this experiment. Each group consisted of six repetitions. One repetition consisted of 6 cages with 5 hens in standard tree-floor batteries, making a total of 30 hens.

**Table 1. Composition and nutrient content of laying hens diet**

<table>
<thead>
<tr>
<th>Components, %</th>
<th>Pre-lay feed</th>
<th>Layer feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>57.32</td>
<td>44.46</td>
</tr>
<tr>
<td>Wheat middlings</td>
<td>6.00</td>
<td>7.00</td>
</tr>
<tr>
<td>Oil</td>
<td>/</td>
<td>2.00</td>
</tr>
<tr>
<td>Full fat soya (extruded)</td>
<td>6.51</td>
<td>15.63</td>
</tr>
<tr>
<td>Soyameal (44% CP)</td>
<td>11.64</td>
<td>9.54</td>
</tr>
<tr>
<td>Sunflower meal (33% CP)</td>
<td>9.5</td>
<td>9.00</td>
</tr>
<tr>
<td>Limestone</td>
<td>6.26</td>
<td>3.25</td>
</tr>
<tr>
<td>Ca particles</td>
<td>/</td>
<td>6.13</td>
</tr>
<tr>
<td>MCP</td>
<td>1.91</td>
<td>1.40</td>
</tr>
<tr>
<td>Salt</td>
<td>0.25</td>
<td>0.28</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.15</td>
<td>0.10</td>
</tr>
<tr>
<td>L-lizin</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>DL Methionine</td>
<td>0.09</td>
<td>0.16</td>
</tr>
<tr>
<td>Premix</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Nutrient content, calculated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>16.5</td>
<td>18.00</td>
</tr>
<tr>
<td>ME MJ/kg</td>
<td>11.52</td>
<td>11.8</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>0.82</td>
<td>0.93</td>
</tr>
<tr>
<td>Methionine (%)</td>
<td>0.41</td>
<td>0.46</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>2.75</td>
<td>4.00</td>
</tr>
<tr>
<td>Available P (%)</td>
<td>0.40</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Hens from both groups consumed a standard diet for pre-lay period (16-18 weeks of hens age) and a diet for laying period. Composition and nutritive value of diets are shown in Table 1. A phytogenic additive based on oregano, citrus and anise essential oils (Biomin® P.E.P. 125 poultry, Biomin GmbH, Herzogenburg,
Austria) was added to the feed for hens in the experimental group in the dosage of 0.125 kg/T of feed. Hens in the control group consumed feed without the additive. The experiment lasted from 16 to 28 weeks of hens age.

Egg production, percentage of second grade eggs and egg weight were determined every week. The egg quality was evaluated at 21, 24, 26 and 28 weeks of age. The following egg quality parameters were determined: egg weight, shell cleanliness, shell weight, shell thickness, shell breaking force, albumen height and yolk colour. Shell cleanliness was assessed by points on a scale from 1 (very dirty) to 5 (completely clean). Shell breaking force was determined by the instrument Egg Force Reader (Orka Food Technology Ltd, Israel). Yolk colour was determined using the Roche fan. Albumen height was measured with a tripod micrometer. On the basis of data on egg weight (M) and albumen height (H), Haugh units were calculated according to formula $HJ=100 \log (H+7.57-1.7M^{0.37})$. Albumen height in measurements for 26 week was not determined, due to technical reasons, and this parameter and Haugh units are not shown.

Statistical analysis was performed using GLM MANOVA and LSD post hoc test, with statistical software Statistica 8 (StatSoft, 2009).

Results and Discussion

Table 2 presents the results of egg production in the period from 19 to 28 weeks of hens age.

Table 2. Production parameters and average egg weight

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatments</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Phytogenic</td>
</tr>
<tr>
<td>Number of eggs per housed laying hen</td>
<td>48.04</td>
<td>48.15</td>
</tr>
<tr>
<td>Number of eggs per average hen</td>
<td>48.58</td>
<td>49.25</td>
</tr>
<tr>
<td>Percentage of second grade eggs</td>
<td>4.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Egg weight (g)</td>
<td>59.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.52&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Values within rows with no common superscript are significantly different.

<sup>*</sup>P<0.05; <sup>**</sup>P<0.01; NS - not significant

Positive effect of the phytogenic additive on egg production are reflected in increased number of eggs per housed and average hen, however these differences were not statistically significant. Differences in laying percentage in favor of the experimental group were statistically significant (P<0.05) in the period from 22 to 24 weeks of hens age. Analysis of the obtained results clearly showed significantly (P<0.05) lower percentage of second grade eggs in hens fed the phytogenic additive.
Findings of other authors (Al-Harthi et al., 2009; Zhou et al., 2009; Çabuk et al., 2006) confirmed increased laying percentage, while researchers such as Florou-Panera et al. (2006), Chowdhury et al. (2005) did not find a significant increase when phytogenic feed additives were used.

The average egg weight (Table 3) was significantly higher (P=0.0001) in the experimental group, which is in agreement with findings obtained by Florou-Panera et al. (2006), Bölükbaşı and Erhan (2007), Bölükbaşı et al. (2009). In their studies addition of phytogenic additives had a positive effect on egg weight. The differences in results of added phytogenic additives usually are attributed to the great diversity of applied treatments (plant species, vegetation period, method of preparation and applications) and different experimental conditions (Perić et al., 2009).

Table 3. The parameters of egg quality

<table>
<thead>
<tr>
<th>Weeks of age</th>
<th>Treatment</th>
<th>Egg weight (g)</th>
<th>Cleanliness</th>
<th>Shell breaking force (kg)</th>
<th>Shell thickness (0.01 mm)</th>
<th>Shell weight (g)</th>
<th>Shell weight (%)</th>
<th>Albumen height (mm)</th>
<th>Yolk colour (Roche)</th>
<th>Haugh unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>A</td>
<td>52.67</td>
<td>4.72</td>
<td>3.22</td>
<td>34.21</td>
<td>5.66</td>
<td>10.79a</td>
<td>10.68</td>
<td>11.97</td>
<td>103.49</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>53.64</td>
<td>4.73</td>
<td>3.23</td>
<td>34.09</td>
<td>5.56</td>
<td>10.29b</td>
<td>10.59</td>
<td>11.79</td>
<td>102.92</td>
</tr>
<tr>
<td>22</td>
<td>A</td>
<td>60.62</td>
<td>4.87</td>
<td>3.15a</td>
<td>35.25a</td>
<td>6.65</td>
<td>10.98</td>
<td>10.46</td>
<td>12.78</td>
<td>101.02</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>61.32</td>
<td>4.80</td>
<td>2.80b</td>
<td>34.65b</td>
<td>6.59</td>
<td>10.75</td>
<td>10.27</td>
<td>12.72</td>
<td>99.81</td>
</tr>
<tr>
<td>26</td>
<td>A</td>
<td>63.18</td>
<td>4.85</td>
<td>2.82</td>
<td>33.43</td>
<td>6.45</td>
<td>10.25</td>
<td>-</td>
<td>10.17</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>63.83</td>
<td>4.90</td>
<td>2.82</td>
<td>34.20</td>
<td>6.55</td>
<td>10.27</td>
<td>-</td>
<td>10.52</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>A</td>
<td>64.26</td>
<td>4.88</td>
<td>2.74</td>
<td>35.38</td>
<td>6.93a</td>
<td>10.81a</td>
<td>9.85</td>
<td>11.59</td>
<td>97.18</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>64.98</td>
<td>4.85</td>
<td>2.83</td>
<td>36.33</td>
<td>7.23b</td>
<td>11.15b</td>
<td>9.58</td>
<td>11.78</td>
<td>94.94</td>
</tr>
</tbody>
</table>

A - control, B - phytogenic additive
\[a, b\] Values within rows with no common superscript are significantly different (P<0.05)

Significant effects of a phytogenic additive on egg quality parameters were not established (Table 3). There was a certain decrease in shell breaking force and shell thickness of the eggs from the experimental group in week 22, but this effect was not repeated in other measurements. In week 28, the phytogenic additive showed a positive effect on shell weight, but a significant increase was not found in other measurements, and aforementioned effect cannot be considered as consistent. Other authors came to similar conclusions. Black cumin, according to the results of Aydin et al. (2008) improved shell thickness and shell breaking force. According to the results Botsoglou et al. (2005), only the addition of saffron, but not rosemary and oregano, affected intensity of yolk colour, while Haugh units and shell
thickess were not affected by any of the examined phytogenic additives. However mangrove leaf and spice mixtures increased Haugh units in a study of Al-Harth et al. (2009). Rosemary (Florou-Panera et al., 2006), oregano essential oil (Florou-Panera et al., 2006) and thyme (Bölükbaşi and Erhan, 2007) did not affect yolk colour and Haugh units.

**Conclusion**

A phytogenic feed additive based on oregano, citrus and anise essentinal oils had a positive impact on production and egg weight. Also, the additive reduced percentage of second grade eggs. Significant effect on the basic parameters of egg quality were not established.

**Acknowledgment**

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**Efekat fitogenog aditiva na proizvodnju i kvalitet konzumnih jaja u ranoj fazi nosivosti**

*M. Vekić, L. Perić, M. Đukić Stojčić, N. Milošević, S. Bjedov, T. Steiner*

**Rezime**

References


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INFLUENCE OF THE PREBIOTIC SALGARD AND A HERB MIXTURE ON PEKIN DUCKLINGS IN ORGANIC POULTRY PRODUCTION

I. GROWTH PERFORMANCE AND BLOOD BIOCHEMICAL PARAMETERS

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Original scientific paper

Abstract: The purpose of this study was to follow out the influence of the prebiotic Salgard and of an herb mixture (rosemary, thyme, basil, oregano and cinnamon) on the growth performance and blood biochemical parameters of Pekin ducklings in an organic production system. In this study, 72 one-day-old Pekin ducklings reared up to the age of 63 days were used. They were divided into 3 groups of 24 birds each and sexed (12 ♂ and 12 ♀) as followed: group I (control) fed a standard feed; group II – fed the same feed supplemented with the prebiotic Salgard at a concentration of 0.15 %, and group III – fed the same feed supplemented with 0.15 % of a herb mixture in an equal proportion (0.03% of each herb – rosemary, thyme, basil, oregano and cinnamon) from the first day of age to the end of the experiment. The individual live weight of the birds and the feed conversion ratio were controlled throughout the experiment at 1, 28 and 63 days of age. By the end of the study, blood serum ASAT, ALAT, GGT, triglycerides (TG), total and HDL cholesterol, and creatinine were assayed. The addition of Salgard to the feed of Pekin ducklings increased live weight with 4.94 % in males and with 4.67 % in females. The addition of the herbal mixture of rosemary, thyme, basil, oregano, cinnamon to the feed had a positive effect on the live weight. It is increased with 3.75 % in males and insignificantly in females. A significant reduction in the blood serum concentrations of triglycerides (P<0.01) and total cholesterol (P<0.01) was established, which could be related with the anti stress effect of the herbal mixture on Pekin ducklings.
Key words: duck, prebiotic Salgard, herb mixture, growth performance, serum biochemical parameters

Introduction

During the last decade, the production of high-quality healthy food from healthy animals, bred without the usage of nutritive antibiotics and anabolic stimulants has steadily become more significant (Eilers, 2006; Wald, 2004; Levic, et al., 2008).

As an alternative to antibiotics ban in the European Union (Regulation 1831/2003/EC), contemporary agriculture uses mostly probiotics, prebiotics, organic acid mixtures, herbs, spices, essential oils and plant extracts that, added to the feed and water, produce a stimulating effect on the productivity, health and welfare of poultry. This is possible due to the inhibiting effect of these natural substances on microbial pathogens in the alimentary tract and their positive effect on the digestion and the growth (Rodenburg et al., 2004, 2005; Wald, 2004; Kijlstra and Eijck, 2006; Levic, et al., 2008; Abd El-Hakim et al., 2009; Van de Weerd et al., 2009; Crandall, et al., 2009).

There are little data about the effects of prebiotics and herbs on health, welfare and growth of Pekin ducklings in organic production systems, thus motivating the current study.

The aim of the current research was to study the influence of the prebiotic Salgard and the herb mixture - rosemary, thyme, basil, oregano and cinnamon on the growth performance traits and blood biochemical parameters.

Material and Methods

Experimental birds and diet. The study was carried out at the poultry farm of Department of Animal Science at the Agricultural University – Plovdiv, in the period from August to October 2008. Three groups of 24 one-day-old ducklings each were formed, sexed and marked by gender (12 ♂ and 12 ♀). Throughout the experiment, all birds were fed ad libitum with diet prepared at the poultry farm. The combined feed of the ducklings from the first group (control) contained the ingredients described in Table 1. The second group’s feed was supplemented with the prebiotic Salgard at a concentration of 0.15 %, while the ducklings from the third group received 0.15 % of the combination: rosemary (Rosmarinus officinalis), thyme (Thymus serpyllum), basil (Ocimum basilicum), oregano (Origanum vulgare L.), cinnamon (Cinnamomum verum) in an equal proportion (0.03% of each herb) from the 1st day of age to the end of the experiment.
The ducklings in the three groups were reared in the same conditions in a closed premise with free access to small yards for walks. Each duckling was provided with 0.205 m² of the total covered area (4.8 birds/m²) and 9.2 m² of the yard. By 14 days of age, the ducklings in the three groups were provided with local brooders. The birds had free access to water for drinking and bathing.

**Table 1. Composition of diets (%) of Pekin ducklings (as-fed basis)**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Starter from 1 to 28 days of age</th>
<th>Finisher from 29 to 63 days of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground yellow maize</td>
<td>48.53</td>
<td>54.90</td>
</tr>
<tr>
<td>Ground wheat</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Soybean meal (44% CP)</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Fish meal (72% CP)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>L – lysine(99% purity)</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td>DL – methionine (99% purity)</td>
<td>0.11</td>
<td>0.1</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.5</td>
<td>1.3</td>
</tr>
</tbody>
</table>

**Calculated analysis**

<table>
<thead>
<tr>
<th>Metric</th>
<th>Starter from 1 to 28 days of age</th>
<th>Finisher from 29 to 63 days of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolisable energy, (MJ/kg)</td>
<td>12.4</td>
<td>12.7</td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>18.6</td>
<td>16</td>
</tr>
<tr>
<td>Crude fibre, %</td>
<td>2.9</td>
<td>2.7</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Linoleic acid, %</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>1</td>
<td>0.85</td>
</tr>
<tr>
<td>Total phosphorous, %</td>
<td>0.69</td>
<td>0.61</td>
</tr>
<tr>
<td>Metabolisable phosphorous, %</td>
<td>0.44</td>
<td>0.37</td>
</tr>
<tr>
<td>Lysine</td>
<td>1</td>
<td>0.75</td>
</tr>
<tr>
<td>Methionine +cystine</td>
<td>0.75</td>
<td>0.66</td>
</tr>
<tr>
<td>Triptophane</td>
<td>0.21</td>
<td>0.17</td>
</tr>
<tr>
<td>Treonine</td>
<td>0.68</td>
<td>0.57</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.12</td>
<td>0.94</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.46</td>
<td>0.4</td>
</tr>
<tr>
<td>Phenylalanine + thyrosine</td>
<td>1.43</td>
<td>1.27</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.6</td>
<td>1.42</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.8</td>
<td>0.67</td>
</tr>
<tr>
<td>Valine</td>
<td>0.9</td>
<td>0.76</td>
</tr>
<tr>
<td>Glycine+serine</td>
<td>1.7</td>
<td>1.43</td>
</tr>
</tbody>
</table>

**Growth performance and slaughter evaluation.** The individual live weight of the ducklings (with ± 0.01 g accuracy), as well as the consumption of feed (with ± 5 g accuracy) were controlled throughout the experiment at 1, 28 and 63 days of age.

At the end of the experiment, slaughter evaluation of the birds was performed with three male ducklings at average gender weight from each group.
The weighing was performed on an electronic scale OHAUS 2000 with ± 0.01 g accuracy.

**Blood biochemical examination.** Blood for analysis (5 ml) was sampled from v. subcutanea ulnaris of six ducks from each group on the 63rd day. The blood was allowed to clot for one hour at room temperature (25°C) and the samples were centrifuged at 2000 g for 10 min.

Blood serum ASAT, ALAT, GGT, triglycerides (TG), total and HDL cholesterol, and creatinine were determined with an automated biochemical analyzer “Cobas mira” at an accredited biochemical lab in the Diagnostic and Consultation Medical Centre “St. George”- Plovdiv.

**Statistical analyses.** Results were expressed as a mean and standard error. Data were subjected to one-way analysis of variance (ANOVA) using GraphPad InStat 3.06 software to determine the level of significance among mean values.

### Results and Discussion

Growth development indicated that at the age of 28 days Pekin ducklings from group I (control) and group II (Salgard addition) attained nearly the same live weight for both sexes (Table 2). In the third group (supplemented with the herb mixture), a lower live weight was observed, compared to group I and II (P<0.001). It is believed that the cause of this was the more difficult adaptation of birds to the feed containing herbs (rosemary, thyme, basil, oregano and cinnamon), which had a strong scent and unusual taste. According to some authors (Loo and Richard, 1992; Frankić et al., 2009) most herbs give feed specific odours and tastes. Jugl-Chizzola et al. (2006) noticed that weaned pigs consumed significantly less feed if it was supplemented with either thyme or oregano. If pigs in this experiment had the possibility to choose among feed with or without these spices, they would choose the non-supplemented feed.

#### Table 2. Body weight gain (BW) of Pekin ducklings

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>♂</td>
<td>52.33±1.15</td>
<td>52.00±1.00</td>
<td>52.08±1.06</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>53.17±1.35</td>
<td>53.42±1.15</td>
<td>53.42±1.08</td>
</tr>
<tr>
<td>28 days</td>
<td>♂</td>
<td>971±27 a1</td>
<td>987±32 a2</td>
<td>836±16 a1,a2</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>991±27 a1</td>
<td>963±27 a2</td>
<td>832±26 a1,a2</td>
</tr>
<tr>
<td>63 days</td>
<td>♂</td>
<td>2105±42</td>
<td>2209±78</td>
<td>2120±53</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>2076±39</td>
<td>2173±54</td>
<td>2154±44</td>
</tr>
</tbody>
</table>

Note: P<0.001 at a1- a1, a2- a2 in the same rank
Therefore, animals most probably needed more time to get accustomed to the taste of their feed, which was observed in the initial period with the ducklings who received the herb-supplemented feed.

In the period between 29 and 63 days of age a tendency for higher growth was observed in birds of both sexes that received the prebiotic Salgard (group II) and the herbal mixture (group III), compared to controls (group I). The intensity of live body weight gain was stronger in the ducklings from the 3rd group, which compensated for their delayed growth in the preceding period. At the experiment’s end, the ducklings that took Salgard (group II) had the highest live weight, followed by those that received the herbal mix with their combined feed (group III), and the control group at the lower end.

We assume that the higher live mass of the ducklings that received Salgard with their combined feed, compared to control ducklings (4.94 % higher weight gain in males and 4.67 % in females) was due to its positive effect on digestion. The released organic acids, included in Salgard, have a strong antibacterial and antifungal effect.

Pekin ducklings from the 3rd group compensated for their delayed growth after the 28th day. This was probably related to accommodation to the unusual smell and taste of the herb-containing feed, as well as to the positive complex effect on the digestion in the poultry. On one hand, the positive effect is due to the oregano essential oil, which improves digestion, resorption and nutrient utilization (Jamroz et al., 2003, 2006; Lee et al., 2003; Bampidis et al., 2005; Mikulski et al., 2008).

Regarding the carcass characteristics, no significant differences between the different groups could be found. In groups I, II and III the grill was 65.74±0.18 %, 65.47±0.63 % and 65.13±2.13 %, respectively, and the brathfertig – 71.75±0.14 %, 69.91±2.61 %, and 71.61±1.19 % (Figure 1).

![Figure 1. Slaughter characteristic of 63-day-old ducklings](image-url)
The positive effects of the used herbal mixture on the live weight of the ducklings could be explained with its antimicrobial, antifungal and antioxidant properties (Faleiro et al., 2005; Hazzit et al., 2006).

According to us, the better feed conversion per unit weight gain in group III, compared to the other two, for the period between 29th and 63rd days of age could result from the improved activity of digestive enzymes, the stimulation of the beneficial and suppression of the pathogenic microflora in the alimentary tracts. As a result, the absorption and utilization of nutrients is reported to be improved (Pasqua et al., 2006; Castillo et al., 2006; Windisch et al., 2008; Frankič et al. 2009).

For the entire period, the best combined feed conversion ratio was observed in ducklings from the group II vs. group I – by 9.03% and vs. group III – by 8.18% (Table 3). In our opinion, this was due to the positive effect of the prebiotic on the normal digestive microflora of birds and the microbicide effect. This is a reason for better utilization of feed, and an improvement in the poultry health, growth, and welfare.

Table 3. Feed intake (FI) and feed conversion ratio efficiency (FCE)

<table>
<thead>
<tr>
<th>Age</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake (FI) - g/bird</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-28 day of age</td>
<td>2276</td>
<td>2165</td>
<td>2130</td>
</tr>
<tr>
<td>29-63 day of age</td>
<td>4808</td>
<td>4782</td>
<td>4913</td>
</tr>
<tr>
<td>1-63 day of age</td>
<td>7084</td>
<td>6947</td>
<td>7043</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feed conversion ratio efficiency (FCE) - FI/ 1 kg BW gain</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1-28 day of age</td>
<td>2452</td>
</tr>
<tr>
<td>29-63 day of age</td>
<td>4337</td>
</tr>
<tr>
<td>1-63 day of age</td>
<td>3478</td>
</tr>
</tbody>
</table>

Regarding the examined blood biochemical indicators, many authors have found that the increase of total serum cholesterol and triglyceride concentrations are reliable indicators of stress in birds (Puvadolpirod and Thaxton, 2000a,b,c; Popova–Ralcheva et al., 2002a,b).
Table 4. Blood serum biochemical parameters in 63-day-old Pekin ducklings (n=6)

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>ASAT, U/L</td>
<td>85.5 ± 9.51</td>
</tr>
<tr>
<td>ALAT, U/L</td>
<td>29.33 ± 0.76</td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>15.35 ± 1.45</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>6.90 ± 0.41 b1</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.25 ± 0.40 b2</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.93 ± 0.23</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>13.67 ± 1.65</td>
</tr>
</tbody>
</table>

Note: P<0.01 at b1, b2 in the same rank

The significantly lower serum concentrations of triglycerides (P<0.01) and total cholesterol (P<0.01) in ducklings who received the herbal mix could be related to the complex antistress, antioxidant and antimicrobial effect of rosemary, thyme and oregano, included in the feed supplement (Faleiro et al., 2005; Hazzit et al., 2006; Windisch et al., 2008) – Table 4. The antioxidant properties of these herbs are due to the effect of their phenols, flavonoids and hydrolyzed tannins, involved in neutralizing free radicals or activating antioxidant enzymes – superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (Halliwell et al., 1995; Craig, 2001; Ćetković et al., 2004; Škerget et al., 2005; Bakirel et al., 2008; Fasseas et al., 2008; Frankič et al., 2009).

Conclusion

1. The addition of Salgard or the herbal mixture (rosemary, thyme, basil, oregano, cinnamon) in a concentration of 0.15 % respectively to the diet of Pekin ducklings contributed to an increase in the live weight and the conversion of feed.

2. The addition of herbal mixture (rosemary, thyme, basil, oregano, cinnamon) decrease the blood serum concentrations of triglycerides (P<0.01) and total cholesterol (P<0.01), which could be related with their anti stress effect.

Acknowledgment

This work was supported by Ministry of Education, Youth and Science of Republic of Bulgaria (Project N 18-08, University Fund for Scientific Research – Agricultural University, Plovdiv)
Uticaj prebiotika Salgard i biljne smeše u ishrani pekinških pačića u organskoj živinarskoj proizvodnji
I. Proizvodni rezultati i biohemijski parametri krvi

V. Gerzilov, N. Bozakova, A. Bochukov, G. Penchev, M. Lyutskanov, S. Popova-Ralcheva, V. Sredkova

Rezime

Cilj ovog ispitivanja je bilo praćenje uticaja prebiotika Salgarda i biljne smeše (ruzmarin, majčina dušica, bosiljak, origano i cimet) na porast i biohemijske parametre krvi pekinških pačića u organskom proizvodnom sistemu.

U ovom istraživanju, 72 jednodnevna pačeta su odgajana do uzrasta od 63 dana. Podeljeni su u 3 grupe od po 24 ptice u svakoj (12 ♂ i 12 ♀) na sledeći način: grupa I (kontrola) hranjena standardnom hranom; grupa II – hranjena istom hranom uz dodatak prebiotika Salgard u koncentraciji od 0.15%, i grupa III – hranjena istom hranom uz dodatak 0.15% biljne smeše u jednakim proporcijama (0.03% svake biljke - ruzmarin, majčina dušica, bosiljak, origano i cimet) od prvog dana do kraja eksperimenta.

Kontrolisani su telesna masa ptica i konverzija hrane tokom čitavog eksperimenta i to 1., 28. i 63. dana uzrasta. Na kraju ispitivanja, krvni serum ASAT, ALAT, GGT, trigliceridi (TG), ukupni i HDL holesterol, i kreatinin su analizirani.

Dodavanje Salgarda u hranu za pekinške pačице je uticalo na povećanje telesne mase za 4.94 % kod muških i za 4.67 % kod ženskih pačića.

Dodavanje biljne smeše koja se sastojala od ruzmarina, majčine dušice, bosiljka, origana i cimeta u hranu je imalo pozitivan uticaj na telesnu masu, koja se povećala za 3.75% kod muških grla, a kod ženskih povećanje nije bilo significantno. Značajno smanjenje u koncentracijama triglicerida (P<0.01) i ukupnog holesterola (P<0.01) u krvnom serumu je utvrđeno, što može biti dovedeno u vezu sa anti stres efektom biljne smeše na pekinške pačiće.

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Fungi on feathers of common clinically healthy birds in Belgrade

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Original scientific paper

Abstract: In the quarantines of the epyzoo technological territory of Belgrade, feathers deriving from live and dead dehydrated exotic birds were samples. Birds were housed in closed disinfected bird shelter facilities with cages. Study was carried out in 4 quarantines of total 22, during 2010, and it included only 5 bird species, 3 birds of each species (Coracias cyanogaste, Acridothere tristis, S. canaria, Pycnonotus cafer and Tockus fasciatus). A total of 15 samples of feathers were analyzed. Samples were placed in the antibiotics solution, for 24 hours at room temperature, and subsequently cultivated on Sabouraud dextrose agar and Potato dextrose agar, in aerobic conditions in the darkness at the temperature of 37°C duration of 5 days, and 3-4 weeks at 20±2 ºC (Scopulariopsis spp.), and in aerobic conditions at room temperature of 20±2 ºC for 5 days (Aspergillus spp., Penicillum spp. and Fusarium spp.) The presence of Scopulariopsis spp., Aspergillus spp., Penicillum spp. and Fusarium spp. was identified. The study showed that commonly healthy birds, as well as dead birds, which died mainly due to exhaustion and dehydration during transportation, can carry various fungi/moulds which contaminate the air, soil and water surrounding their habitats. Most of these birds are sold as closed domesticated pets which are clinically healthy birds, however, they can be important source of potentially pathogen causers which they carry on their body. This finding of fungal species on the body/feathers of birds are naturally suitable place for their transmission, and this also contributes to better understanding of the nature and occurrence of many widespread diseases in transmitting mycoses.

Key words: fungi, birds feather, Belgrade quarantine
Introduction

Most of imported birds, and especially parrots, kept in the house environment as pets, can potentially be dangerous in conditions of direct contact of humans with the feathers (Deshmukh et al., 2004). Bird’s feathers are known carrier of microorganisms and especially of pathogen fungi which are capable to infect humans and animals (Camin et al., 1998; Anbu et al., 2004). In Serbia, mycological studies of the animal feed are done to prevent incidence of diseases and spreading of fungi (moulds) in the free space. Investigation of their prevalence was done in a study of the poultry feed by Krnjaja et al. (2008, 2010). Authors investigated in 2007 a total of 230 samples, and in 2008 total of 234 samples. Dominant presence of Fusarium 56.09 % and 63.40%, respectively was determined, as well as of Aspergillus 54.35 % and 73.62 %. Also Rhizopus, Penicillium, Mucor and Alternaria were isolated.

Numerous cases of aspergillosis in humans were described (Baillot et al., 2001), in exotic birds (Michal and Orosz, 2000), and intensively reared poultry (Ivetić et al., 2003), in chickens after artificial infection (Ivetić et al., 1999), whereas (Spalević et al., 2010) described its incidence in broiler chickens in hatching stations, and (Kureljušić et al., 2010) contributed to diagnostics of aspergilosis.

The following authors report on non-identified white-grey-brown fungi on bird’s feathers and alterations on skin of broiler parent hens in Serbia (Miljković et al., 2006, 2009), Scopulariopsis brevicaulis, was described for the first time as Penicillium brevicaulis Sacc. (Bainer 1907) but anamorph-teleomorph connections with Microascus brevicaulis sp. nov. the teleomorph of Scopulariopsis brevicaulis, and heterothallism in the Microascaceae by Abbott et al. (1998) and Abbot and Lynne, (2001) and its dimorphism was described by (Paula et al., 1987).

Finding of Aspegillus niger, Fusarium spp. Trichophyton spp. Mucor spp., Penicillium spp., Chrysosporium spp. and Scopulariopsis brevicaulis in samples from hoofs horn of 8 investigated horses in Wien was reported by (Apprich et al., 2009).

Scopulariopsis brevicaulis was proven as cause of lack of hair cover on the skin of two goats and it was described by (Ozturk et al., 2009) in turkey.

In a study of the presence of fungal species in poultry feed in Egypt, Scopulariopsis brevicaulis was detected in wheat, soy bean and fish meal, as well as Mucor spp., Aspergillus spp., Penicillium spp., Rhizopus spp., Fusarium spp. and other species (Moharram et al., 1987).

Animal diseases caused by mycological agents have not been described in Serbia as much as diseases present in the environment, on plants, in recreational waters (Matavulj et al., 2005). Authors Muntañola- Cvetković and Ristanović (1977, 1980), are considered as initiators of the research of potential micro fungi in...
the waters of South Adriatic, as a connection between men and waste present on
the sea coast. Some authors study indigenous micro fungi of lakes such as Vlasina
lake (Vukojević et al., 1997), and Sava lake (Ljalević et al., 2000). In all reports
obtained from mentioned studies, presence of Aspergillus, Penicillium, Alternaria,
Trichoderma, Mucor and Rhizopus species, Cladosporium spp. was confirmed as
well as other potential allergens, and also fungi which accompany infections of
immunedeficient persons, are noticed.

In addition to mentioned data referring to their presence, our study as a
pilot experiment can point to the introduction of fungi (moulds) into our
environment through import of otherwise healthy birds. Of course, this finding
explains their natural introduction through bird migration since they know no
borders.

Materials and Methods

Quarantines for imported birds were located within the Belgrade
eyzootiological territory. Total number of birds clinically observed in this study
housed in 4 quarantines was 2260. Birds were commonly healthy, only in 2
quarantine cases deaths of birds during transport were reported, caused by
dehydration during summer months. Larger birds suffered more during transport.
Study was conducted on following bird species: Coracias cyanogaster (Blue-
bellied roller), order Coraciiformes; Acridotheres tristis (Common myna or indian
myna) order Passeriformes; Tockus fasciatus (African pied hornbill) order
Coraciiformes; Familii Fringillidae (S. canaria) genus Serinus, order
Passeriformes; Pycnonotus cafer (Red-vented bulbul) order Passeriformes.

Investigation included healthy birds and birds which died of exhaustion
and lack of water. Feathers were taken, using medical gloves and aseptic tools from
3 birds per species. Four subsamples from each bird consisting of at least five
feathers were plucked aseptically and carefully from the neck area, outside and
inside of the wings and around cloaca. These subsamples were mix to prepare one
composite sample per bird. Collective feather samples per bird were marked
according to the species and packaged into pvc bags with zipper, and transported in
a mobile refrigerator and stored before analysis at +4°C.

Isolation of fungal species was done by cultivation of feather samples kept
in the antibiotic solution for 24 hours (penicillin 10,000 U ml⁻¹, streptomycin 10
mg ml⁻¹, gentamicin sulphate 50 mg ml⁻¹) and subsequently directly cultivated on
selective medium for fungi - Sabouraud dextrose agar, and cultivated aerobic at
20±2 for 5 days. More white colonies of Aspergillus spp. were spotted already
after 18 hours, and after growth period of 48 hours it could be distinguished
macroscopically from colonies of Penicillium spp. Identification was based on

In order to obtain better macro and micro characteristics of Fusarium spp., potato dextrose agar (Nash and Michelle., 1991) was used.

Isolation of Scopulariopsis spp. was done on same mediums for duration of 5 days on 37°C in absolute darkness, and continuous subculturing (5 to 5 days) was realized on potato-dextrose agar at 20±2 ºC for 3-4 weeks (Paula et al., 1987). Obtained culture was microscopically and macroscopically compared to identified and maintained culture of Scopulariopsis brevicaulis isolate number 03-224 of 2009 in Reference Mycologi laboratory, Bristol UK. Microscopic characteristics were obtained by preparation of the native preparation and staining by using laktophenol blue. Species Scopulariopsis brevicaulis has macroscopic characteristics of colonies which is granular surface to powder texture. From the front, colour is white at the beginning and later it turns grey or light brown. Microscopically septa hyphae, conidiophores, annelids and conidia can be seen. Conidiophores are either simple or they branch. Annelids are solitary, in bunches or in pennicilus form: they cylindrical and slightly thickened. Conidia are single cell, round or piriform, smooth, sometimes also coarse, or even barbed surface (Muntañola -Cvetković 1990; Abbott et al., 1998, 2001).

Results and Discussion

Birds in migration know no borders and they carry on their feathers everything present in their environment, either on land or in air. Cafarchia et al. (2006) stated that domestic and wild birds are known carriers of human pathogen fungi. In their research of the presence of yeast in cloacae, included 1726 birds of which 421 (24.39%) were migrating from the territory of Romania, Hungary and Bulgaria.

Our finding showing presence of 80% of fungal species in 15 feather samples from 5 bird species is in accordance to study performed in Kingdom of Bahrain (Qaher et al., 2009), on 10 birds species where they reported that 69.77% of samples were positive in regard to presence of fungi. Also, they determined the presence of keratinophylic fungi, as well as presence of Scopulariopsis spp. in pigeon, parrot, quail, ducks and chicken. Our results are presented in Table 1.
<table>
<thead>
<tr>
<th>Bird species</th>
<th>Fungal genera</th>
<th>Positive samples</th>
<th>Negative samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. cyanogaster</td>
<td>Aspergillus</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fusarium</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Penicillium</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Scopulariopsis</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>A. tristis</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>T. fasciatus</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fringillidae</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. cafer</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Number (%) of isolates (samples)</td>
<td>2 (25%)</td>
<td>2 (25%)</td>
<td>3 (37.5%)</td>
</tr>
</tbody>
</table>

Obtained results indicate that in the environment where birds are kept closed in cages, there may be dust particles in the air, potential carriers of sporogenic pathogens. According to accompanying documentation kept in individual quarantines, it is obvious that certain birds were artificially reared on farms. Birds are transported in cages where they are provided food and water, while travelling from Tanzania, Guinea and Arab Emirates. Obtained finding of fungi is not unusual since feathers can carry all of these agents. Mbata (2009) determined Microsporium gallinae, Microsporium gypseum, Trichophyton mentagrophytes, Candida albicans, Fusarium spp., Scopulariopsis spp., Alternaria alternata, Aspergillus flavus on few wing feathers of chickens reared in warm regions, which are called „featherless broilers“, and had clinical skin superficial mycoses.

Camin et al. (1998) report of the presence of Scopulariopsis brevicaulis and other keratinophylic fungi on sparrow feathers (Sturnus vulgaris), their movement radius is associated with urban and rural parts of France.

On waste sites – land fills surrounding 10 poultry farms where chicken are reared on the waste surrounding farms in the region of Tami Nandu in India, research was carried out on remains of the feathers on presence of keratinolytic fungi. In the soil, the most frequent were Chrysosporium keratinophilum 73%, Microsporium gypseum 64% and other fungi, but Scopulariopsis brevicaulis was also isolated and is presence of 5.64% determined (Anbu et al., 2004).

In addition to soil contamination, they also are transmitted through water, air but also mechanically through ticks.

Yoder et al. (2005) reported of American dog tick, Dermacentor variabilis which was proven to be mechanical vector of yeast (mould) Scopulariopsis brevicaulis. This tick also is transmitter of Rocky mountain spotted fever and within its biological control in the nature Scopulariopsis brevicaulis could not be included. Therefore, the same authors, after extended laboratory investigation, have succeeded in proving endosymbiotic conidial fungus Scopulariopsis brevicaulis...
which has role to protect the tick from other species of entomopathogenic fungus *Metarhizium anisopliae*. (Yoder et al., 2008).

Cages in which birds are housed during the quarantine are presented in (Figure 1a,b,c,d) and pictures of birds (Figure 1e,f,g,h,i) included in the quarantine research were taken from [www.wikipedia.com](http://www.wikipedia.com).

![Figure 1](image)

**Figure 1.** Cages used for housing of birds (a,b,c,i d); *Tockus fasciatus* (e) *S. canaria* (f) *Pycnonotus cafer* (g) *Acridotheres tristis* (h) *Coracias cyanogaste*.(i)

During quarantine, air is disinfected as prevention and food for birds or seeds used in their nutrition are treated with fungicide preparations preventing the development of sporogenic moulds. Of course, it is necessary to exclude the presence of eco-parasites as potential mechanical carriers of fungi.

**Conclusion**

Such researches expend the knowledge of distribution of ubiquitous fungi present so far usually only in and on the soil.
At the same time, such studies help identify the natural habitats of the pathogenic fungi and contribute to our understanding of the epidemiology of infections caused by them.

**Acknowledgment**

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**Gljive na perju obično klinički zdravih ptica u Beogradu**

*B. Miljković, Z. Pavlovski, D. Jovičić, O. Radanović, B. Kureljušić*

**Rezime**


Ispitivanje ukazuje da obično klinički zdrave kao i uginule ptice, najčešće uginule zbog iscrpljenosti i dehidratacije u transportu, mogu da donesu na sebi raznovrsne gljive/plesni kojima se kontaminira vazduh, zemlja i voda u okolini u kome borave. Mikološki je ispitano perje uginulih i perje uzorkovano od živih egzotičnih ptica u karantinu, na epizootiološkom području Beograda.

Većina ovih ptica se prodaju kao zatvoreni kućni ljubimci koji su klinički zdrave ptice ali mogu da budu važan izvor potencijalno patogeni uzročnika kod prenošenja mikoza. Ovaj nalaz gljive na telu/perju ptica otkriva prirodno mesto za njihovo prenošenje, i takođe doprinosi boljem razumevanju prirode i nastanka veoma rasprostranjenih bolesti koje su prouzrokovane navedenim uzročnicima mikoza.
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Fungi on feathers ...


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PARTICIPATION OF MAIN PARTS AND INTERNAL ORGANS IN RABBIT MEAT

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Original scientific paper

Abstract: This study shows the results of examination of main rabbit carcass parts and internal organs. A total of 22 rabbits of mixed breed (Californian and New Zealand rabbits) were examined. The average live mass of the rabbits was 2.467 g varying from 2.000 g to 2.800 g, while the hide participated with 428 g varying from 385 g to 480 g. The head participated with 5.93%, the forelegs and hinde legs participated with 0.8% and 1.94%, and the internal organs participated with 0.51% (lungs) and 17.61% (bowels).

Key words: live mass, average share, head, hide, internal organs

Introduction

In previous years the interest in the production of meat containing less fat, i.e. meat poorer in cholesterol, increased.

Under today’s economic conditions the production of rabbit meat becomes more and more significant. Also, due to rabbit fertility and growth potential the breeding of rabbits becomes more attractive for production of food reserves. The rabbits are very fertile and characterized with a quick and intensive growing, large biological value of the meat and high feed conversion degree of the food. (Hammond and Marshall, 1925; Hammed and Casida, 1969; Hafiez 1970; Urosevic et al.,1986; Urosevic et al.,2000; Kapitan 2006);

The rabbit meat has good nutritional and dietetic value. It is very nutritional and easily digestible with a high content of proteins and a small percentage of fat, which makes it better than beef, pork and lamb meat. The human organism adopts up to 90 % of the proteins in the rabbit meat, while only 82% of the beef meat. (Dale Zote et al.,1986; Dale Zote 2002; Pascual et al.,2004; Polak et al.,2006). That is why it is highly recommended to children, pregnant women, people with cardiac and other diseases of the blood vessels.

In foreign literature the studies of rabbit slaughter values, mainly Californian breed, are numerous. Such studies were conducted by (Kovacevic and Raseta, 1983; Grujic 1985; Trojan and Mach, 1982; Mach et al., 1983; Chavoshki
and Riminskaya, 1982; Skandro et al., 2004; Panic and Petrovic, 1989). Their examination data show a variance of the yield of meat from 49.8% to 53.3%.

Because of the use of different cutting schemes, it is very difficult to compare the results pertaining to cutting of the cooled carcasses. That is why we shall show only the data given by Grujic (1985), who claimed that the front part participated with 22-24%, the back with 34-38%, the thighs 32-36%, the heart and the liver 5-7%.

According to the data of Chavoshki and Raminskaya (1982) who examined Californian breed rabbits with average carcass mass of 3.200 g, the pelvic-thigh part contributed 30-34%, the flank part 20-22%, the forelegs 12-13.5% and the neck-breast part 21-24%. The same authors the head participated with 6.25% and the hide with 11.5%. According to the data of Panic et al. (1986) who examined Californian breed rabbits with average body mass of 3.000 g, the pelvic-thigh part contributed 33.3%, the flank part 25.6%, the forelegs 15.5% and the neck-breast part 25.6%.

Since in our literature there is no data on the share of the basic parts and internal organs in the mass of the carcass of the rabbits, we have decided to set our goal to determine the percentage contribution of some parts and categories of meat in the carcass of rabbits from Macedonia.

**Materials and Methods**

The research of the share of rabbit carcass parts was conducted on 22 slaughtered rabbits of mixed Californian and New Zealand breed.

The rabbits were fed *ad libitum* with granulated rabbit food - palettes that contain alfalfa hay, barley, corn, wheat, soya pods, sunflower pods, premix, salt, vitamins and minerals. Rabbits were not fed twenty hours before slaughtering.

The slaughtering and primary processing were conducted in accordance to all veterinarian and sanitary conditions prescribed by the law. The slaughtering was done by cutting open the blood vessels.

After the skinning, each hide was weighed on an electronic scale to determine the percentage of the hide in total body mass. Then the internal organs of each rabbit (heart, liver, lungs, and intestines) were weighed to determine their separate share in the body mass. After weighing of the carcass with the head and without the head, the carcass was cut in four parts.

The pelvic-thigh part was extracted first by cutting in parallel of the spine by the cranial rim of the bowel bone’s wings. Then the flank part was extracted by a cut going in parallel with the spine by the rim of the last rib.

The back with the ribs was extracted from the foreleg and the neck by a cut going parallel with the spine in the area of the sixth vertebrae cutting the ribs and the inter-rib muscles. Thus the following parts were obtained: pelvic-thigh part,
flank part, forelegs and neck-breast part. All research was conducted by the firm RABBIT Sveti Nikole, R. Macedonia which has its own rabbit farm and rabbits for slaughter. Slaughterhouse operates under the HACCP system. All data were processed according to a variation statistical method (average value, standard deviation, variation coefficient, minimum and maximum value) computed by the UNIVARIATE procedure of the statistical program SAS (SAS Institute 1999).

Results and Discussion

The average values of the live mass before slaughtering and the average mass of some parts and organs of the examined rabbits are given in Table 1. The average mass of the rabbits prior to slaughtering was 2,467 g. The average weight of the head was 124 g, of the hide 428 g, of the lungs 12 g and of the full intestines 445 g. The average share of the carcass with and without the head was 56.18 % and 50.66 % of the total mass of the rabbit (yield), respectively. The highest variation was confirmed for the head and the lungs (Cv 22.83 and 27.50), while the smallest variation was confirmed for the kidneys (Cv 1,10).

Table 1. Average values and variation of the body mass of rabbits before slaughtering structured in certain parts and organs.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>x</th>
<th>Sd</th>
<th>Cv</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live mass (kg)</td>
<td>2.467</td>
<td>0.1045</td>
<td>4.24</td>
<td>2.000</td>
<td>2.800</td>
</tr>
<tr>
<td>Head (kg)</td>
<td>0.124</td>
<td>0.0370</td>
<td>22.83</td>
<td>0.105</td>
<td>0.135</td>
</tr>
<tr>
<td>Hide (kg)</td>
<td>0.428</td>
<td>0.0240</td>
<td>5.81</td>
<td>0.345</td>
<td>0.575</td>
</tr>
<tr>
<td>Kidneys (kg)</td>
<td>0.035</td>
<td>0.0038</td>
<td>1.10</td>
<td>0.010</td>
<td>0.105</td>
</tr>
<tr>
<td>Lungs (kg)</td>
<td>0.012</td>
<td>0.0033</td>
<td>27.50</td>
<td>0.010</td>
<td>0.020</td>
</tr>
<tr>
<td>Liver (kg)</td>
<td>0.078</td>
<td>0.0150</td>
<td>19.23</td>
<td>0.050</td>
<td>0.100</td>
</tr>
<tr>
<td>Heart (kg)</td>
<td>0.060</td>
<td>0.0033</td>
<td>5.50</td>
<td>0.005</td>
<td>0.050</td>
</tr>
<tr>
<td>Full bowels (kg)</td>
<td>0.445</td>
<td>0.0421</td>
<td>9.26</td>
<td>0.405</td>
<td>0.555</td>
</tr>
<tr>
<td>Forelegs (kg)</td>
<td>0.020</td>
<td>3.2252</td>
<td>16.12</td>
<td>0.015</td>
<td>0.060</td>
</tr>
<tr>
<td>Hinder legs (kg)</td>
<td>0.048</td>
<td>14.4871</td>
<td>12.52</td>
<td>0.035</td>
<td>0.100</td>
</tr>
<tr>
<td>Carcass with head (kg)</td>
<td>1.386</td>
<td>0.3650</td>
<td>26.62</td>
<td>1.288</td>
<td>1.478</td>
</tr>
<tr>
<td>Carcass without head (kg)</td>
<td>1.250</td>
<td>0.1000</td>
<td>8.00</td>
<td>1.112</td>
<td>1.480</td>
</tr>
</tbody>
</table>

Obtained results of the share of the parts and organs compared to live mass are slightly higher than those reported by some other authors Mach et.al. (1983) and Skandro et.al (2008) who examined New Zealand white rabbits. The yield results that we obtained are similar to the results of other authors Caklovica et.al. (1986), Omrcen ( 1995), (Skandro et.al. 2004) and Ali (2007) who report yield
values from 40 % to 55 %. The share (in %) of some basic parts and internal organs in the live mass are given in Table 2.

**Table 2. Share in % of some basic parts and internal organs in the live mass**

<table>
<thead>
<tr>
<th>Basic parts and organs</th>
<th>Share (%) in the live mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcass with head</td>
<td>54.96%</td>
</tr>
<tr>
<td>Carcass without head</td>
<td>50.06%</td>
</tr>
<tr>
<td>Head</td>
<td>5.93%</td>
</tr>
<tr>
<td>Hide</td>
<td>17.27%</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.65%</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.51%</td>
</tr>
<tr>
<td>Liver</td>
<td>3.10%</td>
</tr>
<tr>
<td>Heart</td>
<td>2.42%</td>
</tr>
<tr>
<td>Full bowels</td>
<td>17.61%</td>
</tr>
<tr>
<td>Forelegs</td>
<td>0.80%</td>
</tr>
<tr>
<td>Hinder legs</td>
<td>1.94%</td>
</tr>
</tbody>
</table>

As the table shows the largest share in the live mass was determined for cleaned carcass with the head (54.96 %), while the smallest share was determined for forelegs (0.8%) and the hind legs have 1.94 %. In regard to internal organs the smallest share was determined for lungs (0.51%) and the largest share has the full bowels (17.61%).

Our results for the percentage of carcass parts and internal organs of rabbits in the live mass are slightly bigger than the results of other authors Grujic (1985 and Panic et al. (1986) who explored New Zealand white rabbits with smaller average live mass.

The participation of the main meat categories in the mass of the carcass with head on are given in Table 3.

**Table 3. Share of main meat categories of rabbit carcass with head**

<table>
<thead>
<tr>
<th>Share</th>
<th>g</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvic - thigh part</td>
<td>420,5</td>
<td>30.67</td>
</tr>
<tr>
<td>Flank part</td>
<td>398,5</td>
<td>29.04</td>
</tr>
<tr>
<td>Forelegs</td>
<td>300,5</td>
<td>21.94</td>
</tr>
<tr>
<td>Neck - breast part</td>
<td>267,0</td>
<td>18.35</td>
</tr>
</tbody>
</table>
The chart clearly shows that the largest share was determined for thighs (30.67%) and the smallest share for neck-breast part (18.35%).

The results are in accordance with the results of other authors Panic et al. (1986), Ali (2007) and Scandro et al. (2008).

Conclusions

Based on the achieved results we could conclude the following:

The average shares of the carcass with the head and the carcass without the head was 55.65% and 50.66 % of the average rabbit live mass, respectively. Internal organ with smallest share in live mass was lungs with 0.51 % and with largest share of 17.61 % were the full intestines. The shares of the thighs and the flank part were 30.67 % and 29.04 %.

Učesce osnovnih delova trupa i unutrašnjih organa kod kunića

A. Kuzelov, E. Atanasova

Rezime

U radu su izneti rezultati od istraživanja o učešću osnovnih delova unutrašnjih organa u trupovima kunića. Ukupno je ispitano 22 grla zaklanih kunića koji predstavljaju križanci između kalifornijskog i novozelandskog kunića. Prosečna živa masa kunića je iznosila 2467 g sa varijacijama od 2000 do 2800 g. Učešće kože u živoj masi trupa je iznosilo 428 g sa varijacijama od 385 do 480 g. Glava u živoj masi trupa je učestvovala sa 5,93%. Učešće prednjih i zadnjih sepa u živoj masi trupa je iznosilo 0,60% i 1,94%. Od unutrašnjih organa najmanje učešće u živoj masi trupa bilo je kod pluća 0,51% a najveće kod punih creva 17,61%.

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THE PRESENCE OF TOXIGENIC Fusarium SPECIES AND FUSARIOTOXINS DEOXYNIVALENOL AND ZEARALENONE IN WINTER WHEAT

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Original scientific paper

Abstract: The frequency of fungi and mycotoxin concentrations of deoxynivalenol (DON) and zearalenone (ZON) were studied in winter wheat grains harvested in 2009. The most frequently isolated species belonged to genera Alternaria (81.55%) and Fusarium (12%), followed by Rhizopus spp. (3.75%), Acremoniella spp. (1.15%) and other fungi (Acremonium spp., Arthrinium spp., Aspergillus spp., Bipolaris spp., Chaetomium spp., Nigrospora spp., Penicillium spp. and Ramichloridium spp.) isolated in less than 1%. The following species of the genus Fusarium were identified: F. graminearum (82.50%), F. sporotrichioides (5.42%), F. proliferatum (4.17%), F. subglutinans (4.17%), F. poae (1.66%), F. semitectum (1.25%), and F. verticillioides (0.83%). In 100% of wheat grain samples DON was detected (110–1200 μg kg⁻¹, average 490 μg kg⁻¹), while ZON was detected in 10% of samples and in the lower average of 70 μg kg⁻¹ with the limit values ranging from 60 to 80 μg kg⁻¹. Statistically significant positive correlations were established between the concentration of ZON with the frequency of F. graminearum (r = 0.63**) or with the frequency of Fusarium spp. (r = 0.58**). A negative insignificant correlation was determined between the DON level and the percentage of present Fusarium species.

Key words: wheat, moulds, Fusarium spp., DON, ZON

Introduction

The contamination of agricultural commodities by fungi able to produce toxic metabolites is often unavoidable and of worldwide concern. The relatively high intake of raw materials with the diet of livestock can lead to nutrient losses and have adverse effects on animal health and on productivity (Biagi, 2009). Pathogenic microorganisms and their secondary metabolites (mycotoxins) in a general chain of nutrition represent the most important potential risk to animal and
human health. Food safety is an important problem requiring an all-inclusive approach "from field to fork", including all sectors, from the production of livestock feed, primary production, food processing, storage, transport and placing on market (Radin et al., 2005). Wheat is one of the most important crops in Serbia; it is cultivated on approximately 600,000 ha with the average grain yield of 3600 kg ha⁻¹ (Statistical Yearbook, 2010). For livestock nutrition wheat grain can be used as concentrated livestock feed, while whole plants can be used as fodder. During the last twenty years, the increased level of colonization and infection by *Fusarium*, particularly of ripening ears of cereals, has been attracting much attention. Firstly, because of the significant effects on the yield and quality of harvested grains, and secondly, because of the ability of *Fusarium* species to produce a wide range of mycotoxins which can enter the human and animal food chains (Magan et al., 2002).

The genus *Fusarium* is important in agriculture as it includes a number of important plant pathogens that can produce a wide spectrum of mycotoxins (Edwards et al., 2002). Many of toxigenic *Fusarium* species are also major pathogens of cereal plants, causing head blight in wheat and barley, for example, and ear rot in maize. A relationship between the level of crop infection with these pathogens and mycotoxin contamination of harvested grain is to be expected (Placinta et al., 1999). The *Fusarium* mycotoxins are primarily found in cereal grains, where trichothecenes, moniliformin, fumonisins and ZON are the predominant mycotoxins (D’Mello et al., 1999). Many toxigenic *Fusarium* species have been associated with infected wheat grains, but the predominant pathogens found worldwide are *F. graminearum* Schw. (sexual state *Gibberella zeae* (Schw.) Petch), and *F. culmorum* (W. G. Sm) Sacc. (Edwards et al., 2002). These species are primary causing agents of grain fusariosis known as Fusarium head blight (FHB). FHB is most easily recognised on immature ears where one or more spikelets in each ear become prematurely bleached. Sometimes large areas of ears may be affected and, where infection is severe, pink or orange spore masses occur on diseased spikelets. FHB can cause yield losses of 30-70% when conditions favour the disease, but, more importantly, grain from affected crops may be less palatable to stock than healthy grain and may contain mycotoxins (Bai and Shaner, 1994). The most important toxins produced by *F. graminearum* and *F. culmorum* are the trichothecone deoxynivalenol (DON) and the oestrogenic mycotoxin zearalenone (Bottalico, 1998). These mycotoxins have been linked to livestock toxicoses and feed refusal (Lemmens et al., 2004). In nonruminants, DON contaminated feed can reduce growth rates, while zearalenone can cause reproductive problems (Turkington et al., 2002).

Danger from pathogen fungi and their mycotoxins starts with the food chain. Therefore, in order to avoid harmful consequences of the impact of mycotoxins, it is necessary to conduct control of all procedures in the production and processing
of grain. Spike fusariosis in wheat is an economically significant problem in the wheat production in Serbia. The evaluation of grain quality after harvesting is one of the main steps in the control of the grain health status. This is the reason why the incidence of pathogenic fungi was investigated in this study, with a special focus on species of the *Fusarium* genus and its mycotoxins in wheat harvested in 2009.

**Materials and Methods**

**Mycological analysis.** A total of 20 wheat grain samples (1 kg minimum) were collected in the production plots of the Institute for Animal Husbandry in Belgrade-Zemun. Samples were collected successively during the wheat harvest period (June-July, 2009). The average grain moisture content was 14%. Subsamples of each of wheat samples were first rinsed under running water for 2 hours, then surface-disinfected using a commercial solution of sodium hypochlorite (1%) for 1 min, rinsed twice with sterile distilled water, and dried in a laminar flow cabinet. A total of 2000 wheat grains, 10 grains per plate, were arranged on the 2% agar medium in 10-cm Petri dishes. The plates were incubated at room temperature for 7–10 days. Based on macroscopic and microscopic morphological traits the identification of *Fusarium* species was done according to *Nelson et al.* (1983) and *Burgess et al.* (1994), while the remaining fungal genera were determined according to *Ellis* (1971) and *Watanabe et al.* (1994).

**Mycotoxin analysis.** The mycotoxin content in the wheat grains was determined by a direct competitive Enzyme linked immunosorbent assay (ELISA). Five grams of the ground wheat sample were extracted with 25 ml solution of methanol:water (70/30 v/v) for ZON and distilled water for DON. Furthermore, 1 g NaCl was added with the solvent. The samples were blended for 3 minutes, then filtrated through Whatman filter paper 1 and filtrates were collected. Dilution of filtrates was carried out according to the manufacturer's instructions and differed depending on the concentration of toxin in the sample. The ELISA procedure was performed by following the manufacturer's recommendations (Tecna S. r. l., Trieste, Italy). Absorbance was determined using the spectrophotometer Elisa reader BioTek EL x 800\textsuperscript{TM} (Absorbance Microplate Reader) at 450 nm.

**Statistical analysis.** Data obtained for the frequency of *Fusarium* species and concentrations of mycotoxins in 20 investigated wheat grain samples were used for the calculation of Pearson's coefficient of correlation. Significance of interrelations of these two factors was tested at the level of $P_{0.05}$ and $P_{0.01}$.
Results and Discussion

Out of 2000 observed wheat grains within the samples collected immediately after harvest in June-July, 2009, 100% grains were contaminated with various fungal species. The mycobiota associated with wheat grains are shown in Table 1. Based on the fungal frequency 12 filamentous genera were obtained: *Acremoniella*, *Acremonium*, *Alternaria*, *Arthrinium*, *Aspergillus*, *Bipolaris*, *Chaetomium*, *Fusarium*, *Nigrospora*, *Penicillium*, *Ramichloridium* and *Rhizopus*. Species of the genera *Alternaria* and *Fusarium* were predominant (81.55% and 12%, respectively). The presence of species of the following genera was recorded to a smaller extent: *Rhizopus* (3.75%), *Acremoniella* (1.15%), *Ramichloridium* (0.5%), *Acremonium* (0.45%), *Chaetomium* (0.25%), and *Penicillium* (0.15%). Species of the genera *Arthrinium*, *Aspergillus Bipolaris* and *Nigrospora* were present in the smallest percentage (0.05%).

Table 1. Frequency of fungal genera in winter wheat grains

<table>
<thead>
<tr>
<th>Fungal genera</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acremoniella</td>
<td>1.15</td>
</tr>
<tr>
<td>Acremonium</td>
<td>0.45</td>
</tr>
<tr>
<td>Alternaria</td>
<td>81.55</td>
</tr>
<tr>
<td>Arthrinium</td>
<td>0.05</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>0.05</td>
</tr>
<tr>
<td>Bipolaris</td>
<td>0.05</td>
</tr>
<tr>
<td>Chaetomium</td>
<td>0.25</td>
</tr>
<tr>
<td>Fusarium</td>
<td>12.00</td>
</tr>
<tr>
<td>Nigrospora</td>
<td>0.05</td>
</tr>
<tr>
<td>Penicillium</td>
<td>0.15</td>
</tr>
<tr>
<td>Ramichloridium</td>
<td>0.50</td>
</tr>
<tr>
<td>Rhizopus</td>
<td>3.75</td>
</tr>
</tbody>
</table>

Among species of the genus *Fusarium*, the species *F. graminearum* (82.5%) was predominant, and followed by 9.17% of species from section *Liseola* (4.17% *F. proliferatum* (Matsushima) Nirenberg, 4.17% *F. subglutinans* (Wollenw. & Reink.) Nelson, Toussoun & Marasas comb. nov., and 0.83% *F. verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* (Sacc.) Nirenberg), *F. sporotrichioides* Sherb. (5.42%), *F. poae* (Peck) Wollen. (1.66%) and *F. semitectum* Berkeley and Ravenel (1.25%) (Table 2).
Presence of toxigenic *Fusarium* species...

Table 2. Frequency of *Fusarium* species in winter wheat grains

<table>
<thead>
<tr>
<th><em>Fusarium</em> spp.</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. graminearum</em></td>
<td>82.50</td>
</tr>
<tr>
<td><em>F. poae</em></td>
<td>1.66</td>
</tr>
<tr>
<td><em>F. proliferatum</em></td>
<td>4.17</td>
</tr>
<tr>
<td><em>F. sporotrichioides</em></td>
<td>5.42</td>
</tr>
<tr>
<td><em>F. semitectum</em></td>
<td>1.25</td>
</tr>
<tr>
<td><em>F. subglutinans</em></td>
<td>4.17</td>
</tr>
<tr>
<td><em>F. verticillioides</em></td>
<td>0.83</td>
</tr>
</tbody>
</table>

DON and ZON were detected in 100% and 10% wheat samples, respectively. Co-occurrence of DON and ZON was found in 10% of the samples analysed. The DON mycotoxin analysis determined the average concentration of 490 μg kg⁻¹ with the limit values of 110 to 1200 μg kg⁻¹. ZON was present in the lowest average concentration of 70 μg kg⁻¹ with limits ranging from 60 to 80 μg kg⁻¹ (Table 3).

Table 3. Concentrations of *Fusarium* mycotoxins in winter wheat grains

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Frequency (%)</th>
<th>Mycotoxin concentration (μg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
</tr>
<tr>
<td>DON</td>
<td>100</td>
<td>490</td>
</tr>
<tr>
<td>ZON</td>
<td>10</td>
<td>70</td>
</tr>
</tbody>
</table>

Statistically significant positive correlations were established between the ZON concentration and the frequency of *F. graminearum/Fusarium* spp. (r = 0.63**, r = 0.58**, respectively). A negative insignificant correlation was determined between DON and the percentage of the present *Fusarium* species.

The obtained results of microbiological analyses of wheat grain samples are in accordance with previously published results (*Bočarov-Stančić*, 1996; *Bočarov-Stančić*, 1998; *Bočarov-Stančić et al.*, 2000; *Balaž et al.*, 2003; *Bagi et al.*, 2005; *Stanković et al.*, 2007; *Krnjaja et al.*, 2008; *Lević et al.*, 2008b, 2009) or results of similar studies carried out in Serbia.

According to results achieved by *Bočarov-Stančić* (1996, 1998) and *Bočarov-Stančić et al.* (2000) out of *Fusarium* species determined on observed wheat grain, the species *F. graminearum* had the highest frequency. In accordance with the two-year studies (2001-2002) on the mycopopulation of durum wheat seed samples, the presence of the species of the genus *Alternaria* ranged from 25.1% (2001) to 69.3% (2002), while species of the genus *Fusarium* were less present and their presence varied from 7.4% (2001) to 20.6% (2002) (*Balaž et al.*, 2003). *Bagi et al.* (2005) performed a three-year study (2002-2004) on mycopopulations on the durum wheat seeds and also established more significant presence of species of the
genus *Alternaria* (12.3% in 2004 to 28.8% in 2003) in relation to species of the genus *Fusarium* whose presence varied from 7.4% (2002) to 10.8% (2003). According to *Stanković et al.* (2007) two-year results showed that the greatest number of wheat grain samples was infected with species of the genera *Fusarium* (81.8 and 65.0%), and *Alternaria* (36.3 and 17.5%) with the intensity ranging from 9.4 to 84.0% in 2005 and from 23.4 to 80.6% in 2006. Out of 13 identified species belonging to the genus *Fusarium*, *F. graminearum* had the highest frequency (35.2 and 12.5%) and the intensity up to 67.2%, and 21.9%, in 2005 and 2006, respectively, followed by *F. poae*, but only in 2005 (20.4%), and *F. proliferatum* in 2006 (19.7%) (*Stanković et al.*, 2007). Agroecological conditions in Serbia appeared favourable for pathogenic and toxigenic *Fusarium* species of a wider scale, which in some years, as was the case in 2005, could cause the decrease in the grain yield by 38% and the increase in mycotoxin contamination of wheat by 50% (*Lević et al.*, 2008a). Under such conditions, in the last decade, *F. graminearum* and *F. poae* (>16%), and *F. proliferatum, F. verticillioides* and *F. subglutinans* (6–15%) were species most often determined in wheat (*Lević et al.*, 2009).

According to data of the Republic Hydrometeorological Service of Serbia, during May of 2009, when wheat was in a pheno-phase susceptible to fusarioses (especially in the first decade of May), mean daily temperature was, on the average, 19.6°C with small precipitation sums (35 mm on the average). Cooler days in the beginning and at the end of this month, as well as, a very small precipitation sum did not favour the development of *Fusarium* species under field conditions.

Although DON is probably the most distributed mycotoxin in food and feed all over the world, data on the presence of this mycotoxin in wheat in our country are very scarce. According to data obtained by *Jurić et al.* (2007), studies carried out on wheat samples in Vojvodina during 2004 and 2005 showed that 41.6% of samples were contaminated with DON, whose concentrations varied from 57 to 1840 µg kg⁻¹. Studies mycotoxin contamination in wheat grains in Serbia showed the presence of DON in 33.3–50.0% of the samples, and the concentration of mycotoxins on average ranged from 124 to 1235 µg kg⁻¹, depending on the conditions in the year of the study (*Jajić et al.*, 2008). According to *Stanković et al.* (2008) Serbian isolates of *F. graminearum* have high potential for DON production (up to 45,260 µg kg⁻¹). In Serbia, ZON is one of the most important fusariotoxins that caused mycotoxicoses of domestic animals and is one of the most frequent contaminant of feed mixtures and their components (*Bočarov-Stančić et al.*, 1995; *Lević et al.*, 2004). *Bočarov-Stančić* (1996) recorded a low level of ZON in observed food samples made of wheat, while the level of ZON in feed samples made of wheat amounted to 800 µg kg⁻¹ (*Bočarov-Stančić et al.*, 2000). *Stojanović et al.* (2005a) performed mycotoxological studies on four different wheat grain fractions (healthy, dark, slightly *Fusarium*-infected and considerable *Fusarium-*
Presence of toxigenic Fusarium species ... 69

infected) of three wheat varieties originating from two locations and established that the ZON concentration varied from 0 to 500 µg kg\(^{-1}\). By studying grain samples of six wheat varieties (location 1) and five varieties (location 2), which were grouped into considerably to slightly Fusarium-infected grains, it was established that 77% of samples was contaminated with ZON in the concentrations of 150 to 1600 µg kg\(^{-1}\) (Stojanović et al., 2005b). According to Pancladi et al. (2004) in grain samples from three studied cultivars of durum wheat inoculated in the field with *F. graminearum* and *F. culmorum* and untreated by fungicides, the DON concentration ranged from 0.500 to 1.040 mg kg\(^{-1}\).

Reported correlations between FHB severity, *Fusarium* frequency in grain and mycotoxin concentrations are variably. Cromey et al. (2002) established that the incidence of FHB, levels of *Fusarium* infection and mycotoxins were very closely related in samples from a particular crop, but there were differences between crops. The frequency of visibly infected grains, FHB, grains contaminated with *Fusarium*, and mycotoxin levels also differed markedly between cultivars in the 1999/2000 survey in New Zealand. In investigated samples per particular crop, levels of DON mycotoxin were higher compared to levels of fusariotoxin nivalenol (NIV), however this was not constant in samples of different crops (Cromey et al., 2002). A high correlation was found between the percentage of *Fusarium* damaged kernels and amounts of DON in wheat grain samples in consecutive years 1986-1989 in Poland. Taking into consideration their frequency and concentration, the most important mycotoxin detected in field samples of wheat grains was DON, occurring in 60-65% of samples in 1998 and 89-95% of samples in 1999 (Tomczak et al., 2002).

In our studies a negative correlation between DON mycotoxin and the incidence of *Fusarium* species was established, which is similar to results of De Nijs et al. (1996) who established that there was no relationship between the counts of total fungi and high levels of the mycotoxins DON and ZON.

**Conclusion**

In agroecological conditions of Serbia, toxigenic *Fusarium* species and their mycotoxins are special hazard in the food chain. Results presented in this research impose constant need for monitoring the incidence of *Fusarium* species in the field and also later during storage and processing of grain. Mycotoxicological analyses are also one of the basic methods in the assessment of grain quality, as well as, of the quality of grain products. A regular application of microbiological and mycotoxicological methods, as well as, their improvement and development, are prerequisites for defining adequate prevention measures, i.e. the improvement of quality of life, primarily of human and animal health.
Acknowledgment

Research was financed by the Ministry of Science and Technological Development of Republic of Serbia within project TR-20046.

**Prisustvo toksigenih *Fusarium* vrsta i fuzariotoksinsa deoksinivalenola i zearalenona u ozimoj pšenici**

*V. Krnjaja, S. Stanković, J. Lević*

**Rezime**

Učestalost gljiva i koncentracija mikotoksina deoksinivalenola (DON) i zearalenona (ZON) je proučavana u zrnu ozime pšenice požnjevenom 2009. godine. Najčešće izolovane vrste gljiva pripadale su rodovima *Alternaria* (81,55%) i *Fusarium* (12%), a zatim su sledili *Rhizopus* spp. (3,75%), *Acremoniella* spp. (1,15%) i druge gljive (*Acremonium* spp., *Arthrinium* spp., *Aspergillus* spp., *Bipolaris* spp., *Chaetomium* spp., *Nigrospora* spp., *Penicillium* spp. i *Ramichloridium* spp.) izolovane u manje od 1%. Unutar roda *Fusarium* identifikovane su sledeće vrste: *F. graminearum* (82,50%), *F. sporotrichioides* (5,42%), *F. proliferatum* (4,17%), *F. subglutinans* (4,17%), *F. poae* (1,66%), *F. semitectum* (1,25%) i *F. verticillioides* (0,83%). U 100% uzoraka zrna pšenice DON je bio detektovan u koncentracijama od 110 do 1200 μg kg⁻¹, sa prosečnom koncentracijom od 490 μg kg⁻¹. ZON je bio detektovan u 10% uzoraka u koncentracijama od 60 do 80 μg kg⁻¹, sa prosečnom koncentracijom od 70 μg kg⁻¹. Statistički značajna pozitivna korelacija utvrđena je između koncentracije ZON i frekvencije *Fusarium* spp. (r = 0.58**) i frekvencije *F. graminearum* (r = 0.63**). Negativna korelacija, statistički nesignifikanta, utvrđena je između nivoa DON-a i procentualne zastupljenosti *Fusarium* vrsta.

**References**


Presence of toxigenic *Fusarium* species ...


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INFLUENCE OF AGE AND WEIGHT OF LANDRACE GILTS AT FERTILE INSEMINATION ON LITTER SIZE AND LONGEVITY

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Original scientific paper

Abstract: The relationship between age and weight of Landrace gilts both litter size and longevity then culling has been analyzed. The data structure included 1,615 gilts which have been divided in three groups of age at fertile insemination (180-210 days; 211-230 days and 231-260 days) and three groups of weights at the same time (90-114 kg; 115-130 kg and 131-170 kg). Management and the nutrition of gilts and sows have been treated at common level of production. Analysis included data from 4 farms and the period of two years production (2009 and 2010). MME – mixed model has been installed to use FYS (Farm, Year and Seasons) and litter as fixed effect and sire influence as random. Significant influence of age and weight on litter size has been recognized. Also those two factors had significant effect on longevity and production of sows. The best results were determined in gilts introduced at the age of 231-260 days and weight class of 131-170 kg. Those factors had significant influence on longevity and life production. Genetic and phenotypic correlations showed high and significant value. All results have been interpreted in 8 tables and 4 graphs.

Key words: pigs, reproduction, longevity, age, weights, correlation.

Introduction

From economical and biological point of view there is the common question of influence of age and weight at fertile insemination on litter size and longevity of sows. Of course between those two factors there is high genetic relationship and significant influence on latter results. Since the sows use the same amount of food, the question is how many piglets can be weaned per sow per year or what is life production. Those two parameters are correlated to the age and the weight of gilts at fertile insemination.

The purpose of this paper was to analyze relationship and influence of the age and the weight of Landrace gilts at insemination in first five litters. We
Materials and Methods

To analyze influence of the age and the weight of Landrace gilts on litter size, culling rate and longevity of sows in production we used 1,615 gilts. All measurements have been done at 4 farms during 2 years and 8 seasons. All analyses included 5 litters.

We used following MME- mixed model analysis:

\[ Y_{ijkl} = u + FYS_i + A_{ij} + S_{ijk} + E_{ijkl} \]

Differences between FYS (Farms, Years and Seasons), litters as fixed effects and random sire effects.

Gilts were divided into three classes according to their age (180-210 days; 211-230 days and 231-260 days), and in three classes according to their weight (90-114 kg; 115-130 kg and 131-170 kg) at insemination. The number of gilts in one of those classes is shown in Table 1.

Table 1. Number of gilts with the age and the weight at fertile insemination

<table>
<thead>
<tr>
<th>No of gilts</th>
<th>Age at fertile insemination, days</th>
<th>Weight at fertile insemination, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>180-210</td>
<td>211-230</td>
</tr>
<tr>
<td>1615</td>
<td>434</td>
<td>682</td>
</tr>
</tbody>
</table>

Results and Discussion

All data obtained in the study was interpreted in table 2-8 and 4 graphs.
### Table 2. Effect of age at mating on the number of piglets per litter in Landrace

<table>
<thead>
<tr>
<th>Litter</th>
<th>Age at mating</th>
<th>180-210</th>
<th>211-230</th>
<th>231-260</th>
<th>180-210</th>
<th>211-230</th>
<th>231-260</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alive born piglets</td>
<td></td>
<td></td>
<td></td>
<td>Alive piglets at day 5&lt;sup&gt;th&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \bar{x} ) ( \sigma )</td>
<td>( \bar{x} ) ( \sigma )</td>
<td>( \bar{x} ) ( \sigma )</td>
<td>( \bar{x} ) ( \sigma )</td>
<td>( \bar{x} ) ( \sigma )</td>
<td>( \bar{x} ) ( \sigma )</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8.86 2.44</td>
<td>9.48 2.08</td>
<td>10.32 2.09</td>
<td>8.52 2.28</td>
<td>9.32 2.10</td>
<td>10.15 2.12</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9.02 2.29</td>
<td>9.44 2.14</td>
<td>10.60 2.08</td>
<td>8.64 2.31</td>
<td>9.14 2.08</td>
<td>10.30 2.02</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9.28 2.24</td>
<td>9.36 2.17</td>
<td>10.64 1.86</td>
<td>8.92 2.20</td>
<td>9.13 2.14</td>
<td>10.34 1.64</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9.36 2.28</td>
<td>9.52 2.32</td>
<td>10.71 1.44</td>
<td>9.04 2.27</td>
<td>9.24 2.02</td>
<td>10.42 1.76</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9.40 2.09</td>
<td>9.48 2.44</td>
<td>10.56 1.87</td>
<td>9.00 2.34</td>
<td>9.20 2.07</td>
<td>10.22 1.70</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>9.18 2.44</td>
<td>9.46 2.17</td>
<td>10.57 2.04</td>
<td>8.82 2.38</td>
<td>9.21 2.12</td>
<td>10.30 1.84</td>
<td></td>
</tr>
</tbody>
</table>

**Graph 1: Effect of age at mating on number of piglets per litter**

![Graph 1: Effect of age at mating on number of piglets per litter](image-url)
As we can see the effect of litters showed the same tendency in all classes. But, the differences between youngest and oldest gilts at insemination were statistically significant. Weight of gilts showed the same tendency. Similar trend and conclusion for Yorkshire breed was presented by Vidović and Lehocki (1998), Merks (2006), Vidović et al. (2011). Standard deviation also followed size of litter and there was no significant difference.

Table 3. Effect of weight at mating on number of piglets per litter

<table>
<thead>
<tr>
<th>Litter</th>
<th>Weights at mating</th>
<th>Live born piglets</th>
<th>Live piglets at day 5th</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90-114</td>
<td>115-130</td>
<td>131-170</td>
</tr>
<tr>
<td></td>
<td>(\bar{x})</td>
<td>(\sigma)</td>
<td>(\bar{x})</td>
</tr>
<tr>
<td>1</td>
<td>8.64</td>
<td>2.74</td>
<td>9.32</td>
</tr>
<tr>
<td>2</td>
<td>8.87</td>
<td>2.38</td>
<td>9.40</td>
</tr>
<tr>
<td>3</td>
<td>9.13</td>
<td>2.44</td>
<td>9.33</td>
</tr>
<tr>
<td>4</td>
<td>9.27</td>
<td>2.54</td>
<td>9.50</td>
</tr>
<tr>
<td>5</td>
<td>9.38</td>
<td>2.50</td>
<td>9.42</td>
</tr>
<tr>
<td>Average</td>
<td>9.06</td>
<td>2.54</td>
<td>9.39</td>
</tr>
</tbody>
</table>
The differences between age and weight effects on litter of litter size and longevity of sows and their life production are not significant. The genetic and phenotypic correlations were high and positive. The best result were registered in females in the last class of age (231-260 days) and weight (131-170 kg). It is optimum size and age to include gilts in regular production level.
The gilts from the third class (age 231-260 day and weight of 131-170 kg) at first farrowing had between 190-220 kg of weight. With these sizes they display no syndrome of second litter. It means they have no more than 11 empty days and bigger litter then first one.

Effect of weight on litter size and longevity was significant. The best results were recorded in case of older and heavier gilts. The difference of age and weight effect on litter size and live production and longevity of sows wasn’t significant. Standard variation decreased with the increase of age and weight of gilts at mating. It confirmed that the best weight and age, according to final results, were in class of the oldest and heavier gilts.

Table 4. Number of stillborn and weaned piglets in different classes of gilts

<table>
<thead>
<tr>
<th>Litter</th>
<th>Age at insemination gilts, days</th>
<th>Stillborn</th>
<th>Weaned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><code>X σ</code> <code>X σ</code> <code>X σ</code> <code>X σ</code> <code>X σ</code> <code>X σ</code></td>
<td><code>X σ</code> <code>X σ</code> <code>X σ</code> <code>X σ</code> <code>X σ</code> <code>X σ</code></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.8 1.6 0.8 1.4 1.1 2.0 7.96 2.14 9.02 2.02 9.52 1.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.0 2.3 0.9 1.3 1.2 2.3 8.12 2.28 9.12 1.98 9.54 1.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.1 1.0 0.8 1.6 1.4 1.9 8.32 2.56 8.92 1.89 9.59 1.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.8 1.3 1.0 1.7 1.1 1.8 9.32 2.27 8.76 1.86 9.82 1.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.8 1.2 1.0 2.0 1.2 1.8 9.28 2.17 8.89 1.45 9.82 1.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.9 1.6 0.9 1.3 1.2 1.8 8.60 2.08 8.94 1.86 9.67 1.60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Stillborn piglets showed no significant difference between litters. More piglets in the litter are followed with more stillborn. It could be consequence of technology and high selection pressure on number of alive piglets.

Number of weaned piglets is significantly higher in class of older and heavier gilts. These figures lead us to the conclusion to enter older and heavier gilts into production. The similar trend has been established by Knap (1998), then Vidović and Lehocki (1998), Šalehar (1985), Kovač and Šalehar (1985) showed similar trends.
Table 5. Relative replacement rate, %, according to age and weight of gilts at fertile insemination

<table>
<thead>
<tr>
<th>Litter</th>
<th>Age at insemination, days (Replacement rate, %)</th>
<th>Weight at insemination, kg (Replacement rate, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>180-210</td>
<td>211-230</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>σ</td>
</tr>
<tr>
<td>1 – 2</td>
<td>30</td>
<td>23</td>
</tr>
<tr>
<td>2 - 3</td>
<td>27</td>
<td>20</td>
</tr>
<tr>
<td>3 - 4</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>4 - 5</td>
<td>8</td>
<td>23</td>
</tr>
</tbody>
</table>

Younger and less heavy gilts are not capable to maintain production continuously. They show effect of next litter. It means that they have lost weight of more than 40 kg in the previous litter and have more than 12 empty days. In that case we are forced to cull them from production. Their life production is shorter, economically pure (Table 6). This result means we have to enter older and heavier gilts (third class in regard to age and weight).

Table 6. Genetic (above) and phenotypic (under diagonal) correlations between certain traits

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Weight</th>
<th>Live born</th>
<th>Weaned</th>
<th>Stillborn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-</td>
<td>0.89</td>
<td>0.67</td>
<td>0.61</td>
<td>0.10</td>
</tr>
<tr>
<td>Weight</td>
<td>0.51</td>
<td>-</td>
<td>0.64</td>
<td>0.62</td>
<td>0.14</td>
</tr>
<tr>
<td>Live born</td>
<td>0.57</td>
<td>0.77</td>
<td>-</td>
<td>0.91</td>
<td>0.10</td>
</tr>
<tr>
<td>Weaned piglets</td>
<td>0.47</td>
<td>0.51</td>
<td>0.74</td>
<td>-</td>
<td>0.08</td>
</tr>
<tr>
<td>Stillborn</td>
<td>0.08</td>
<td>0.07</td>
<td>0.37</td>
<td>0.31</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 7. Heritability estimates for live born in different litters

<table>
<thead>
<tr>
<th>Litter</th>
<th>Age at mating</th>
<th>Weight at mating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>180-214</td>
<td>215-230</td>
</tr>
<tr>
<td></td>
<td>live born piglets</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$h^2$  $Sh^2$</td>
<td>$h^2$  $Sh^2$</td>
</tr>
<tr>
<td>1</td>
<td>.07  .23</td>
<td>.07  .27</td>
</tr>
<tr>
<td>2</td>
<td>.08  .34</td>
<td>.08  .33</td>
</tr>
<tr>
<td>3</td>
<td>.09  .38</td>
<td>.09  .37</td>
</tr>
<tr>
<td>4</td>
<td>.07  .41</td>
<td>.06  .45</td>
</tr>
<tr>
<td>5</td>
<td>.09  .44</td>
<td>.09  .47</td>
</tr>
</tbody>
</table>

Table 8. Number of piglets per sow for first five litters at different gilt classes

<table>
<thead>
<tr>
<th>Traits</th>
<th>Age at insemination, days</th>
<th>Weight at insemination, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>180-210</td>
<td>211-230</td>
</tr>
<tr>
<td>Live born</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>33.9</td>
<td>40.8</td>
</tr>
<tr>
<td>Weaned</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30.2</td>
<td>37.7</td>
</tr>
</tbody>
</table>

Genetic and phenotypic correlations confirm previous conclusion to select older and heavier gilts for higher number of piglets per sow per litter, life production and better economic benefit.

Conclusion

Effects of age and weight of gilts at fertile insemination on litter size traits and longevity of sows has been analyzed on Landrace population. The significantly best results were established in gilts with age of 231-260 days of age and weight of 131-170 kg.
Those age and weight of gilts had smallest culling rate during production and highest number of live born and weaned piglets. These groups also have remained longer in the production.

These results have been confirmed with significantly high and positive genetic and phenotypic correlations.

Standard deviation showed expected variation according to trend and correlation between analyzed traits.

We can recommend as optimal age and weight of gilts the third class (231-260 days and 131-170 kg) of gilts as economically the best one. To follow up this result it is necessarily to use new feeding technology and modern management system of production.

**Uticaj uzrasta i mase landras nazimica pri inseminaciji na veličinu legla i dugovečnost**

*V. Vidović, Lj. Štrbac, D. Lukač, M. Stupar*

**Rezime**

Istraživanja su izvedena na 1.615 plotkinja, lociranih na 4 farme, u periodu od 2 godine, tj. 8 sezona (2009. i 2010.). Cilj rada je bio ispitati uticaj starosti i težine Landras nazimica pri fertilnom osemenjavanju na rezultate plodnosti (živo, mrtvo i zalućenih prasadi) u prvih 5 uzastopnih prašenja kao i dugovečnost krmača. Oba faktora podeljena su u tri klase. Za korekciju uticaja razlika između farmi godina i sezona, te prašenja po redu, kao sistematski uticaji i razlika između očeva, kao slučajan uticaj na ispitivane osobine, korišćen je mešoviti model analize.

Dobijeni rezultati interpretirani su u 8 tabela. Može se uočiti da je uticaj oba faktora (dobi i težine) bio signifikantan na veličinu legla kao i dugovečnost odnosno životnu proizvodnju krmača. Ovakvi uticaji imaju i značajne ekonomske efekte na proizvodnju u praksi. U zaključku, najoptimalnije vreme uvođenja nazimica u fertilnu oplodnju jeste u uzrastu od 231-260 dana i pri težini od 131-170 kg telesne mase. Vrednosti genetskih i fenotipskih korelacija potvrđuju ovakav zaključak. Istovremeno, ekonomski gledano je najpovoljniji uzrast kada grlo treba uvesti u proizvodnju bilo da se radi o nucleus ili komercijalnoj farmi. Ocene heritabilnosti, i ako beznačajne za ovakvu vrstu analize, za ispitivana svojstva pokazali su stabilnost bez signifikantnih razlika između pojedinih klasa. Ako međutim znamo, da je heritabilnost za broj ovuliranih jajnih ĉelija na nivou (h2 - 0,40), što ga svrstava u grupu srednje naslednih, tada dolazimo do zaključka da je pored navedena dva efekta posebno važna i tehnologija i režim hranjenja plotkinja
u pojedinim reproduktivnim fazama. Ovo stoga da se izazove fertilan estrus, preživi što više embriona i optimalne težine prasadi kao i kontinuitet u produktivnosti krmača sa optimalnim remontom.

References


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ESTIMATION OF HERITABILITY COEFFICIENTS OF NUMBER OF BORN ALIVE PIGLETS IN THE FIRST THREE FARROWINGS SWEDISH LANDRACE SOWS

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Abstract: The main objective of this study was to evaluate phenotypic and genetic variation of the number of piglets born alive (NBA) Swedish Landrace sows (SL) in three consecutive parities under the influence of sires, year and season of mating. The study included: 618 litters in the first, 470 in the second and 403 litter in the third farrowing. Testing the homogeneity of variance was performed with Levene’s test. The data were analyzed using analysis of variance (Statistica Ver. 6.0., 2003). Heritability coefficients were evaluated by intra-class correlation. Swedish Landrace sires have influenced on the variability of the NBA's daughter in the first and second farrowing (p<0.05), but not in the third farrowing. Year and season of mating was not influenced on the variability of the NBA (p>0.05). The estimated heritability coefficients for the NBA were the highest in the second (0.123), then the first (0.092) and lowest in the third farrowing (0.030).

Key words: sows, litter size, heritability, sires, year, season

Introduction

Reproductive traits such as litter size, litter weight at birth and survival rates of piglets to weaning, are the most important economic trait in swine production (Yen et al., 1987). The objective of modern pig breeding is to exhaust the genetic potential in reproduction performance of sows regarding to litter size and number of weaned piglets per litter (Wähner and Brüssow, 2009). Litter size is one of the most important reproductive traits. It is a low heritable trait and the influence of environmental factors on its expression is significantly expressed, because it is necessary 8 to 10 generations of selection to increase litter size by one piglet (Kosovac et al., 1996).

Knowledge of genetic parameters of reproductive traits is an essential requirement in estimating the breeding value, selection, implementation and select
the best method of breeding, because only in this way can the right to genetic improvement of the herd. Heritability in the narrow sense determines the method and evaluate the effect of selection, because of additive gene effect has more influence on the expression of quantitative traits (Kosovac et al., 1996). A large number of tests of litter size in different populations of pigs showing a low coefficient of heritability of 10% and a relatively high variance of 25% (Petrović et al., 1997).

There are differences in the estimated values of heritability coefficient for the same trait depending on the method used (Petrović et al., 1997). Roehe and Kennedy (1995) are using multiple-trait animal model and determine low heritability coefficient values that were increased from first to fourth parities (0.086, 0.096, 0.116 and 0.141) Landrace sows. According to the results Tholen et al. (1995), heritability coefficients for number of live born piglets in two herds, in the first three parities were of 0.09 to 0.16.

Data analysis sought to determine how sires, year and season of mating influence the phenotypic and genetic variability of traits of litter size (number of piglets born alive). The aim was to estimate heritability coefficient for the number of live born piglets in three consecutive parities.

**Materials and Methods**

The study uses data on fertility of sows of Swedish Landrace (SL), since 2000, by 2008. year from a commercial type farms. After calculating the distribution of number of daughter by father and setting the criteria of "a minimum of 10 daughters by sires", made the formation of subsamples that include the daughters of the ten selected sires with balanced distribution of litters by farrowing ordinal numbers. The number of analyzed litters at farrowing is: 618 in the first, 470 in the second and 403 litters in the third farrowing. The trait of number of born alive piglets (NBA) in this study involved a number of live born piglets, increased body weight of 800 grams.

Data processing was performed using the statistical package Statistica Ver. 6.0. (StatSoft Inc., 2003). Were applied a standard statistical methods. Depending of the observed factors in the analysis of variance was used mathematical and statistical model:

\[ Y_{ijkl} = \mu + N_i + G_j + S_k + E_{ijkl}, \]

where: \( Y_{ijkl} \)- number of piglets born alive, \( \mu \)- average value, \( N_i \)- random effect of sires, \( G_j \)- fixed effect of the year of mating, \( S_k \)- fixed effect of the season of mating and \( E_{ijkl} \)- random error.

Testing the homogeneity of variance, or whether the data meet the requirements for the application of the methodology was performed with Levene's
test (Petrie and Watson, 2006). Testing (comparison) the average values obtained by descriptive statistical analysis was done by t-test.

Estimates of heritability coefficients was performed by intra-class correlation of paternal half-sisters in the first, second and third farrowing sows. The analysis included nine consecutive years and all four seasons of mating. Standard error of heritability was calculated using the form Končar and Simić (1978).

**Results and Discussion**

Descriptive statistical parameters of number of born alive piglets in sows of Swedish Landrace by farrowing are shown in Table 1.

Number of live born piglets is low, but increases from first to third parities (8.44 to 9.97). Relative indicator of variability (CV = 25.94 to 29.66) points to the relative homogeneity of the trait of the number of live born piglets in the first three parities.

| Table 1. Descriptive statistical parameters of number of born alive piglets (NBA) of Swedish Landrace sows in the first three parities |
|---------------------------------|-------|-------|-------|
| **Parameter** | **1** | **2** | **3** |
| n | 618 | 470 | 403 |
| $\bar{x}$ | $8.44^{A}$ | $9.44^{B,a}$ | $9.97^{B,b}$ |
| $S\bar{x}$ | 0.10 | 0.12 | 0.13 |
| CV | 29.66 | 27.48 | 25.94 |
| SD | 2.50 | 2.59 | 2.58 |

Differences between mean values marked with different letters are statistically significant: AB= p<0.001, ab= p<0.01

Comparing the average values NBA between farrowing exhibited a statistically highly significant difference (p<0.001) between the litter size of primiparous sows and litter sows in the second and third farrowing, while the difference in litter size between the second and third parities is significant (p<0.01). Levene’s test of homogeneity of variance by the factors of the model (Table 2) indicates that the Swedish Landrace sows in the third farrowing there was a statistically lower significant difference (p<0.05) between the variances by year of mating. Value variance properties of the number of live born piglets for year of mating in the third farrowing from 2.93 (year 9) to 16.25 (year 1). In all other cases, in all three parities, the variance in the observed factors (sires, year and season) are homogeneous, and among them there was no statistically significant difference.
Homogeneity of variance determined by the factors of the model justifies the use of methods of analysis of variance to access the observed impact on litter size.

Analysis of variance (Table 3) shows that the Swedish Landrace sires influenced on the variation of the number of live born piglets in the first and second, but not in the third farrowing daughters. Year and season of mating within individual farrowing did not significantly affect on the variation of the NBA (p>0.05). The results are consistent with research Radojković et al. (2007) that found that the boars influenced on the variation of the number of live born piglets in first litter daughter, and year and season were not influenced significantly on the variation of these traits.

Table 2. Results of Levene's variance homogeneity test of number of born alive piglets per factors involved in the model observed by the ordinal number of farrowing

<table>
<thead>
<tr>
<th>Order of farrowing</th>
<th>Factor</th>
<th>MSef</th>
<th>MSg</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sire</td>
<td>3.222736</td>
<td>2.180420</td>
<td>1.478034</td>
<td>0.152285</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>4.063620</td>
<td>2.246363</td>
<td>1.808977</td>
<td>0.072552</td>
</tr>
<tr>
<td></td>
<td>Season</td>
<td>2.476417</td>
<td>2.216010</td>
<td>1.117512</td>
<td>0.341273</td>
</tr>
<tr>
<td>2</td>
<td>Sire</td>
<td>2.946778</td>
<td>2.510424</td>
<td>1.173817</td>
<td>0.309749</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>2.725185</td>
<td>2.500661</td>
<td>1.089786</td>
<td>0.368862</td>
</tr>
<tr>
<td></td>
<td>Season</td>
<td>0.560710</td>
<td>2.486074</td>
<td>0.225540</td>
<td>0.878634</td>
</tr>
<tr>
<td>3</td>
<td>Sire</td>
<td>2.698323</td>
<td>2.393963</td>
<td>1.127136</td>
<td>0.342107</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>4.953463</td>
<td>2.412145</td>
<td>2.053551</td>
<td>0.039377</td>
</tr>
<tr>
<td></td>
<td>Season</td>
<td>5.923849</td>
<td>2.492488</td>
<td>2.376681</td>
<td>0.069517</td>
</tr>
</tbody>
</table>

MSef- average squared effects; MSg- average square error; F- value; p- significance

The estimated heritability coefficients were low, but the calculated values were different at farrowing (Table 3). Heritability coefficients for number of born alive piglets in the first three parities were: 0.092, 0.123 and 0.030.

Variance between fathers was highest in the second farrowing ($\sigma^2_{BS}=0.206$), and lowest in the third ($\sigma^2_{BS} = 0.050$) when the variance within the sires, so between the daughters was the largest ($\sigma^2_{WS} = 6.679$). In the third farrowing reduced the average square between sires (MS$_{BS} = 8.559$), increased the average of squares within the sires (MS$_{WS} = 6.679$) and reduced the average number of daughters (k = 37.692) compared with the second farrowing. This resulted on the reduction of variance between sires ($\sigma^2_{BS}=0.050$). Variations of the number of born alive piglets in the third farrowing increased between the daughters.
of the same sire, so the standard deviation (SD) was from 1.98 (sire 13449) to 4.01 piglets (sire 757).

In research conducted a calculated value of the coefficient of heritability for number of born alive piglets in the first farrowing sows SL was low but slightly higher \( (h^2 = 0.092 \pm 0.054) \) with less error than are found Radojković et al. (2005, \( h^2 = 0.083 \pm 0.058 \)). The results are consistent with research Petrović et al. (1998) who found that heritability coefficient was 0.092 ± 0.021. The obtained values of heritability for litter size in Landrace were lower in the first and second, and higher in the third farrowing than they found Irgang et al. (1994). Compared with the results of the coefficient of heritability for NBA Landrace sows (Kim, 2001), the estimated value of heritability in this study are higher in the first and second, and lower in the third farrowing. The values of the coefficient of heritability for number of born alive piglets in this study are lower than the results of Suarez et al. (2004) who determine, in the first three Landrace sows farrowing, the coefficients of heritability of 0.16 to 0.27.

Table 3. Results of the analysis of variance and the estimated coefficients of heritability of NBA of sows purebred SL in the first, second and third farrowing

<table>
<thead>
<tr>
<th>R</th>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>( \sigma^2 )</th>
<th>k</th>
<th>( h^2 \pm Sh^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Between sires</td>
<td>9</td>
<td>130.578</td>
<td>14.509</td>
<td>2.345*</td>
<td>0.146</td>
<td></td>
<td>0.092±0.054</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>8</td>
<td>38.686</td>
<td>4.836</td>
<td>0.782ns</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Season</td>
<td>3</td>
<td>1.701</td>
<td>0.567</td>
<td>0.092ns</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Within sires</td>
<td>597</td>
<td>3693.074</td>
<td>6.186</td>
<td>-</td>
<td>6.186</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sum</td>
<td>617</td>
<td>3864.039</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Between sires</td>
<td>9</td>
<td>139.446</td>
<td>15.494</td>
<td>2.378*</td>
<td>0.206</td>
<td></td>
<td>0.123±0.070</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>8</td>
<td>67.974</td>
<td>8.497</td>
<td>1.304ns</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Season</td>
<td>3</td>
<td>24.654</td>
<td>8.218</td>
<td>1.261ns</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Within sires</td>
<td>449</td>
<td>2925.875</td>
<td>6.516</td>
<td>-</td>
<td>6.516</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sum</td>
<td>469</td>
<td>3157.949</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Between sires</td>
<td>9</td>
<td>77.034</td>
<td>8.559</td>
<td>1.281ns</td>
<td>0.050</td>
<td></td>
<td>0.030±0.045</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>8</td>
<td>13.635</td>
<td>1.704</td>
<td>0.255ns</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Season</td>
<td>3</td>
<td>43.356</td>
<td>14.452</td>
<td>2.164ns</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Within sires</td>
<td>382</td>
<td>2551.488</td>
<td>6.679</td>
<td>-</td>
<td>6.679</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sum</td>
<td>402</td>
<td>2685.513</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns >0,05; *<0,05
Comparison of results of heritability coefficients between farrowing, shows that the heritability of number of born alive piglets in the second farrowing is largest. Explanation of increased heritability in the second farrowing may be a weaker selection criteria applied to the primiparous sows. In fact, after the first farrowing sows are not yet completed growth and are not fully expressed their reproductive potential (Radojković et al., 2005). As a consequence, an increase of genetic variability in the second farrowing. The estimated value of heritability of born alive piglets in the third farrowing is lower than in the second farrowing. The reason may be tightening criteria for selection of sows after second farrowing, causing a reduction in additive genetic variation. The conclusions are supported by the research Radojković et al. (2005). Conclusions about the reasons for decrease of heritability in the third farrowing similar to the results Irgang et al. (1994). According to some researchers, the coefficient of heritability of litter size (NBA) increased from first to third parities (cited Kosovac et al., 1996), which is in contrast with the results of this study. Number of born alive piglets in this study involved a number of live born piglets greater body mass of 800 grams. It was due to a reduced variability in these traits in the studied herd. With increasing age of sows and ordinal number of farrowing (from first to third) increased variance within the sires and increased at varying NBA between daughters of the same father, which influenced on the coefficient of heritability.

**Conclusion**

In this paper study the evaluation of phenotypic and genetic variation of the number of piglets born alive (NBA) Swedish Landrace sows (SL) in three consecutive parities under the influence of sires, year and season of mating. Based on the results of this study can be seen that the average of number of piglets born alive (NBA) is low, but increased from first to third parities (8.44-9.97).

Results of analysis of variance showed a statistically significant effect of sires Swedish Landrace on the variability and average expression of the NBA daughter in the first and second farrowing (p<0.05). Year and season of mating did not affect on the variability of the NBA (p>0.05).

Heritability coefficients for number of born alive piglets in the first three parities were: 0.092, 0.123 and 0.030.
Procena koeficijenta herabiliteta za broj životorodene prasadi u prva tri prašenja krmača švedskog landrasa

R. Savić, M. Petrović, Č. Radović

Rezime

Osnovni cilj rada bio je da se oceni fenotipska i genetska varijabilnost broja životorodene prasadi (NBA) krmača švedskog landrasa (SL) u tri uzastopna prašenja pod uticajem očeva, godine i sezone pripusta. Istraživanjem je bilo obuhvaćeno: 618 legala u prvom, 470 u drugom i 403 legla u trećem prašenju. Testiranje homogenosti varijanse izvršeno je testom Levene-a. Dobijeni podaci su obrađeni metodom analize varijanse (Statistica Ver. 6.0., 2003). Koeficijenti herabiliteta su ocenjeni metodom intra-klasne korelacije. Očevi švedskog landrasa uticali su na varijabilnost NBA kćeri u prvom i drugom prašenju (p<0.05), ali ne i u trećem prašenju. Godina i sezona pripusta nisu uticale na varijabilnost NBA (p>0.05). Procenjeni koeficijenti herabiliteta za NBA su bili najviši u drugom (0.123), zatim u prvom (0.092) i najniži u trećem prašenju (0.030).

References


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MYCOBIONTA IN FEED FOR FARmed SEA BASS
(Dicentrarchus labrax)

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Original scientific paper

Abstract: Aquaculture producers, feed manufactures, farmers and distributors and their feed quality-control are, nowadays, placed in the centre of feed safety issues due to possible repercussions of residues in food. The aim of this preliminary study was to evaluate fungi contamination in 87 samples finished fish feed samples for sea bass (52 extruded feed and 35 pelleted), were randomly collected from different factories in Portugal. All extruded samples revealed to be negative for fungi contamination. Concerning to 35 pelleted samples, mould counts were around 1.0 ×10 to 6.5 ×10² cfu.g⁻¹. Six moulds genera were recovered. Aspergillus flavus had the highest incidence appearing in 35 samples (100 %), with a range of 1.0 ×10⁻² – 1.5 ×10³ and a mean value of 5.3 ×10⁻². In the second order were the moulds from the specie Aspergillus niger in 34 samples (97 %) with a range of 1.0 ×10 – 4.5 ×10⁻² and a mean value of 1.6×10⁻² Aspergillus glaucus had a percentage of 74 % with a levels ranging between 1.0 ×10 to 2.5 ×10². Penicillium and Cladosporium both recovered from 25 samples (71.4 %) with a range of 1.0 ×10² – 6.5 ×10² and 1.2 ×10² – 2.0 ×10³ and a mean of 1.2 ×10⁻² and 9.8 ×10⁻¹, respectively. To the fifth order was mould from the genera Fusarium contamination from 22 samples (62.8 %) with a range of 1.0 ×10 – 1.9 ×10² and a mean of 7.1 ×10¹.

Key words: aquaculture, fungi, fish feed, quality control

Introduction

Information about fungi associated with food and feeds is important in assessing risk of mycotoxin contamination. They are usually in form of dry complex feeds composed of plant (cereal seeds, bran, rapeseed or soybean meal or...
cake, legume seeds) and animal components (meat-bone and fish meal, poultry off-
fall, meat, powdered milk, animal fats) supplemented with vitamins and minerals (Zmyslowska and Lewandowska., 2000). This type of raw materials have been associated with contaminants produced by moulds during the initial stages of the crop production.

Feed manufacturing is concerned with the physical transformation of a written formulation into a compounded “edible” diet. A wide variety of techniques exist for the manufacture of complete aquaculture feeds (straight mixing/blending; flaking; dry or steam compaction pelleting; extrusion/expansion pelleting; and so on). In our study the samples analysed were processed using extrusion/expansion pelleting (dry, moist or rehydratable expanded pellets). According to FAO/WHO (2004), the efficiency of manufacturing process employed will depend on the feeding habit of the fish to be fed (ie. benthic, pelagic or surface feeder; visual or olfactory feeder; moist or dry diet feeder; rapid or slow feeder) and its physical feed requirements (ie. feed size, buoyancy, texture, palatability, and desired water stability) for all stages of the culture cycle. These technical factors will have then to be balanced with the market value of the cultured species and the availability of cash funds, feed ingredients and services.

Aquatic animals cannot digest starch effectively resulting in excessive excrement which can causes physiological problems such as excessive gas, bloating diarrhoea and these apart, from affecting the growth of the fish, will also lead to water pollution. The extrusion, high temperature and short duration process, cooks the raw materials, eliminating microorganisms including pathogens, and feed became more digestible. The use of extruded floating fish feed comes with a host of advantages in terms of digestion, growth, water protection, zero water pollution, optimized labour usage and zero wastage of raw materials, encouraging the use of this type of feed by fish farmers (Hashimoto et al., 2003).

During manufacturing, feeds can be contaminated with mould spores, especially when the cereal grains are ground and the feeds are pelleted (Suárez, 1999). Mould contamination not only causes deterioration of food and feeds, but also can adversely affect the health of humans and animals, once they may produce toxic metabolites (Joseph, 1971; Cole and Cox, 1981). The filamentous moulds most commonly found in stored cereal grains are Aspergillus, Penicillium and Fusarium species (Abarca et al, 1994; Joosten et al, 2001; Martins et al, 2001). Aspergillus is common contaminants of oilseed, crops, such as cottonseed, peanut meal and corn. Wheat, sunflower, soybean, fish meal, and nutritionally complete feeds can also be contaminated with aflatoxins and other mycotoxins.

The contamination of agriculture commodities with toxigenic fungi under favourable conditions may lead to mycotoxin build-up reaching to injurious levels for farm animals and human health. It can cause impairment of immune and acquired resistance to infections causing health problems which lead to economic and productivity losses Effective post-harvest management of stored commodities
requires clear monitoring criteria and effective implementation in relation to abiotic and biotic factors, hygiene and monitoring to ensure that mycotoxin contamination is minimised and that stored grain can proceed through the food chain for processing (Magan and Aldred, 2007).

The aim of this study was to make a preliminary screening of mycological analysis in fish feed using standard method of microbiology.

**Material and Methods**

A total of 87 samples finished fish feed samples for sea bass (52 extruded feed and 35 pellet), were randomly collected from different factories in Portugal. All packaged samples were aseptically transported to laboratory and tested within 24 hours after collection or, if necessary, they were stored for 2-3 days in paper bags at room temperature (22-25°C).

Ten grams of each sample were homogenized for 3 min in 90 ml (10⁻¹ suspension) peptone water (Oxoid, code CM 9, Basingstoke, England). Ten-fold dilutions were prepared till 10⁻³.

From each dilution in peptone water 1 ml was spread onto plates of two media: Dichloran Rose Bengal Chlortetracycline Agar (DRBC) and Sabourad’s agar with choramphenicol (BD Diagnostic Systems) with 0.25 ml per plate; the plates were incubated at 25°C in the upright position for 5 days according to the King et al. (1984).

Taxonomic identification of all colonies considered as different was achieved through macroscopic and microscopic studies. Aspergillus species were identified according to Pitt and Hocking (1985) and Raper and Fennell (1965). Other moulds genera were identified according to Domsch and Gams (1980).

**Results and Discussion**

The results obtained in this first survey showed that of fifty two extruded samples analyzed, none were contaminated by fungi. Otherwise, the 35 pellet samples presented fungi contamination (Table 1).

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Nº of analysed samples</th>
<th>Positive samples</th>
<th>Percentage (%)</th>
<th>Fungal counts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;1×10¹</td>
</tr>
<tr>
<td>Extruded</td>
<td>52</td>
<td>0/52</td>
<td>0</td>
<td>52</td>
</tr>
<tr>
<td>Pelleted</td>
<td>35</td>
<td>35/35</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. Number and percentage of positive fish fed samples analysed
Table 2. Frequency and average count of mould genera from 35 fish pellet feed samples

<table>
<thead>
<tr>
<th>Moulds</th>
<th>Frequency of positive samples</th>
<th>Percentage of mould genera frequencies (%)</th>
<th>Range</th>
<th>Average counts cfu g⁻¹ (*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus flavus</td>
<td>35</td>
<td>100</td>
<td>1.0 ×10² – 1.5 ×10³</td>
<td>5.3 ×10²</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>34</td>
<td>97</td>
<td>1.0 ×10⁻ – 4.5 ×10²</td>
<td>1.6 ×10³</td>
</tr>
<tr>
<td>Aspergillus glaucus</td>
<td>26</td>
<td>74</td>
<td>1.0 ×10⁻ – 2.5 ×10²</td>
<td>8.7 ×10¹</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td>25</td>
<td>71.4</td>
<td>1.0 ×10² – 6.5 ×10²</td>
<td>1.2 ×10²</td>
</tr>
<tr>
<td>Cladosporium spp.</td>
<td>25</td>
<td>71.4</td>
<td>1.2 ×10⁻ – 2.0 ×10²</td>
<td>9.8 ×10¹</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>22</td>
<td>62.8</td>
<td>1.0 ×10⁻ – 1.9 ×10³</td>
<td>7.1 ×10¹</td>
</tr>
</tbody>
</table>

Legend: (*) cfu- colony forming units per g of sample

Total number of cultivable fungi varied from 4.5 ×10¹ to 4.1 ×10³ CFUs g⁻¹ (colonies forming units). The counts should not exceed the values of 10⁵ CFU⁻¹.

The most predominant genera identified were *Aspergillus*, *Penicillium*, *Cladosporium* and *Fusarium*. *Aspergillus flavus* had the highest incidence appearing in 35 samples (100 %), with a range of 1.0 ×10⁻ – 1.5 ×10³ and a mean value of 5.3 ×10². In the second order were the moulds from the specie *Aspergillus niger* in 34 samples (97 %) with a range of 1.0 ×10⁻ – 4.5 ×10² and a mean value of 1.6 ×10³. *Aspergillus glaucus* had a percentage of 74 % with a levels ranging between 1.0 ×10⁻ to 2.5 ×10². Other genera were isolated at lower frequency, *Penicillium* and *Cladosporium* both recovered from 25 samples (71.4 %) with a range of 1.0 ×10² – 6.5 ×10² and 1.2 ×10² – 2.0 ×10³ and a mean of 1.2 ×10² and 9.8 ×10¹, respectively. To the fifth order was mould from the genera *Fusarium* contamination from 22 samples (62.8 %) with a range of 1.0 ×10⁻ – 1.9 ×10³ and a mean of 7.1 ×10¹. The *Fusarium* percentage counts were the lowest one with 72 % (Table 2).

This is the first study in Portugal identifying and enumerating fungi infecting fish farmed feed. Hence, it is very difficult to compare the results from this study with those from other authors in Portugal or by international researchers. However, many reports about mycodiversity have been published earlier at different animal feed (bovine, swine, poultry). The results obtained in this study have provided, for the first time, information about the presence and distribution of mycotoxigenic fungi in fish feed.

Ones that the composition in fish feed as many ingredients in common (wheat, soy, barley, maize, sunflower seed and fat) with other animal feed, we opted to discuss the results of this screening with the results found for other authors in different feed.

In a previous study effectuated in feed by *Almeida et al. (2007)* they found contamination with levels ranging between 1.7 log₁₀ cfu/g up to 4.6 log₁₀ cfu/g in samples of corn and levels 1.1 x10² to 5.0x 10⁴ in feeds (bovine, swine and poultry). Regarding others previous studies effectuated by others authors, *Martins*...
and Martins (2001) it was concluded that the levels and frequency of mycobiota contamination are decreasing judging the results obtained in the last ten years, in Portugal. According to Almeida et al. (2010), in 31 samples of rat feed mould counts ranged from 3 to 4.2 log_{10} cfu/g, highlighted once again the low levels among this kind of contamination.

Dalcero et al (1998) the presence of mycobiota in 180 samples of poultry had mean value from 1x 10^3 to 9.5x 10^4 CFU/g for the Aspergillus spp. and 1.2 x 10^3 to 2.5 x10^5 cfu/g for the Penicillium spp. These results agree with those obtained by screening effectuated by Martins and Martins (2001).

In 2010 a similar study done by Saleemi et al., also showed that the fungi contamination was low. Data related to the mould incidence in maize kernels from Swat Valley (Shah et al 2010) are in agreement with results found in corn-based food by Almeida et al (2007).

The total fungal loads in finished feed samples analyzed by Almeida et al (2007) were 10^4 cfug^{-1} which is higher than the ones found in Iraq of 10^5 (Shareef, 2010).

The knowledge of mycobiota and its evolution on feed chain is an essential tool to manage production of animal production systems. These include cultural practices of cereals in fields as well as harvest, transport and storage conditions. Physical, chemical or biological treatments of contaminated feed have poor efficacies and are not economically viable. Organic and inorganic adsorbents could be used to decrease the deleterious effects of contaminated animal feeds, they bind to mycotoxins and decrease their bioavailability which shows a great deal of promise in strategies that attenuate mycotoxin-induced toxicosis. The high affinity and high adsorption capacity of yeast-derived glucomannan preparations make their use as adjuncts for controlling naturally occurring mycotoxins in feeds attractive. The problem is that they have not yet been allowed for such purpose in the European Union (Jouany, 2007).

Conclusion

Plant ingredients pose a high risk for mycotoxin contamination. Since the aquafeed industry is moving towards using more plant ingredients, both risk assessment of mycotoxins as well as the development of appropriate protection strategies will become an integral part of aquaculture nutrition. The presence of toxigenic fungi does not indicate the mycotoxin production, because the toxins may persist long after vegetative growth has occurred and the moulds have died. Therefore, the presence of determinates fungi implicates a potential risk for animal health (Bueno et al., 2001).

Good quality of the products used and proper hygiene of the technological processes decrease the risk of microbiological contamination of fish feeds.
According to Zmyslowska and Lewandowska (2000), storage conditions, especially temperature and humidity represent another important factor affecting microbiological quality of feeds. Improper storage temperature may prolong survival of the micro-organisms present in the feed, or even enhance their multiplication and production of toxic substances.

**Mikobiota u hrani za brancina koji se gaji u ribnjacima** *(Dicentrarchus labrax)*

*I. Almeida, H. M. Martins, S. Santos, S.G. Freitas, F. Bernardo*

**Rezime**

Proizvođači u akvakulturi, proizvođači hrane, farmeri i distributeri i njihove kontrole kvaliteta hrane, danas, se nalaze u centru svih pitanja koja se odnose na bezbednost hrane zbog mogućih posledica rezidua u hrani. Cilj ovog preliminarnog istraživanja je bio da se uradi procena kontaminacije gljivama 87 uzoraka gotove hrane za brancina (52 ekstrudiranih uzoraka i 35 peletiranih), koji su slučajno odabrani iz različitih fabrika u Portugaliji. Svi ekstrudirani uzorci su se pokazali negativni u smislu kontaminacije gljivama. U vezi sa 35 peletiranih uzoraka, broj plesni je bio oko $1.0 \times 10^1$ do $6.5 \times 10^2$ cfu.g$^{-1}$. Određeno je šest rodova plesni. Za vrstu *Aspergillus flavus* je utvrđena najveća učestalost i to u 35 uzoraka (100 %), u opsegu od $1.0 \times 10^2$ – $1.5 \times 10^3$ i srednjom vrednošću od $5.3 \times 10^2$. Na drugom mestu je bila vrsta *Aspergillus niger* u 34 uzorka (97 %) opsega od $1.0 \times 10^1$ – $4.5 \times 10^2$ i srednjom vrednošću od $1.6 \times 10^2$. *Aspergillus glaucus* je imao procenat od 74 % sa nivoima u opsegu od $1.0 \times 10^1$ to $2.5 \times 10^2$. *Penicillium* i *Cladosporium* su registrovani u 25 uzoraka (71.4 %) u opsegu od $1.0 \times 10^2$ – $6.5 \times 10^2$ i $1.2 \times 10^5$ – $2.0 \times 10^5$ i srednjom vrednošću od $1.2 \times 10^5$ i $9.8 \times 10^1$, respektivno. Na petom mestu se nalazila vrsta iz roda *Fusarium* u 22 uzorka (62.8 %) u opsegu od $1.0 \times 10^1$ – $1.9 \times 10^2$ i srednjom vrednošću od $7.1 \times 10^1$.

**References**


Mycobiota in feed ...

Mycotoxins in the food chain. Int. Journal of Food Microbiology, 119, 131-139.

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DETERMINATION OF SHELF LIFE OF MARINATED CARP FILLETS

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Original scientific paper

Abstract: In the present study, sensory, microbiological and chemical changes during manufacturing and storage of marinated and baked fillets were investigated. For this purpose, the fillets were divided in to three groups. One group was control and the other two groups were marinated with two different marination mixes (group A and group B). The control fillets were cooked at 150 °C for 55 minutes. The marinated groups were cooked after holding at +4 °C for 6 hours. Central temperatures of cooked fillets were chilled to +4 °C and vacuum packaged. Vacuumed fillets stored at +4 °C. Then fillets were analysed for sensory, microbiological (mesophilic aerobic bacteria, psycrophilic aerobic bacteria, mesophilic anaerobic bacteria, yeast and mould counts) and chemical (pH, moisture, total volatile – nitrogen, tiobarbituric acid and salt) on 0, 7 th,14 th,28 th,42 nd,56 th,70 th,84 th and 98 th days of storage. In the microbiological analysis, counts of total mesophilic aerobic bacteria, psycrophilic aerobic bacteria, mesophilic anaerobic bacteria, yeast and mould counts were determined as < 10 cfu/g in all groups during the storage. There was no significant difference among the groups in pH, moisture, TVB-N and salt level (p>0.05). The TBA value increased during the storage period in control group. As for the TBA value, the difference between control group and group A and group B was significant (p<0.05). When fillets have been investigated from sensory characteristics, A and B products found better than control group throughout the storage period.

Key words: carp fillet, marination, baking, sensory, microbiological and chemical quality

Introduction

Fresh seafood is a highly perishable product and spoilage developing in aerobically stored fish typically consists of Gram-negative psychrophilic non-fermenting rods. Thus, under aerobic ice storage, the flora is composed almost
exclusively of *Pseudomonas* spp. and *Shewanella putrefaciens* (Davies et al., 2001). The *Enterobacteriaceae* count is considered as another index of fish quality because it is related to storage in ice, washing and evisceration (Emblem et al., 2001). A monitoring of these microorganisms has been suggested as a measure of fish quality. Also, risk management decisions should take into account the whole food chain from primary production to consumption, and should be implemented in the context of appropriate food safety infrastructures, for instance regulatory enforcement, food product tracing and traceability systems. In the fish processing chain managing risks should be based on scientific knowledge of the microbiological hazards and the understanding of the primary production, processing and manufacturing technologies and handling during food preparation, storage and transport, retail and catering (Davies et al., 2001).

Protein represents the major component of fish feeds. It is a source of energy for the fish, but also a medium for micro-organisms, especially proteolytic bacteria and ammonifiers. Good quality of the products used and proper hygiene of the technological processes decrease the risk of microbiological contamination of fish feeds.

Fresh fish is consumed in different patterns (fried, baked, steamed etc.); it can also be consumed by transformation into different fish products (Cardinall et al., 2004). Several technological processes can be employed in order to preserve the freshness of fish flesh.

Sauce compositions prepared with natural additives improve the flavor, aroma, appearance, color and texture features of food; they also preserve the food against microbial, chemical and physical effects (Altuğ et al., 2001). In addition, they facilitate and improve technological process (Damarlı et al., 1992). Fish fat becomes easily oxidized as it includes high level of unsaturated fats, natural catalysts in its structure (i.e. hem pigment), and abundance of vitamins A and D. Some spices in the sauce improve the aroma and flavor of food; they also have antimicrobial and antioxidant characteristics (Damarlı et al., 1992).

In our country, the sales figures of food made of sea products which are readily consumable are not high. It is extremely important that fish is transformed into ready or semi-ready products so that the animal-based protein gap can be closed and consumption of fish harvested during hunting season can be ensured in periods other than their seasons. In our region *Cyprinus carpio* is produced abundantly but not preferred by the public as its organoleptic features are low. This study was conducted so as to improve organoleptic features of *Cyprinus carpio* in our region and lengthen its shelf life, so that a contribution can be made to the economy.
Material and Methods

**Raw materials and process.** Material of the study was *Cyprinus carpio* (*Cyprinus carpio* Linnaeus, 1758) hunted in Keban dam lake. The study consisted of three trials and in every trial fish with approximately 15 kgs live weight was used. Fish hunted from Keban dam lake were transported to the laboratory in cold chain. Their heads were chopped, skins were stripped and internal organs were taken out. Then fillets cleaned out from muscles, fishbones and bones were divided into 64 parts with similar thickness, each of which weighed 70-80 grams. Two different types of sauces were used in the study (A and B). Prepared fillets were separated in to three groups. The first group was assigned as the control group. Three percent salting process was applied to control group samples. Second group was left in the sauce with formula A (Table 1) for 6 hours at + 4°C, whereas third group was left in the sauce with formula B (Table1) for 6 hours at + 4°C. The weight of the sauce used was 20% of the fish weight. Fillets placed on a tray were baked for 55 minutes in the oven (*Arçelik, MF 2009*) at 150°C. Central temperature of the fillets was measured with K probed thermocouple (*HI 9057 KJT* thermocouple, Hanna instruments, Portugal). Organoleptic appearance of the product was also taken into account during baking. After the baking process was completed, the tray was covered with aluminum foil and fillets were rapidly cooled in deep freezer until their central temperature fell to + 4°C. The cold fillets were packaged with vacuum. Vacuumed fillets were preserved at + 4°C and examined on days 0, 7, 14, 28, 42, 56, 70, 84 and 98 in terms of organoleptic, microbiological and chemical features.

**Table 1. Sauce combinations**

<table>
<thead>
<tr>
<th></th>
<th>A Group</th>
<th></th>
<th>B Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount(g)</td>
<td>Rate(%)</td>
<td>Amount(g)</td>
</tr>
<tr>
<td>Tomato Paste</td>
<td>200</td>
<td>20</td>
<td>300</td>
</tr>
<tr>
<td>Lemon juice</td>
<td>200</td>
<td>20</td>
<td>300</td>
</tr>
<tr>
<td>Oil</td>
<td>200</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Garlic</td>
<td>100</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Onion</td>
<td>-</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>Thyme</td>
<td>10</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Red Pepper</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Black Pepper</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Cumin</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Salt</td>
<td>30</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Water</td>
<td>240</td>
<td>24</td>
<td>80</td>
</tr>
</tbody>
</table>
Physical and chemical analysis. pH values of the samples were determined with pH meter (EDT, GP 353) (AOAC, 1990). The method reported by Varlik (1993) was employed in determination of TVB-N amount of the samples. The amount of malonaldehyde formed with fat oxidation in samples was calculated in mgs (Tarladgis, 1960). Salt level of the samples was determined according to Mohr method (55). Moisture was determined according to TSE (1974).

Microbiological analysis. For all microbiological counts, 10 g of sample were taken and transferred into 90 ml 0.1% peptone water (Difco, 0118-17-0) and homogenized. From the 10⁻¹ dilution, other decimal dilutions were prepared. Plate Count Agar (Oxoid CM 325) was used in order to count mesophilic aerobe bacteria of the samples. Implanted plates were incubated at 35°C for 48 hours and colonies formed were counted. Wort Agar (Merck 1.10130) was employed for counting yeasts in the samples. Plates were incubated at 30°C for 5 days and the colonies formed were counted. Sabouraud Dextrose Agar (Acumedia 7150 A) was used for counting mould in the samples. Plates were incubated at 30°C for 5 days and the colonies formed were counted. Brewer Agar (Oxoid) was used to count the total mesophilic anaerobe microorganisms. Plates were incubated at 30 ± 1°C under anaerobe conditions for 3 days. Colonies formed at the end of incubation were counted. Plate Count Agar (Oxoid CM 325) was used in order to count the total psychrophilic aerobe bacteria. Plates were incubated at 5°C for 10 days and colonies were counted (Harrigan et al., 1976).

Sensory analysis. Samples were examined in terms of crispiness, flavor, saltiness, appearance and total assessment. For this purpose samples were analyzed by a group of 8 panelists against preset criteria. The samples were scored from 1 to 5, where 1 very bad, 2 bad, 3 normal, 4 good and 5 very good (Kurtcan and Gönül, 1987). The samples were evaluated color, smell, flavor, appearance, crispiness, saltiness and total assessment.

Statistical analysis. Analysis of the data was conducted using Statistical Analysis System (SAS) package programmed. Values between groups and within group-between days were compared. Data were subjected to variance analysis in accordance with 3 x 11 x 3 x 1 factorial design and in terms of fix effects and inter-variable interactions so that “repetition number x sampling time x test groups x number of samples examined at one instance from each test group”. According to General Linear Models (GLM) procedure, Fisher’s smallest squares average (LSD) test was used. Standard deviation figures of all averages were calculated (Anonim, 1996). Alpha value was determined as 0.05.
Results and Discussion

Raw material of microbiological analysis are given in Table 2. Total mesophilic aerobic bacteria, total mesophilic anaerobe bacteria, total psychophilic aerobic bacteria, yeast and mould were determined to be <10 kob/g on all sampling days in all groups.

Table 2. Raw and sauced fillets of number microorganisms (log_{10} kob/g) (n=3)

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>T.F.</th>
<th>Sauced Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Mezophilic aerobic</td>
<td>4.16</td>
<td>3.62</td>
</tr>
<tr>
<td>Psicrophilic aerobic</td>
<td>4.25</td>
<td>3.79</td>
</tr>
<tr>
<td>Mezophilic anaerobic</td>
<td>1.74</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Yeast</td>
<td>3.71</td>
<td>2.56</td>
</tr>
<tr>
<td>Mould</td>
<td>3.77</td>
<td>2.77</td>
</tr>
</tbody>
</table>

a : Raw Fillet, b : A Groub, c : B Groub

Changes detected during the production and preservation of fish fillets are given in figure 1. The pH value of the prepared sauce was determined as 5.63 in group A and 4.8 in group B. 6.51 pH value found in fresh fillet decreases after saucing process and becomes 5.89 in samples from group A and 5.35 in samples from group B. irregular pH changes were observed in all three groups during preservation (Figure 1.a.). It was found out that the difference between groups and in the same group-between days was not significant (p>0.05).
The humidity changes of samples are given in the figure 1.b. It was determined that changes between groups and within all three groups during production and preservation periods was insignificant (p>0.05).

TVB-N is used for determination of the spoilage level of fish during the storage period. TVB-N values detected in control group samples were higher compared to the other two groups; this value was 11.56 mg/100 g on day 0, and increased to 23.53 mg/100 g on day 98. During production and preservation of fillets, TVB-N values of samples form groups A and B increased slower compared to the control group (Figure 1.c). In statistical analysis, it was determined that the difference between days within the same group was not significant (p>0.05) in terms of TVB-N values. When a comparison was made between groups, it was found out that the difference between control group and group B on day 56 was statistically
significant, but difference on other days was insignificant (p>0.05). When samples are statistically evaluated, it was found out that difference between groups was not significant (p>0.05), but when days within a group are compared, it was found out that the difference between raw fillet and sauced fillets was significant (p<0.05), whereas difference between other days was insignificant (p>0.05).

The TBA value of raw material was found to be 0.153 mg/1000 g. This value decreased to 0.104 mg/1000 g in group A after saucing process, whereas it increased to 0.612 mg/1000 g in group B. On the day 0 of preservation, TBA values was found as 0.143 mg/1000 g in control group and group B, whereas it was 0.161 mg/1000 g in group A. TBA value in control group increased regularly during preservation. TBA number in sauced groups was found to be close to each other; it was found to be 0.703 mg/1000 g on day 98 of preservation in group A and 0.688 mg/1000 g in group B(Figure 1. d.). Statistically speaking, it was determined that the difference between days within group in groups A and B were not significant (p>0.05), whereas the difference between days 0, 7, 14, 28 and 42 and other groups was significant (p<0.05). It was also fund out that the difference between control group and groups A and B on days 84 and 98 was significant (p<0.05). TBA numbers detected in samples of group B were rather low in all preservation days compared to the samples in group A, whereas the results were statistically examined the difference turned out to be insignificant (p>0.05).

Changes detected during the production and preservation of fish fillets in determined sensory quality values are given in figure 2.

As a result of sensory evaluation of samples, when the scores they received in terms of total assessment, it can be seen that the lowest scores belonged to control group, whereas the highest scores were represented by group A. Samples were evaluated in terms of colour, smell, flavour, appearance and total assessment; as a result, it was detected that the difference between control group and groups A and B was statistically significant (p<0.05), whereas in terms of crispiness and saltiness, the difference between groups remained insignificant (p>0.05).

Total number of mesophilic aerobe bacteria in fresh fillet was found as 4.16 log_{10} kob/g on average. Patr et al., (2001) determined 6.70x10^4 kob/g total number of mesophilic aerobe bacteria in carpio fillet in the beginning, which is higher than the value obtained in this study. Values can differ depending on contaminations during preparation of the fillets used in experiments.

As regards preservation of fish flesh using different methods (using potassium sorbate, curing, marination etc.) there is lots of information in the literature; however, there are few resources on such preservation of same carp fillets (using thermal process and saucing).

Rosnes et al., (1999), applied thermal process at 70 °C for 15 minutes after they vacuumed sauced trout fillets and preserved them at 4 and 10 °C (sous-vide method). At the end of day 42, researchers found the total number of mesophilic aerobe bacteria to be < 1 log_{10} kob/g of fillets kept at 4 °C. These values are in agreement with the values that we found. The same study determined this value to
be $6 \log_{10}$ kob/g on day 17 and above $8 \log_{10}$ kob/g on day 42 in fish stored at 10 °C. Applied temperature level was lower and preservation period was shorter compared to those employed in our study.

Figure 2. a: Changes in color of during storage and production. b: Changes in smell of during storage and production. c: Changes in flavor of during storage and production. d: Changes in appearance of during storage and production. e: Changes in crispiness of during storage and production. f: Changes in saltiness of during storage and production. g: Changes in total assessment of during storage and production. T.F.: Raw Filet. S.S: Marination.
Bergslien (1996); preserved trout fillets at 2 °C to which 10 minutes of thermal process was applied at 65 °C. On day 7 of preservation, the total number of mesophilic aerobe bacteria was found to be above 5 log_{10} kob/g. However, even the value determined in fresh fillet (4.16 log_{10} kob/g) in the study is lower than this value, which was found as <10 kob/g on day 7 of the study. In their study, Simpson et al., (1994) applied 65 °C (71 and 105 minutes) and 75 °C (37 and 40 minutes) thermal process to sauced spaghetti and sauced meat, preserved it at 5 and 15 °C, and checked the total number of mesophilic aerobe, mesophilic anaerobe and lactic acid bacteria. In the study, they determined that samples preserved at 5 °C maintained their quality for more than 35 days, whereas samples maintained at 15 °C lost their organoleptic features turned sour on day 14. This finding can be attributed to the fact that microorganisms injured by the effect of heat but which became active again at preservation temperature proliferated.

Sauced trout fillets were preserved at 2 °C for 45 days by applying 3,3 minutes of thermal process at 90 °C (Gonzalez, 2004). In the study, psychophilic aerobe bacteria number found as 5 log_{10} kob/g in fresh fillet decreased below 1 log_{10} kob/g. When the product obtained with the same method is preserved at 10 °C, psychophilic aerobe bacteria number was again found to be below 1 log_{10} kob/g. Although the temperature and heating period was low, findings obtained coincide with the findings of our study. In the same research (Gonzalez, 2004), number of mesophilic aerobe in fresh fillet were found as 4.4 log_{10} kob/g, whereas number of mesophilic anaerobe es was found as 1.5 log_{10} kob/g. However, this value is higher than the findings obtained in this study. Sauced trout fillets were exposed to 5 minutes of thermal process at 70 °C and preserved at 10 °C; at the end of day 45, number of mesophilic aerobe bacteria was found as above 7.5 log_{10} kob/g. applied temperature, heating period and preservation temperature cause difference in terms of microbial quality of the product.

Gonzalez et al., (2005) subjected the salmon fillets to which olive oil and salt was added to 5 minutes of thermal process at 65°C and 10 and 15 minutes of thermal processes at 90 °C, and preserved them at 2 and 10°C. In fresh fillet, number of mesophilic aerobe bacteria determined as 4.77 log_{10} kob/g decreased to 2 log_{10} kob/g at the end of day 45 in fillets which were treated with 90°C for 10 minutes. This finding differs from ours. It is believed that this difference can be explained by the difference in method, applied temperature and additives included in the fillets.

Sardine fish were filled in jars after they were marinated with different sauces; when the central temperature reached 70 °C, they were subjected to thermal process for 20 minutes. In the study, samples were preserved at 4 °C and examined for 6 months in terms of total number of mesophilic aerobe, psychophilic aerobe, lactic acid bacteria, yeasts and fungi. In the end, it was found out that the number of mentioned microorganisms was below <10 kob/g (Kilinç and Çaklı, 2005). This
study is in agreement with our findings although it yielded partially different results.

Generally, preservation period of sauced fish fillets changes according to the microbial load in the beginning, applied temperature, packaging type and preservation temperature.

Determination of total volatile basic nitrogen (TVB-N) used in finding the freshness of fish flesh and fish products is an important parameter. The value that we found in fillet (12.03 mg/100 g) is close to the value found by Patr et al., (2001) for fresh carp, which was 11.67 mg/100 g. In addition, if we compare with the values obtained by researchers using different types of fish, the findings reached by Turan and Erkoyuncu (1997), 4.20 mg/100 g, Yapar and Yetim (2000) for anchovy fillets 7.10 mg/100g, are much lower than our findings. However, they are lower than the findings of Tunç (1994), Metin (1995) and İzgi (1996) for fresh trout (15.33 mg/100 g and 17.97 mg/100 g). Throughout the study, TVB-N amounts obtained during preservation from all three groups did not transcend the suggested criteria and, it was concluded that, taking these criteria into account, they were in good quality. Thermal process, vacuum packaging and preservation at +4 °C did not cause much increase in TVB-N amount. Kaya (1994) found TVB-N amount at cured trout and bonito preserved at room temperature to be 48.6 mg/100 g on day 5; the findings for salmon was 45.2 mg/100 g. the same researcher (Kaya, 1994) found that if preserved in refrigerator for 50 days, trout produced 58.4 mg/100 g, bonito produced 57.6 mg/100 g and salmon produced 55.8 mg/100 g TVB-N. Cardinall et al., (2004) reported that TVB-N amount of cured and vacuum-packaged Atlantic salmons which were preserved at +4°C for 2-3 weeks was found as 22.4 mg/100 g.

TBA number was found as 0.153 mg malonaldehyde/1000 g in the carp fillet which was used in this study; this number showed a relative increase during production and preservation. However, the increase in both groups was lower than the increase observed in control group. Antioxidant lycopene in tomato paste and citric acid in lemon juice may have prevented oxidation in sauced groups. It is believed that the increase in TBA number of control group can be attributed to the fact that it did not include any sauces. Vacuumed packaging prevents oxidation of products (Ariyani 2000); however, there can be a small amount of oxygen in the vacuum package which can trigger oxygen oxidation. The reason for which TBA number remained high in control group was that there was no such barriers which could prevent oxidation at the initial phase. During the study, TBA numbers found in all three groups were found to lower than suggested levels (7-8 mg malonaldehyde/1000 g).

Taking into all organoleptic evaluation criteria, samples of group A got higher scores than group B, and control group samples received the lowest scores. This result is attributed to the effect and composition of sauces.
Conclusion

The reason for using *Cyprinus carpio* in the research is that it is a less costly, abundant, and easily treatable type of fish. Turkey has favorable climatic condition for carp growing. However, hunting season of carp matches with the season when sea fish is most abundant, which is an important disadvantage for carp growing. In this case, carp loses its competitive power and marketed at prices far below its value. For this reason, *Cyprinus carpio* which cannot be consumed fresh can be turned into various products and its consumption outside the production season can be popularized. Three basic criteria must be observed so as to ensure microbial safety in sauced products; namely, thermal process at proper temperatures, rapid cooling of the product to a proper temperature and its preservation at low temperature. Thermal process applied to fillets is economic and free from chemical residues; it also terminates the microorganisms which cause spoilage and improves organoleptic characteristics of the product, which are regarded as its advantages.

It has been determined that carp fillets, which were preserved at 4°C after being sauced, baked and vacuum packaged, maintained their organoleptic, microbiological and chemical quality for at least 98 days.

Acknowledgements

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Određivanje roka trajanja mariniranih fileta šarana

Ö. Pelin Can, A. Arslan

Rezime

U ovom istraživanju, ispitivane su senzorne, mikrobiološke i hemijske promene tokom procesa proizvodnje i skladištenja mariniranih i pečenih fileta. U tu svrhu, fileti su podeljeni u tri grupe. Jedna grupa je bila kontrola, a druge dve su bile marinirane korišćenjem dve različite smeše za mariniranje (grupa A i grupa B). Kontrolni fileti su kuvani na 150°C u trajanju od 55 minuta. Marinirane grupe su kuvane nakon čuvanja na 4°C u trajanju od 6 sati. Centralna temperatura skuvanih fileta je smanjena, ohlađena na 4 °C i fileti su vakumirani. Vakumirani fileti su
O. Pelin Can and A. Arslan

sclkadišteni na 4 °C. Nakon toga fileti su analizirani, i to: senzorne, mikrobiološke (mezofilne aerobne bakterije, psihrofilne aerobne bakterije, mezofilne anaerobne bakterije, broj plesni) i hemijske osobine (pH, vлага, ukupni isparljivi – azot, tiobarbiturna kiselina i so) 0, 7.,14.,28.,42.,56.,70., 84. i 98. dana skladištenja. U mikrobiološkoj analizi, brojevi mezofilnih aerobnih bakterija, psihrofilnih aerobnih bakterija, mezofilnih anaerobnih bakterija, plesni, kvasaca su određivani kao <10 cfu/g u svim grupama tokom skladištenja. Nije bilo signifikantne razlike među grupama u pH, sadržaju vlage, TVB-N i nivou soli (p>0.05). TBA vrednost se povećala tokom skladištenja u kontrolnoj grupi. U vezi sa TBA vrednošću, razlika između kontrolne grupe i grupa A i B je bila signifikantna (p<0.05). U ispitivanju senzornih karakteristika fileta, A i B proizvodi su imali bolje osobine tokom čitavog perioda skladištenja.

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IMPACT OF ANIMAL HEALTH MANAGEMENT ON ORGANIC PIG FARMING IN GREECE

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Abstract: In Greece, organic pig farming started in 2002 and since then made significant steps forward due to the extended interesting of Greek consumer for organic products during last decade. This report aims at updating information about organic pig farming in Greece, relating production system and most health risk factors. Furthermore, a veterinary health management program is proposed.

The most common health problems that occur in the Greek organic pig farming are respiratory problems, gastrointestinal problems, claw and skin problems, parasitic infections and piglet mortality. A veterinary health management program in organic pig farms, including use of alternatives to antibiotics (prebiotics, probiotics and phytogenics), antiparasitics, appropriate vaccinations (e.g. against E. coli, M. hyo, PRRSV) and the appropriate disinfection program (e.g. rodent management with rodenticides) could be an useful tool for prevention and control diseases as well as for improvement of growth performance.

In conclusion, the productivity growth in Greek organic pig farms can be increased by improving the living conditions of animals and the implementation of preventive measures (e.g. vaccination programs, treatments with phototherapeutics) that reduce mortality rates (especially during the winter months). For this reason the application of a herd health management program is necessary in organic pig farms.

Key words: organic farming, pig, risk factors, Greece

Introduction

Greece has a comparative advantage over other countries in terms of organic farming, due to favorable pedo-climatic conditions, rich natural resources in mountainous and semi-mountainous areas, the rich biodiversity of plant life (with a significant number of endemic plants in the different geographical districts) and extensive farming, which can easily be converted to organic. Organic animal
farming is directly related to organic agriculture farming because the nutritional needs of animals other than grazing and covered with organic feed.

The importance of organic pork in Europe has been rising during the last years. The National Greek projects of organic pig farming started in 2002. Most of EU countries had an increasing number of organically produced pigs and Greece had one of the most enormous increases in Europe (Lampkin et al., 2007). That is why in the beginning of 2005 Greece has become the second largest organic pig producer in the EU with over 100,000 pigs (Table 1). With the exception of Greece (13% national production share in 2005) the market share of organic pig production in Europe amount to less than 2% (Llorens Abando and Rohner-Thilen, 2007). The contribution of organic pig farming in total Greek organic livestock farming was minimal until in 2004, but then, has grown significantly, reaching a participation rate to 15% (DOA, 2009). In 2002, the total number of organic pigs was just 1,288. Since then and until 2005 a steady increase was observed while, in 2006-2007 a significant increase was noticed, reaching the number of 175,000 organic pigs (DOA, 2009).

Table 1. Number of organic pigs from 2002 to 2007 in Greece.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of organic pigs</td>
<td>1,288</td>
<td>3,628</td>
<td>4,469</td>
<td>126,003</td>
<td>110,096</td>
<td>175,004</td>
</tr>
<tr>
<td>Total number of pigs</td>
<td>1,288</td>
<td>3,628</td>
<td>4,469</td>
<td>126,003</td>
<td>110,096</td>
<td>175,004</td>
</tr>
<tr>
<td>Fattening pigs</td>
<td>1,238</td>
<td>1,648</td>
<td>1,856</td>
<td>**</td>
<td>87,669</td>
<td>69,180</td>
</tr>
<tr>
<td>Sows</td>
<td>18</td>
<td>752</td>
<td>2,557</td>
<td>**</td>
<td>20,345</td>
<td>18,200</td>
</tr>
<tr>
<td>Other pigs <em>(</em>)</td>
<td>32</td>
<td>1,228</td>
<td>56</td>
<td>**</td>
<td>2,082</td>
<td>87,624</td>
</tr>
</tbody>
</table>

(*) Including estimated young animals, (**) there is not published data


The National Greek projects of organic pig farming started in 2002. The contribution of organic pig farming in total Greek organic livestock farming was minimal until in 2004, but then, has grown significantly, reaching a participation rate to 15%. In 2002, the total number of organic pigs was just 1,288. Since then and until 2005 a steady increase was observed while, in 2006-2007 a significant increase was noticed, reaching the number of 175,000 organic pigs (DOA, 2009).

Most Greek consumers do not seem to be aware of the meaning of balanced nutrition, but food borne hazards is the greatest worry (Zervas, 2007).
Their concern for consuming safe food seems to affect their preferences for specific types of market, according to value and trust criteria. This selection is also affected by personal criteria, according to which many consumers seem to assess possible causes of food borne hazards and ways to avoid them as well. Disease prevention is a key point in organic livestock production. Health management by identifying and controlling the level of risk factors may therefore be particularly relevant for organic farmers (Arsenos et al., 2004).

The aim of this study is to provide information about organic pig farming in Greece, relating production system, genetic background, diets and most health risk factors. Furthermore, in present study a herd health management program is proposed.

**Genetic, diets, health problems.** Swine breeds that are usually preferred in the Greek organic pig farming are types of Greek domestic swine and various domestic pigs derived from crossbreeding between male Greek domestic pigs and improved breeds, such as Large White and Landrace.

According to the legislation in force about organic pig farming, all animals should originate from organic farms, except the cases that are required to renew the herd, so it is allowed to entrance conventional pigs in a percentage of 20% or in a percentage of 40% in cases of increasing the capacity or of changing of breeding stock. Therefore, the different Greek domestic swine breeds (which still exist today in many mountainous areas) can be the initial breeding stock (sows and boars) of organic pig farms, that after a short period of just 4 months, could be a grandparent nucleus of sows which will be kept in the farm for producing its own organic gilts. In organic pig farming, organic feed have to be used, preferably from the same unit or another organic unit. However, it can be used to 30% transition feed, except where the feed from the same farm where the percentage is until 100%. Therefore, the function pig units, which produce their organic feed, could contribute significantly to securing the required quantities of feed and at the same time reducing production costs.

The most common health problems that occur in the Greek organic pig farming are respiratory problems, gastrointestinal problems, claw and skin problems, parasitic infections and piglet mortality. The housing condition of organic farming may predispose animals to various infectious micro-organisms, normally no longer present indoors because of the strict hygienic measures that are taken.

Causes of high piglet mortality are skin traumas or crushing of piglets by the sow and diarrhea syndromes in suckling and weaning piglets. Other health problems in weaned pigs are respiratory diseases, arthritis and endoparasites. The risk factors suggested for diarrhea in weaned pigs are related to feed quality and hygiene of outdoor area, indoor pens and wallowing holes. In Europe, studies reported that neonatal diarrhea is uncommon in organic pig production, possibly
due to routine vaccination with \textit{Clostridium perfringens} and enteritis (Feenstra, 2000), but diarrhea is noticed in finishing pigs (Baumgartner et al., 2003). Therefore the improvement of animal welfare and housing conditions, e.g. the construction of simple and well designed facilities, including batches with infrared lamp for piglets, can significantly reduce piglets’ mortality (especially during the winter months). Common are also respiratory problems, with usually sign the pneumonia, mainly in growing and finishing pigs. Causes of pneumonia usually involve pathogens such as \textit{Mycoplasma Hyopneumoniae}, \textit{Actinobacillus pleuropneumoniae} and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). Studies in Austria showed similar finding (Baumgartner et al., 2003).

Furthermore, in sows most common problems are leg problems (lameness, hoof injuries and abscesses), reproductive disorders (variations in litter size and abortions or returns-to-oestrus) and poor body condition. Apart from that, poor body condition and reproduction problems are frequently observed in the sows, and stone chewing in pregnant sows. Important risk factors for leg problems in sows are considered to be genetic factors affecting leg strength, diseases in legs and hooves, ground condition in outdoor areas and management in the mating area (increased social activity resulting in trauma). Poor mating management regarding oestrus and pregnancy testing, synchronization of oestrus in sow batches and poor body condition are regarded as important risk factors for reproductive problems in the herd. Similar findings are referred from other studies in organic pig farms in Europe (Feenstra, 2000; Vaarst et al., 2000; Kampshof and Steverink, 2001).

Another common situation formulate identified are the various ectoparasites and endoparasites because of non-parasitic programs prophylaxis regimes. More frequently parasites in Greek organic pig farms are \textit{Sarcoptes scabei}, \textit{Trichuris suis} and \textit{Ascaris suum}. Especially \textit{Ascaris suum} causes growth retardation and “milk spot” lesions in the liver. Pigs need some place for rooting which is one of the basic needs of them. Rooting helps against tail biting but paddocks are a very suitable environment for \textit{Ascaris suum}, \textit{Trichuris suis} and maybe also for other helminths. Endoparasites and ectoparasites must be considered one of the major constrains for welfare and health as well as economy in organic swine production, especially during post weaning period (Mejer et al., 1999; Nansen and Roepstorff, 1999; Leeb and Baumgartner, 2000; Vermeer et al., 2000). In Europe, studies have shown high prevalences of helminth infestations in organic outdoor pig production (Roepstorff et al., 1992; Carstensen et al., 2002).

An important risk factor in organic pig production is the more frequent, compared with conventional swine industry, in contact with rodents. Rodents are actors - tank of several pathogens, some of which are hazardous to public health, such as \textit{Trichinella} spp., \textit{Toxoplasma gondii}, \textit{Salmonella} spp., \textit{Campylobacter} and \textit{Leprospira} spp. \textit{Salmonella} and \textit{Campylobacter} spp. are the most important causes of bacterial gastroenteritis in humans and are responsible for food-borne diseases.
Impact of animal health management

(Mead et al., 1999; Tauxe, 2002). Wild rodents may spread zoonotic bacteria between farms (Gratz et al., 1994; Hiett et al., 2004; Leirs et al., 2004). A comparison of the Salmonella-seroprevalence in Danish organic, free-range, conventional and breeding pig herds (Wingstrand et al., 1999) showed that the risk of meat juice samples being seropositive was higher for organic and free-range than for conventional herds. This risk may be even greater in organic production, where contact with livestock is more likely and rodenticides are used less often. Organic farmers should apply effective rodent management that is in line with organic principles to protect livestock and human health (Meerburg et al., 2004).

**Animal health management.** In order to prevent the above diseases, it is suggested the replacement animals in organic farms to derived from breeding organic or conventional farms, which are 'free' from parasitic and respiratory diseases. Furthermore, it is important especially for the conventional farms to apply the necessary preventive antiparasitic and vaccination programs.

Disease prevalence and veterinary treatments in organic animal production differ from conventional systems. According to the European Commission (EC) regulation on organic agricultural production (EC, 2004), disease prevention is based on the principles that the feeding, housing and care of the animals should limit animal health problems so that they can be controlled mainly by prevention. If, despite these preventive measurements, treatment should be necessary, phytotherapeutic and homeopathic products should be used in preference to chemically synthesized allopathic veterinary medicinal products, provided they are therapeutically effective. Most important current EC organic regulations for the animal treatment are:

A) First of all there is a treatment requirement. Sick animals must "immediately" get treated. Thus, a farmer acts adversely to the regulation, if he does not give treatment to his ill livestock.

B) The organic farming favours complementary medicine, because these are to support the defense mechanism of the organism, without leaving chemical residues in dung and food. Homeopathic and phytotherapeutic products are to be preferred provided that they actually have a therapeutic effect on the animal species and the illness. But: “chemically-synthesized allopathic veterinary medicinal products or antibiotics may be used under the responsibility of a veterinarian” if the alternative treatment “is unlikely to be effective”.

C) The doubling of the legal withdrawal period for chemical drugs in the organic farming is to improve the desired consumer protection: “The withdrawal period... is to be twice the legal withdrawal period or, in a case in which this period is not specified, 48 hours.” The duplication of the withdrawal period and the 48-hours rule only concerns the allopathic veterinary remedies, thus everything that is not ranked among the homoeopathics. This would concern also the
phytotherapeutics. The 48-hours rule concerns as well medicinal products with a legal withdrawal period of 0 days.

D) Finally, the number of “chemically-synthesised allopathic veterinary” treatment courses is restricted. This means: Two treatment courses in fattening pigs and fowl inhibit the marketing as an organic product.

In recent decades, prebiotics, probiotics and phytogenics (also referred to as botanicals or phytobiotics) have been used as potential alternatives to antibiotics in swine diets (Mroz, 2005; Windisch et al., 2008; Jacela et al., 2010). The proposed benefits from probiotics are improved digestion, stimulation of gastrointestinal immunity, and increased resistance to infectious diseases of the gut (Doyle, 2001; Zimmermann et al., 2001). For example, probiotics containing Bacillus cereus var Toyoi, Bacillus licheniformis or Bacillus subtilis spores have in sows and their litters had positive effect on health status and performance characteristics of sows as well as of weaning, growing and finishing pigs, as well as on the reduction of incidence and severity of Post Weaning Diarrhoea Syndrome (PWDS), (caused mainly by enterotoxigenic E. coli - ETEC strains) (Alexopoulos et al., 2001, 2004a,b; Kyriakis et al., 1999, 2003). Phytogenics have significant antimicrobial activity against bacteria (especially Gram negative bacterial species, like Salmonella spp. and E. coli) yeasts, moulds (Tsiloyiannis et al., 2001a,b; Øverland et al., 2007; Creus et al., 2007; Piva and Grilli, 2007; Papatsiros et al., 2009) and parasites (like Ascaris suum) (van Krimpen et al. 2010). These products potentially provide also antioxidative effects, enhance palatability, improve gut functions, or promote growth (Partanen, 2001; Namkung et al., 2004; Franco et al., 2005; Kasprowicz-Potocka et al., 2009).

In conclusion, the above alternatives to antibiotics could be a useful tool for prevention and control diseases (especially PMWS) as well as for improvement of growth performance in organic pigs (Castro, 2005; Jacela et al., 2010).

Vaccinations against porcine parvovirus, Escherichia coli, Mycoplasma hyopneumoniae, PRRSV and Aujeszky have to be applied in regions with large number of conventional swine farms. In European countries some of the above vaccinations have good results in prevention of diseases (Hämeenoja, 2001; Baumgartner et al., 2001). The preventive vaccinations programs are important especially in regions, where there are near to organic farms and other conventional swine farms.

Conclusion

Productivity growth in Greek organic pig farms can be increased by improving the living conditions of animals and the implementation of preventive measures (e.g., vaccination programs, treatments with phototherapeutics) that reduce mortality rates (especially during the winter months). Disease prevention is
a key point in organic livestock production and for this reason the application of a health management program in organic pig farming is suggested. Furthermore, to ensure food safety of organic products, monitoring risk factors for diseases and to control these risks factors as a means to prevent diseases, are suggested the implementation of health management based on Hazard Analysis Critical Control Point (HACCP).

Uticaj upravljanja zdravstvenim stanjem životinja na organskim svinjarskim farmama u Grčkoj

V.G. Papatsiros

Rezime

U Grčkoj, organsko stočarstvo je počelo u 2002. godini, i od tada su učinjeni značajni koraci u pravcu povećane zainteresovanosti grčkih potrošača za organske proizvode tokom poslednje decenije. Ovaj rad ima za cilj ažuriranje informacija koje se odnose na organsku proizvodnju svinja u Grčkoj, koje se odnose na proizvodni sistem i najvažnije i najčešće faktore zdravstvenih rizika. Takođe, u ovom istraživanju predlaže se program upravljanja zdravljem životinja.

Najčešći zdravstveni problemi koji se pojavljuju na grčkim farmama za organsku proizvodnju svinja su respiratorni problemi, gastrointestinalni problemi, problemi sa papcima i kožom, parazitske infekcije i mortalitet prasadi. Program upravljanja veterinarskim zdravljem životinja na organskim farmama za proizvodnju farmi, uključujući i korišćenje alternativa za antibiotike (prebiotici, probiotici i fitogenici), antiparazitika, odgovarajućih vakcina (npr. protiv *E. coli*, *M. hyo*, PRRSV) i adekvatni program dezinfekcije (npr. kontrola štetoca korišćenjem deratizacionih preparata) može biti koristan instrument za prevenciju i kontrolu bolesti, kao i za poboljšanje proizvodnih performansi i porasta.

Kao zaključak, povećanje produktivnosti na grčkim organskim farmama za proizvodnju svinja se može ostvariti poboljšanjem uslova života životinja i implementacijom preventivnih mera (npr., program vakcinacije, tretmani sa fototerapeuticima) koji smanjuju stopu smrtnosti (posebno tokom zimskih meseci). Iz tog razloga primena programa upravljanja zdravljem zapata je neophodna na organskoj farmi za proizvodnju svinja.

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Instruction for authors


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Example 1

**TABLE EGGS OF KNOWN ORIGIN AND GUARANTEED QUALITY - BRAND EGG**

Authors, Times New Roman, font size 12, bold

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Invited paper

Example 2

**THE EFFECT OF PARAGENETIC FACTORS ON REPRODUCTIVE TRAITS OF SIMMENTAL COWS**

M. D. Petrović¹, Z. Skalicki², V. Bogdanović², M. M. Petrović³

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Original scientific paper

use ¹,², ... numbers in suffix to refer to addresses of authors, under affiliations of authors should be mentioned e-mail of corresponding author and category of paper, Times New Roman, font size 9

Original scientific paper should contain following paragraphs with single spacing (title of paragraphs should be in Times New Roman 14 bold, except for Abstract and Key words where font size is 11 bold):

**Abstract:** 250 words
Key words: state key words (not more than 6)

Introduction - present the review of previous research and objective of the paper.

Materials and Methods - state methods applied in the paper.

Results and Discussion - present investigation results separately from discussion or together in one paragraph. Presentation of the results should be precise and without repetitions, and include the evaluation of significant differences and other parameters.

Text and titles of tables, figures and graphs, Times New Roman, font size 9, bold, in the following form:

Table 1. Least square means for the reproductive traits of cows

Tables and figures should be numbered and with adequate title and legend, width and height not exceeding 12 cm and 17 cm, respectively. Tables should be prepared according to instruction for forming of tables in Office Word. Each column in table must have heading and, when necessary, abbreviations should be explained in the legend/footnote.

Conclusion - containing the most important issues of the paper

Acknowledgment - for example:
Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 6885.

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Konzumna jaja poznatog porekla i garantovanog kvaliteta - brand jaja

Z. Pavlovski, Z. Škrbić, M. Lukić

Summary - should contain the most important issues of the paper. It should be in English, and Serbian for domestic authors (min. 250 words).

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5 – 7th October, 2011

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