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IS IT POSSIBLE TO OBTAIN ZERO ESTIMATES OF GENETIC DIVERSITY? A CASE STUDY OF THE NIGERIAN INDIGENEOUS GOAT BREEDS AT THE *β*-LACTOGLOBULIN GENE LOCUS

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Original scientific paper

Abstract: The current investigation was conducted to appraise the genetic diversity and genetic distance of three goat populations namely; Red Sokoto, Sahel and West African Dwarf (WAD), in Nigeria, making use of blood samples collected from 20, 20 and 20 individual from which blood DNAs were extraction, respectively. The DNAs extracted were used to study polymorphism at the β-lactoglobulin gene locus using RLFP-PCR process. Results revealed that the mean total number of alleles was 1 while the effective number of alleles was also 1. The percentage of polymorphic locus was 0% while Shannon's information index, observed homozygousity, expected heterozygosity, unbiased expected heterozygosity and inbreeding coefficient (F) were all observed to be 0.000. The pairwise Fst was 0.000 between all the breeds of goats. Variation within and between the populations of goats was 0% at p>0.05. The genetic distance between the goat breeds was 0.000. The present study revealed that RLFP-PCR may not be a powerful tool for the study of the β-lactoglobulin gene locus and hence other methodologies should be employed for a broader judgment on the genetic status of the goat population at the locus.

Key words: *β*-lactoglobulin, RLFP-PCR, genetic diversity, Nigerian indigenous goats and DNA.

Introduction

All over the world, indigenous small ruminant breeds such as goats are playing key roles in the lives of people *(Pollot and Wilson, 2009)*. In most economically emergent countries Nigeria inclusive, no serious consideration is given to sheep and goat genetic assets management policies *(Wilson, 1990)*.

Inadequacy or lack of these kinds of policies in most cases, have led to reduced outputs, unsystematic copulation and decline of genetic differences *(Kosgey et al., 2006; Groenevald et al., 2010)*. As a result of the inadequate national breeding programmes, animal genetic diversity records are inadequate in most developing countries *(Guimarães et al., 2007; FAO 2008)*.

The goat is a domesticated subspecies of the wild goat originating from Eastern Europe and southwest Asia (Hirst, 2008). It belongs to the lineage *Bovidae* and is very much interrelated to the sheep as they belong to the subfamily *Caprinae*. Food and Agricultural Organization *(FAOSTAT, 2008)* reported that there are 861.9 million goats worldwide out of which 33.8% are found in Africa *(FAOSTAT, 2008)*. Goats have specific weight ranges for each recognized breed which varies from 20 to 27kg for smaller goat does to over 140kg for males of bigger breeds for instance the Boer *(Taylor and Field, 1999)*. The different strains or bloodlines within the breeds also have diverse established sizes. Naturally, most goats have two horns which come in various sizes and shapes being breed dependent.

β-lactoglobulin is a protein found in mammals with the exception of the humans, rodent and lagomorphs milks. In mature cattle milk having a concentration of 3.2g/l, *β*-lactoglobulin represents roughly 10% of the combined milk proteins, and roughly 50-60% of the combined whey proteins. It is the most copious protein existing in the whey portion of sheep, goat and cattle milk *(Hinz et al., 2012)*. Owing to its great quantity and comparative simplicity of cleansing, bovine *β*-lactoglobulin has served as a brand protein for numerous biophysical studies of folding, stability and self-association. *β*-lactoglobulin is in the lipocalin family of proteins. The earliest reported atomic level resolution composition of *β*-lactoglobulin solved by X-ray crystallography for bovine *β*-lactoglobulin *(Papiz et al., 1986)*, revealed extraordinary resemblance to retinol-binding protein and led to the categorization of *β*-lactoglobulin as a lipocalin.

More than two heritable variants of *β*-lactoglobulin are recognized over the years with the most common variants labelled A and B *(Godovac-Zimmermann et al. 1996)*. These variants have identical amount of amino acids (162), but vary at positions 64 and 118 in two amino acids. At position 64 and 118, variant A has an aspartic acid residue and valine residue in that order whereas variant B has glycine in location 64 and alanine in location 118. The make up of *β*-lactoglobulin is composed of 15% α-helix, 50% β-sheet, and 15-20% reverse turn *(Sawyer and Kontopidis, 2000)*. Under physiological circumstances, *β*-lactoglobulin is in equilibrium amid monomers and non-covalent dimers. Protein concentration, pH, ionic strength, and temperature all have an effect on this equilibrium and as a result, the amount of monomers and non-covalent dimers in solution *(Mercadante et al., 2012)*. In spite of the structural similarities between variants A and B, quite a lot of dissimilarities occur in their physical and chemical properties especially as it relates to isoelectric point *(Yan et al., 2013)*, stability of native dimers *(Mercadante*

et al., 2012), thermal denaturation temperature *(Manderson et al., 1999),* denaturation reaction rate *(O'kennedy et al., 2006),* vulnerability to chemicals *(Bouhallab et al., 2004; Boye et al., 2004),* and attraction for fatty acids *(Loch et al., 2013).*

β-lactoglobulin unlike the other main whey protein of milk; α-lactalbumin, has no clear cut function. However, it is one of the whey proteins that has been most evaluated and is known to connect hydrophobic ligands like fatty acids or vitamins, suggesting a responsibility in the conveyance of retinol from the mother to the neonate in view of the fact that it is homologous with serum retinol-binding protein, and its capacity to bind retinol *in vitro (Puyol et al. 1991). McMeekin et al. (1949), Lišková et al. (2011)* and *Le Maux et al. (2012)* had all reported on the binding of sodium dodecyl sulfate (SDS) to native *β*-lactoglobulin. *β*-lactoglobulin has also been shown to demonstrate an ability to bind other hydrophobic ligands such as cholesterol, curcumin, fatty acid in addition to their derivatives, aromatic compounds, catechin, and cations. *β*-lactoglobulin has been shown to be able to bind iron *(Roth-Walter et al., 2014),* and thus might have a role in combating pathogens. The natural functions of the complicated protein/ligand are still exploratory. Assumed roles could be an enhancement in fatty acid assimilation *(Solène Le et al., 2014),* adjustment of the kinetics of the enzymatic hydrolysis of protein *(Mandalari et al. 2009),* safeguarding of susceptible ligands to counter oxidation or additional stresses *(Solène Le et al., 2014),* and adjustment of the bioaccessibility of the ligands *(Riihimäki-Lampén, 2009).* Furthermore, in food produce, the binding and biological properties of *β*-lactoglobulin /ligand complexes possibly will be affected by the constitution of *β*-lactoglobulin, and/or the occurrence of additional proteins with the capacity of contending with *β*-lactoglobulin for ligand binding.

Ballister et al. (2005) amplified and sequenced the leading six exons containing the whole coding section of the *β*-lactoglobulin gene in eleven goat breeds of Spain, France, Italy, Switzerland, Senegal and Asia in a bid to ascertain the different genetic variants. Recent studies on *β*-lactoglobulin polymorphism have also been carried out on Egyptian goat breeds; Barki, Damascus and their crossbred *(El-Hanafy et al., 2010)* and indigenous Ardi, Habsi and Harri goats of Saudi-Arabia *(El-Hanafy et al., 2015),* Honamli, Hair and Saanen goats of Turkey *(Özgecan et al., 2012),* and the small east African goat breeds; Samburu and Narok *(Lekerpes et al., 2014).* There is however a dearth of literature on similar works using the Nigerian indigenous goat breeds. This may have been brought about by the non-involvement in biotechnologically oriented researches as a result of their high cost, inadequate governmental incentives on research and general lack of interest *(Udeh, 2015).* This work was therefore embarked upon to provide information on the genetic diversity at the *β*-lactoglobulin gene locus of indigenous Nigerian goat breeds using RFLP- PCR methods.

Materials and Methods

Animals used for the experiment and their brief description

Blood samples were collected from 6O goats belonging to three different breeds (20 each from Sahel, Red Sokoto and West African Dwarf). The animals were sampled at two locations in Nigeria namely; Ibadan where the West African Dwarf goats were sampled, and the National Animal Production Research Institute (NAPRI), Shika, Zaria, Nigeria where the other three breeds were sampled. The WAD goat is evolved from the short-legged goats and is well adapted to the humid tropical conditions after many years of adaptation and natural selection *(Leak et al., 2002)*. The goats are predominantly raised under backyard systems. The Sahelian goat derives its name from the Sahel region of Africa and is most suited to desert or semi arid environments; it is intolerant of areas with high humidity. Populations of this goat breed can also be found elsewhere in the world in areas which provide suitable environments such as Australia *(Deneice, 2011)*. The Sokoto Red goat falls within the savannah group of goat with a somewhat diminutive size which infers probable cross breeding with forest or dwarf goats prior to selection in its current locale *(DAGRIS, 2007)*. The Red Sokoto goat is the predominant indigenous breed in the northern part of the country *(Onyenwe et al., 2005)*.

DNA extraction and RAPD-polymerase chain reaction

DNA was extracted from whole blood using a ZYMOBeadTM Genomic DNA kit (ZYMO Research Corporation). β-Lactoglobulin genotypes were identified as described by *Feligini et al. (1998)* and *Anton et al. (1999)*. In the first step, the 120 bp fragment of the goat β-lactoglobulin gene was amplified using forward primer 5-CAACTCAAGGTCCCTCTCCA-3 and reverse primer 5-CTTCAGCTCC TCCAGGTACA-3. PCR amplifications was performed in a reaction mixtures of 25 μL containing 12.5 μL of 2× PCR Master Mix (ZymoBIOMICS™ PCR PreMix), 0.5 μM of each primer, and 25-75 ng genomic DNA. Amplification was performed in a Biologix Thermal Cycler (TC1000-G), programmed for an initial denaturation at 95° C for 10 minutes, followed by 35 cycles each with denaturing at 93° C for 15 seconds, annealing at 60° C for 30 seconds, extension at 72° C for 30 seconds, and a final extension at 72° C for 10 minutes. In the second step, the 105 bp fragment of the goat β-lactoglobulin gene was amplified using forward primer 5-TCAGGACCCCGGAGGTGGACAAC-3 and reverse primer 5-CCTCCAGCTGGGTCGGGTTGAAG-3. The cycling programme began with an initial denaturation step (1 min at 94° C) followed by 30 cycles consisting of 15 seconds at 94° C, 1 minute at 60° C, 10 seconds at 72° C, and final elongation for 10 minutes at 72° C. The same PCR reaction mixtures used

in the first step was used for amplification. In both cases, PCR products (12 μL) were digested with 8 U of *Ras*a and 10 U of Mspl restriction enzyme in a 20 μL total reaction volume for 2 hours at 37° C. The restriction fragments was directly analyzed by electrophoresis using a 3 % agarose gel in 1× TAE buffer, stained with ethidium bromide, and visualized under Ultra Violet (UV) light to detect amplification.

Statistical analysis

The measurement of genetic diversity including number of alleles (Na), effective number of allele (Ne), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected heterozygosity (uHe), Shannon's information index (I), % polymorphic locus, fixation index (F), Analysis of Molecular Variance (AMOVA) and Nei's unbiased genetic distance *(Nei, 1978)* was estimated using GenAlEx 6.2 software *(Peakall and Smouse, 2008)*.

Results

Genetic diversity of β-lactoglobulin gene locus across Sahel, Red Sokoto and West African Dwarf goat

The various parameters of genetic differentiation at the β-lactoglobulin gene locus of indigenous Nigerian goats such as number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected heterozygozity (uHe), Shannon's information index (I) and fixation index (F) are presented in Table 1. The entire genetic diversity parameters analyzed resulted in zero (0) values, except the number of alleles (1).

Table 1. Genetic differentiation at the *β*-lactoglobulin Locus of three Nigerian goat breeds

Goat population	N	Na	Ne	I	Ho	He	uHe	F	%P
Sahel	20	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WAD	20	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Red Sokoto	20	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	20	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

SE = standard error, Na = number of alleles, Ne = number of effective alleles, I – Shannon's information index, Ho = observed heterozygosity, He = expected heterozygosity, uHe = unbiased expected heterozygosity, F = fixation index, %P = percentage of polymorphic locus, WAD = West African Dwarf.

The pairwise F-statistics (Fst) values measured for the three breeds of goat are presented in Table 2. The Fst value showed zero level genetic differentiation among the breeds. It was 0.00 between all the breeds of goats.

Table 2. Pairwise Fst values presented for different goat breeds

	Sahel goat	WAD goat	Red Sokoto goat
Sahel goat	0.00		
WAD goat	0.00	0.00	
Red Sokoto goat	0.00	0.00	0.00

Variation within and between populations of goat breeds was estimated using AMOVA (Table 3). The results revealed that no proportion (0 %) of the observed variance (0.00 at $p>0.01$) occurred both within the breeds and between the breeds.

Table 3. Summary of AMOVA table showing variation within and between goat populations

Source of variation	df	SS	MS	Estimate of variance component	% variation	P-value
Among population	2	1.172	0.00	0.00	0.00	0.00
Among individual	57	16.144	0.00	0.00	0.00	
Within animal	60	19.000	0.00	0.00	0.00	0.00
Total	119	36.316		0.00	0.00	

Genetic distance and identity of the three goat populations studied

The genetic distance of the indigenous goats is shown in Table 4. The result revealed zero (0) values for genetic distance between all the three goats examined. The genetic distance between the goat breeds indicated no genetic similarity or differences between the goats breeds studied. No cluster analysis based on Nei's standard distance matrix was obtained due to the monomorphic nature of alleles.

Table 4. Pairwise population matrix of Nei genetic identity and Nei genetic distance of the three goat breeds

	Sahel goat	WAD goat	Red Sokoto goat
Sahel goat	1	0.00	0.00
WAD goat	0.00	1	0.00
Red Sokoto goat	0.00	0.00	1

Discussion

The observed number of alleles (Na) at a locus and the genetic distance values (Table 1) indicate genetic differences at that locus and this will suggest the suitability of the locus to be used for the genetic analysis of diversity in goats. The observed number of alleles in this study suggests that the locus is not suitable for the genetic analysis of the three Nigerian goats; or, the methodology used might not have been appropriate particularly at the *β*-lactoglobulin gene locus. *Barker et al. (2001)* had opined that for studies of genetic diversity and genetic distance inside and between populations, microsatellite markers ought to have at least four alleles which might assist in reducing errors of estimation. This was not the situation in the present study and probably explains the results obtained. The effective number of alleles (Ne) was 1. The locus used to analyse the diversity in the Nigerian indigenous goats was not greatly enlightening making it not to be effective in genetic diversity studies. This is attributable to the actuality that if a locus has a PIC estimate < 0.5, that locus is said to be not polymorphic; a locus with PIC estimates ranging from 0.25 to 0.5 is said to be an average polymorphic marker *(Vanhala et al., 1998)*. In the present study, the locus was not polymorphic at all.

The observed mean heterozygosity value (0.00) was similar to the expected mean heterozygosity for all the investigated goats. This is in total disagreement with earlier reports on the domestic goat breeds *(Barker et al., 2001; Behl et al., 2003; Kumar et al., 2005; Aggarwal et al., 2007; Dixit et al., 2008; Kumar et al., 2009; Hassen et al., 2012; Hassen et al., 2016)*. Clearly, extensive bio-ecological selection for acclimatization to the different climatic conditions of the country and the existence of interbreeding due to free movement of animals for grazing and other purposes have not contributed to a large extent to the genetic diversity of the goats at the studied locus.

The population of goats used in this study was observed to be genetically similar (absence of genetic diversity) at the *β*-lactoglobulin gene locus (He = 0). This might be due to the monomorphic nature of the *β*-lactoglobulin gene allele at the gene locus. The high degree of similarity (no genetic distance) at the *β*-lactoglobulin locus encourages complete panmixis; that is, the three populations are interbreeding freely (complete sharing of genetic material). Genetic diversity is a measure of the degree of heterozygosity and allelic variation in a population. A low level of heterozygosity as observed in the present study may lead to genetic similarity (loss of genetic diversity). This zero level of heterozygosity can be caused by the occurrence of null allele which is the allele that fails to proliferate during polymerase chain reaction with a given genetic marker primers site *(Pemberton et al., 1995)*. When the sample size is not large enough, the Wahlund effect (the incidence of lesser quantity of heterozygote in the population than is expected because of subdivision of the population), and inbreeding *(Kumar et al.,*

2006) could also lead to this situation. Low degree of heterozygosity might also be expected if species are isolated with consequent deficit of unexploited genetic capability, while a high level of mean heterozygosity at a locus possibly will be expected to associate with high levels of genetic variation at locus with significant value for adaptive response to environmental changes *(Kotzé and Muller, 1994)*.

The Shannon information index revealed no genetic diversity across the populations. The Shannon information index of the Nigerian goat breeds is much lower than the 3.5 set for high species evenness and richness *(Krebs, 1989)*. This means that the goats have very low species richness and evenness at the *β*-lactoglobulin gene locus. This low species richness could be associated with very high level of heterozygote deficiency among the goats population sampled and this could be ascribed to the system of management in use by the goats farmers *(Mukesh et al., 2006)*. The mean Shannon's index (I) of 0.00 is an indication that equitability in the genetic distribution of the Nigerian indigenous goat breeds has been seriously disturbed and eroded hence exhibiting very low genetic diversity. Low amount of genetic diversity has been reported to increase susceptibility of populations to devastating situations like disease and pest outbreaks, and also the expression of negative and disadvantageous alleles or even, loss of over-dominance *(Bizhan et al., 2010)*.

The populations' genetic differentiation was studied base on fixation indice (F). The within breed deficit or excess in heterozygosity value was assessed by the inbreeding coefficients which was 0.00. The locus having positive values (even though zero), indicates that the goat population is just at equilibrium with there being no more than expected number of homozygotes or heterozygotes in the goat breeds studied. The average F revealed that the majority of the overall genetic variation did not correspond to any difference between individuals within goat populations. Elevated F values means that there is significant extent of inbreeding and genetic differentiation between goat populations. The goat populations studied are almost slipping into that. According to *Wang (1996)*, estimates of inbreeding of less than 0.5 or nearer to zero may have occurred because of the absence of mating among close relations and/or within individuals. The values obtained in this study at the locus studied reflect the presence of close relative matings. The results therefore disagrees with those observed for Asian goats *(Barker et al., 2001)*, Indian goats *(Kumar et al., 2005; Aggarwal et al., 2007; Dixit et al., 2008; Dixit et al., 2010)* and indigenous goats of Albanian *(Hoda et al., 2011)*.

The analysis of molecular variance revealed that 0% of all the dissimilarity was present amongst and within the goat populations making the result to be at variance with the partitioning of variance reported *(Vahidi et al., 2014)*. Although the Nigerian goat population's genetic dissimilarity was computed using molecular data, no genetic distance was really observed between the three goats at the locus studied. This made it impossible to generate or create a phylogenetic tree.

Conclusion

The result of this study probably describes the first endeavour to evaluate the molecular genetic differences of the Nigerian indigenous goat populations at the *β*-lactoglobulin locus. The results reveal the presence of very low or no genetic difference among the goat population at the *β*-lactoglobulin locus and such low or zero variation within breed will not provide an excellent basis for designing genetic improvement programme.

Da li je moguće dobiti nultu procenu genetičke raznovrsnosti? Studija slučaja nigerijskih autohtonih rasa koza na lokusu gena β-laktoglobulinskog

Emeka A. Ezewudo, Geka R. Abubakar, Stephen Sunday A. Egena, Olushola J. Alabi

Rezime

Ispitivanje je sprovedeno radi procene genetičke raznovrsnosti i genetske udaljenosti kod tri populacije koza: Red Sokoto, Sahel i zapadno-afričke patuljaste rase (West African Dvarf WAD), u Nigeriji, koristeći uzorke krvi prikupljene od 20, 20 i 20 pojedinačnih grla iz kojih su krvne DNK ekstrahovane, respektivno. DNK ekstrahovana korišćena je za ispitivanje polimorfizma na lokusu β-laktoglobulinskog gena koristeći RLFP-PCR proces. Rezultati pokazuju da je srednji ukupan broj alela bio 1, dok je efektivni broj alela takođe bio 1. Procenat polimorfnog lokusa bio je 0%, dok je Shannonov indeks informacija registrovao homozigotnost, očekivana heterozigotnost, nepristrasnu očekivanu heterozigotnost i koeficijent inbridinga (F), svi na 0.000. Parni Fst je bio 0.000 između svih vrsta koza. Varijacija unutar i između populacije koza iznosila je 0% kod p> 0,05. Genetska udaljenost između rasa koza iznosila je 0.000. Ova studija je otkrila da RLFP-PCR možda nije moćan alat za proučavanje lokusa β-laktoglobulinskog gena i stoga bi trebalo koristiti druge metodologije za širu procenu genetičkog statusa populacije koza na lokusu.

Ključne reči: β-laktoglobulin, RLFP-PCR, genetička raznovrsnost, nigerijske autohtone koze, DNK.

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FERTILITY TRAITS OF AUTOCHTHONOUS BREEDS OF MANGALITSA, MORAVKA AND RESAVKA

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Abstract: Objective of this paper was to evaluate phenotypic variability of fertility traits of indigenous breeds of Mangalitsa (Swallow-Belly Strain Mangalitsa–SBSM), Moravka breed (M) and Resavka (R). Indigenous pig populations are usually constituted by a quite low number of active boars and sows. Their pedigree information is lacking or absent, complete phenotypic description is usually not available for most of these populations that are very well adapted to specific local agro-climatic environments. In controlled herds in period of four years, the average age at first farrowing (AFF) was 18.5 months with large variability of 5.9 months for Swallow belly Mangalitsa. Less AFF (14.3 months) and less variability (4.4 months), in relation to SBSM, determined for Moravka breed while for Resavka determined age of 14.7 months with at least variability for this trait from 4.4 months. The average number of piglets born alive (for a period of four years) of SBSM was the lowest from 4.37 to 4.81; in case of M and R, this value was significantly ($P<0.001$) higher (from 5.75 to 8.17 and for R breed 6.40 to 9.00). With average duration of suckling period (duration of lactation - DL) of 52.92 days in the first year for the breed SBSM with the lowest number of reared piglets (NRP=4.10) whereas the DL for M and R breeds was shorter (from 45.75 to 52.03 day) with a higher NRP (from 4.57 to 8.92 reared piglets).

Key words: pig, indigenous breeds, reproductive parameters, parity, piglets

Introduction

Pig production in the Republic of Serbia has a long tradition. During the 19th century, pigs were the main export product. In Republic of Serbia Šiška is our

most primitive breed of pigs, created by domestication of wild pigs, it most resembles a wild boar, not only in appearance and features, but also lifestyle. Šiška once had enormous significance, in the relatively recent past (eighteenth century), not only in Serbia but also Croatia, Slovenia, Hungary, Romania and Bulgaria. In the 19th century Šiška pushed Šumadinka. Šumadinka is improved Šiška reared in slightly better conditions. Both of these breeds are permanently lost in their original form. Šumadinka which served as the basis for creating Mangalitsa. Today in Serbia there are three local indigenous pig breeds: Mangalitsa, Moravka and Resavka breeds. Mangalitsa was very popular in Vojvodina (especially in Srem) and Hungary in the period from the 19th century until the fifties, and recently farming of this breed has been restored. In the Republic of Serbia there are three Mangalitsa breed strains, the Swallow-belly Strain (Srem Black Mangalitsa or Bođanovci pig), white and Subotica strain. In Hungary and Romania there is also so called red strain of this breed. Swallow belly Mangalitsa developed in the area of Srem (near Ruma, village of Bođanovci, the residents of this village in Srem are called "Lasans"). It is late maturating breed. The sexual maturity is reached at the age of 8-12 months, breeding maturity at the age of 15-18 months, and growth stops at the age of 3-4 years. Reproductive ability is poorly expressed. Mangalitsa is typical fatty pig breed, it has in carcass sides 65-70% of fat and approx. 28 % meat (*Petrović et al., 2009*). Moravka and Resavka, were reared in the same region of Serbia around rivers Velika Morava, Mlava and Resava. Moravka and Resavka are breeds of combined production abilities. Moravka was created by crossing Šumadinka and Berkshire (unplanned) while the Black Slavonian pig breed was created deliberately. It was completely black without any marks. Resavka and Moravka breeds were created simultaneously, in a similar manner, but in smaller numbers. The only difference is the colour, as the Moravka is black, Resavka breed pigs are spotted (white-yellow-black). It was created as a result of non-systematic crossing with Šumadinka, Berkshire and Yorkshire for whom there are no relevant data. Moravka and Resavka is more fertile with litters of 6 to 8 piglets (*Živković and Kostić, 1952; Petrović et al., 2007a*) and meatier than Mangalitsa (30.00 : 24.85 % meat; *Petrović et al., 2007b*). Also Moravka and Resavka are in danger of being extinct, which means that it is necessary to work on their preservation and sustainable use. It is known that by extinction of one breed or strain also the genetic diversity contained within them is lost. Importance of these breeds reflects in genes which provide excellent ability of adapting to breeding conditions, good vitality and resistance to diseases. The breed is very resistant and well adapted to extensive housing conditions, animals of this breed need only a simple shelter from rain and snow.

Material and Methods

Investigation included sows three registered native, autochthonous breeds: Swallow-Belly Strain Mangalitsa (SBSM; n=356), Moravka (M; n=93) and Resavka (R; n=45). Of the reproductive traits, were monitored in controlled herds for a period of four years, are included the average age at first farrowing (AFF), number of live born piglets (NLB), number of stillborn piglets (NSB), duration of suckling period (DL) and number of weaned piglets (NWP). Determination of the status of vulnerability is one of the important indicators of the state of the locally adapted breeds in the Republic of Serbia. Defining the status of endangered breeds depends on numerous factors, including: the number of reproductive-age males and females, level of breeding in relationship, the effects of reproduction and population trends. Calculation of the effective population size (Ne), is carried out according to the formula:

$Ne = 4NmNf / N$

Where:

Ne - effective population size

Nm - number of reproductive-age males

Nf - number of reproductive-age females

N - total number of reproductive-age individuals

Data on numbers of Mangalitsa, Moravka and Resavka obtained from Main breeding organization.

Results and Discussion

In Table 1, we see that the age at first farrowing varied highly statistically ($P<0.001$) between breeds while in regard to the number of live born and reared piglet, highly statistically significant difference was found with respect to fertility of Mangalitsa breed. No differences ($P>0.05$) were established between Moravka and Resavka for the mentioned traits. Also no differences were determined between the studied breeds in the number of stillborn piglets and duration of suckling period.

Table 1. Reproductive traits of indigenous breeds

Breed/Trait	Age at first farrowing	Number of live born piglets	Number of stillborn piglets	Duration of suckling period	Number of reared piglets
Mangalitsa; n=356	556 ±176.65[a]	4.65 ±1.62[a]	0.30 ±0.77	50.04 ±6.03	4.33 ±1.74[a]
Moravka; n=93	428 ±132.46[b]	7.31 ±2.52[b]	0.49 ±0.94	48.73 ±9.08	7.11 ±2.67[b]
Resavka; n=45	441 ±87.86[c]	7.96 ±1.76[b]	0.11 ±0.11	48.11 ±10.48	6.96 ±3.01[b]

a, b, c Means in column with different superscript are significantly different at $P<0.001$

For the fertility traits, by years, a statistically significant difference was found only for the duration of lactation ($P<0.05$), while for other traits no statistically significant difference was recorded.

Table 2. Mangalitsa fertility traits by years of study

Year/Trait	Number of live born piglets	Number of stillborn piglets	Duration of suckling period	Number of reared piglets
I	4.37±1.36	0.33±0.47	52.92± 2.56	4.10±1.42
II	4.56±1.63	0.31±0.88	50.73±6.52	4.37±1.53
III	4.81±1.59	0.34±0.87	49.38±7.29	4.26±2.00
IV	4.74±1.75	0.24±0.63	48.34±4.17	4.49±1.81

Table 3. Moravka fertility traits by years of study

Year/Trait	Number of live born piglets	Number of stillborn piglets	Duration of suckling period	Number of reared piglets
I	6.00±3.24	0.92±1.27	52.03± 4.47	6.00±3.24
II	5.75±2.08	0.88±1.36	51.25±14.25	5.38±2.53
III	9.08±1.68	0.25±0.45	45.92±2.71	8.92±1.62
IV	8.17±2.08	0.42±0.67	46.83±3.41	8.08±1.98

Table 4. Resavka fertility traits by years of study

Year/Trait	Number of live born piglets	Number of stillborn piglets	Duration of suckling period	Number of reared piglets
I	6.71±1.60	0.44±0.68	50.00±5.33	4.57±2.82
II	9.14±1.35	0.14±0.38	46.14±10.78	7.86±3.72
III	9.00±1.20	0.13±0.35	45.75±3.20	8.88±1.36
IV	6.40±0.89	0.36±0.73	52.00±2.35	6.00±1.73

In regard to parities, a statistically significant difference was established for the mentioned traits, except for number of stillborn piglets.

Table 5. Mangalitsa fertility traits by parity

Parity/Trait	Number of live born piglets	Number of stillborn piglets	Duration of suckling period	Number of reared piglets
1.	3.67±1.44	0.48±1.11	51.49±6.10	3.25±1.61
2.	4.45±1.53	0.26±0.73	49.23±6.37	4.28±1.65
3.	4.90±1.83	0.41±0.73	48.43±6.33	4.59±2.06
4.	4.72±1.47	0.23±0.54	49.19±5.04	4.49±1.59
5.	5.21±1.64	0.14±0.36	48.89±4.40	4.89±1.37
6.	5.36±1.26	0.74±1.15	49.32±3.93	5.14±1.28
7.	5.37±1.38	0.26±0.56	51.95±5.73	5.05±1.22
8.	5.79±1.25	0.37±0.27	55.00±6.92	4.93±1.98
9.	5.28±1.18	0.50±1.04	50.61±7.37	4.72±1.45

When we talk about autochthonous breeds most research was in the field of traits of growth and carcass quality at least in the area of reproduction. Mangalitsa is domestic primitive breed, created from the former Šumadinka and is "fatty" pig breed in regard to the production type. Mangalitsa gives on average of 4.60-6.64 piglets (*Petrović et al., 2013; Egerszegi et al., 2003*), with strong maternal instincts. In our research, Swallow-Belly Strain Mangalitsa (SBSM) and Moravka (M) had higher age and greater variability of the first parity compared to data presented in the Annual Report of the Institute for Animal Husbandry (2015) for SBSM=508.92 ± 127.56 days and M = 373.29 ± 10.19 days. Results obtained in the experimental farm of the Faculty of Agriculture in Zemun (*Belić, 1951*) show the average fertility of SBSM from 4.42 to 5.80 piglets in the period from 1945 to 1950, which is in accordance with our research and research of *Petrović et al. (2013).* In relation to our research, significantly higher average fertility (6.64 piglets) in 74 SBSM litters have been determined by *Egerszegi et al. (2003).* Moravka and Resavka are breeds of combined production abilities with more liveborn piglets and meat in carcass sides. Sexual maturity of gilts is possible at the age of 5-6 and breeding maturity at the age of 10-12 months. According to our research on Moravka breed, average value and the variation in fertility (M = 7.20 ± 2.04 piglets) were found in the study *Petrović et al. (2007).* Increased fertility (7.82 ± 2.06 and 8.17 ± 2.08 live born piglets) according to research of *Živković and Kostić (1952)* and in *Annual Report of the Institute for Animal Husbandry (2015).* In comparison with our results for the same length of suckling period (50 days) and Annual Report of the University of Belgrade - Faculty of Agriculture (2010) a larger number of weaned pigs (4.92 ± 2.24 pigs reared in 24 litters) is reported. *Petrović et al. (2013),* for longer duration of suckling period, report less reared piglets (4.09 ± 1.91reared piglets) compared to our research. For Resavka breed, when it comes to fertility, we have very little data, i.e. there is only one recent report (*Annual Report of the Institute for Animal Husbandry, 2015*), the research of *Živković and Kostić (1952),* and *Lalević (1952)* as well as a textbook by Belić (1951) which state that the Resavka is characterized by good fertility, with 6 - 8 piglets. In our study, an increasing number of piglets was determined in 37 litters, compared to data stated in the *Annual Report of the Institute for Animal Husbandry (2015)* for 5 litters stating average 6.40 live born piglets and 6.00 reared piglets with suckling period of 52 days.

In-situ protection includes the preservation, maintenance or recovery of populations and species in their natural habitats. Endangered species can be protected only if their natural habitats are protected, to which they are inextricably linked. On the basis of the Law (*Official Gazette of the Republic of Serbia,* 2015) on incentives in agriculture and rural development is defined maximum amounts of incentives per head for breeding gilts, boars and sows of Mangalitsa, Moravka and Resavka. The tendency is that the number of breeders of indigenous breeds of pigs is increasing. Observing the the degree of endangered (data obtained from the main

breeding organizations in the Republic of Serbia) locally adapted breeds of pigs have the status of highly endangered (Mangalitsa FAO / EAAP - high risk; Ne = 174.68) and status of critically endangered populations (Moravka FAO / EAAP - critically endangered; Ne = 13.71 and Resavka FAO / EAAP - critically endangered; Ne = 2.66). *Ex – situ* protection of endangered species does not exist in Serbia.

Conclusion

The age at first farrowing varied highly statistically between breeds while in regard to the number of live born and reared piglet, highly statistically significant difference was found with respect to fertility of Mangalitsa breed. No differences were established between Moravka and Resavka for the mentioned traits. Also no differences were determined between the studied breeds in the number of stillborn piglets and duration of suckling period. For the fertility traits, by years, a statistically significant difference was found only for the duration of lactation ($P<0.05$), while for other traits no statistically significant difference was recorded. The result was expected because it is the preservation of animal resources.

In the recent decades Europe one number of indigenous breeds pig is lost. Local pig populations are usually constituted by a quite low number of active boars and sows, pedigree information is lacking or absent, complete phenotypic description is not available for most of these populations that are very well adapted to specific local agro-climatic environments. However, only few cases of successful local breed chains exist in Europe, importance of these breeds reflects in genes which provide excellent ability of adapting to breeding conditions good vitality and resistance to diseases, and the vast majority of them remain an untapped potential. In terms of scientific substantiation, their performances and products are practically untapped and market potential of their products unexploited. Due to the extremely slow weight gain and high feed conversion, breeding of mentioned indigenous breeds may be economical justified only if the breeders are in the organic production system (applied pasture feeding system during most of the year as it was in the past) and receive state subsidy for this kind of production and preservation of the genes. It is necessary as soon as possible to form Gene bank and have *Ex – situ* protection of endangered breeds of pigs. Locally adapted breeds of pigs have the status of highly endangered and status of critically endangered populations. For their protection the following measures should be taken:

- continuous monitoring of trends and population structure,
- strict implementation of the breeding program and program of selection measures

- continuation of activities on the identification and description of the breed, of production characteristics and molecular-genetic typing,
- to establish gene bank and provide continuous storage of genetic material,
- intensive activities on the promotion of breed and their products,
- improve animal recording for these breeds
- to develop conservation programs by integrating with the conservation programs within the "*agro-forestry*" system.

Plodnost autohtonih rasa mangulice, moravke i resavke

Čedomir Radović, Milica Petrović, Nenad Katanić, Dragan Radojković, Radomir Savić, Marija Gogić, Nenad Terzić

Rezime

Cilj ovog rada je bio da se utvrdi fenotipska varijabilnost osobina plodnosti autohtonih rasa mangulice (lasasta mangulica-SBSM), moravke (M) i resavke (R). Autohtone populacije svinja obično čini prilično mali broj aktivnih nerasta i krmača. Informacije o poreklu često nedostaju ili su nedostupne, kompletan fenotipski opis obično nije dostupan za većinu ovih populacija koje su vrlo dobro prilagođene specifičnim lokalnim agro-klimatskim uslovima. Kod kontrolisanih zapata, u periodu od četiri godine, prosečan uzrast pri prvom prašenju (AFF) iznosio je 18,5 meseci sa velikom varijacijom od 5,9 meseci kod lasaste mangulice. Manje vrednosti AFF (14,3 meseca) i manja varijabilnost (4,4 meseca), u odnosu na SBSM, utvrđen je za moravku, dok je za resavku utvrđen uzrast od 14,7 meseca sa najmanjom varijabilnošću za ovu osobinu od 4,4 meseca. Prosečan broj živo rođene prasadi (u trajanju od četiri godine) kod SBSM, je bio najniži od 4,37 do 4,81; kod svinja M i R, ova vrednost je značajno (P <0,001) viša (od 5,75 do 8,17 i 6,40 do 9,00 respektivno). Prosečno trajanje perioda sisanja (trajanje laktacije - DL) od 52,92 dana u prvoj godini za rasu SBSM, sa najnižim brojem odgajenih prasadi (NRP = 4,10), dok je DL za M i R rase kraći (45,75 i 52,03 respektivno) sa većom vrednošću NRP (od 4,57 do 8,92 odgajanih prasadi).

Ključne reči: svinje, autohtone rase, reproduktivni parametri, paritet, prasad

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BLOOD PARAMETERS, CARCASS AND MEAT QUALITY OF SLAUGHTER PIGS WITH AND WITHOUT LIVER MILK SPOTS

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Abstract: The aim of this study was to determine the influence of liver milk spots on hematological, carcass and meat quality parameters in slaughter pigs. A total of 120 pigs with a live weight of approximately 115 kg and six months old were examined. Any signs of liver milk spots were recorded as present or absent according to *Welfare Quality® protocol (2009)*. A complete blood picture was investigated. The following carcass quality parameters were measured: live, hot and cold carcass weights, dressing percentage, backfat thickness and meatiness. pH and temperature measurements were performed 45 minutes postmortem. Pork quality classes (PSE – pale, soft and exudative, normal, DFD – dark, firm and dry meat) were determined according to *Adzitey and Nurul (2011)* using pH_{45} value. Pigs with liver milk spots had significantly higher middle-sized cell count (monocytes, eosinophils, and basophils) and neutrophils count, but significantly lower red blood cell count, hemoglobin concentration, hematocrit and MCV than unaffected pigs. The same group of pigs had significantly lower live weight, hot carcass weight, cold carcass weight, dressing percentage and meatiness compared to the pigs free of milk spot lesions. Pigs showing liver milk spots had significantly higher pH_{45} value and incidence of DFD meat than pigs without pathological lesions in the livers. In conclusion, assessment of liver milk spots at slaughter line has potential to serve not only as an indirect measure of pig health and welfare, but also for the carcass and pork quality.

Key words: DFD meat, liver milk spots, slaughter pigs, slaughterhouse

Introduction

Although a number of studies have previously reported that ascariasis results in significant economic losses to the pig industry, the occurrence of this helminth infection both under conditions of intensive and extensive pig breeding remains high (*Vlaminck et al., 2015*). This can be ascribed to the facts that, in most cases, ascariasis occur in a subclinical form and that exposure of animals to this parasite cannot be unambiguously diagnosed (*Vlaminck et al., 2015*). Ascariasis can be identified in slaughtered pigs through the presence of milk spots – whitish healing foci which result from *Ascaris suum* larval migration through the liver stroma (*Sanchez-Vazquez et al., 2012*). Liver milk spots, along with pneumonia and pleurisy, are the most frequent pathological lesions in pig organs observed at the slaughter line (*Sanchez-Vazquez et al., 2011, 2012; Čobanović et al., 2015, 2016a*). It has been reported that housing conditions (e.g. outdoor/indoor farming, floor type, type of bedding, high stocking density), management practices (e.g. cleaning and disinfection procedures, type of feeding) and seasonal variations (e.g. temperature and relative humidity) play an important role in the development of *Ascaris suum* and in the subsequent presence of milk spots in the liver (*Sanchez-Vazquez et al., 2010*). The presence of *Ascaris suum* infection and liver milk spots can lead to the following negative effects: (1) farm economic losses attributed to decreased daily weight gain, anthelmintic treatment costs, depressed growth rates and feed conversion efficiency (*Sanchez-Vazquez et al., 2010*), (2) changes in hematological values (*Makinde et al., 1996; Zanga et al., 2003; Wieczorek et al., 2006*), (3) slaughterhouse operator losses due to trimmings and disposal of organs unsuitable for human consumption (*Pyz-Lukasik and Prost, 1999*), (4) lower carcass and pork quality (*Theodoropoulos et al., 2004; Knecht et al., 2011, 2012*).
A number of studies revealed that pork quality is affected by many different factors, such as feeding, slaughter weight and gender, pre-slaughter and slaughter conditions (*Šefer et al., 2015; Rocha et al., 2016; Đorđević et al., 2016; Čobanović et al., 2016b, 2016c*). However, only one published article is available in the literature about the relationship between liver milk spots and pork quality (*Theodoropoulos et al., 2004*). Therefore, the aim of this study was to determine the impact of liver milk spots on hematological, carcass and meat quality parameters in slaughter pigs.

Material and Methods

A total of 120 slaughter pigs with an average live weight of approximately 115 kg and about six months old were examined. All the animals were of the same breed (Yorkshire x Landrace crossbreeds) and fattened on the same commercial

farm under identical conditions. They were all exposed to the same condition of pre-slaughter treatments and were killed at the same slaughterhouse

The livers of slaughtered pigs were removed from the slaughter line and visually appraised and palpated for milk spots according to the *Welfare Quality® protocol (2009)*. The complete assessment of liver milk spots scores was performed by a single trained investigator. The percentage of affected livers was calculated as the percentage of pig livers on which was detected the presence of at least one milk spot lesion.

Immediately after the onset of bleeding, blood samples were collected from each pig. They were kept refrigerated (4±1°C) until processed immediately on arrival at the laboratory. The vacutainers (2 mL) coated with EDTA were used to measure hematological parameters including white blood cells, lymphocytes, middle-sized cells (monocytes, eosinophils and basophils), neutrophils, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelet count. The proportions of lymphocytes, middle-sized cells and neutrophils were calculated as a percentage of leukocyte concentration on the same device. The indicators of the hematological profile were analyzed by an automatic hematological analyzer Abacus junior vet (Diatron MI PLC, Hungary).

The carcases were weighed immediately after splitting and final washing to obtain the hot carcass weight and re-weighed 24 hours after chilling at 4°C to determine the weight of the cooled carcass. The dressing percentage was calculated as: (hot carcass weight ÷ live weight) x 100. Carcass backfat thickness was measured with a metal ruler (accuracy of 1.0 mm) at two points (between the 13th and 15th dorsal vertebrae - fat carcass thickness on the back; and over M. *gluteus medius* - fat carcass thickness at the sacrum). Meatiness (%) was calculated according to *Official Gazette (1985)* based on hot carcass weight and the sum of carcass fat thickness on the back and at the sacrum. The pH and temperature of the M. *longissimus dorsi* were measured 45 minutes after slaughter on the left half of the carcass at the level of the 10th and 11th ribs using a pH-meter “Testo 205” (Testo AG, Lenzkirch, Germany). Pork quality classes (PSE meat, normal meat, DFD meat) were determined according to *Adzitey and Nurul (2011)* using pH_{45} value. The carcasses showing pH_{45} values lower than 6.0 were classified as PSE meat, while the carcasses showing pH_{45} values higher than 6.4 were classified as DFD meat. The carcasses with pH_{45} between 6.0 and 6.4 were classified as normal pork quality.

Statistical analysis of the results was conducted using software SPSS version 23.00 for Windows (*SPSS, 2015*). According to the presence of liver milk spots, the pigs were allocated to two groups: 1) the group of pigs with liver milk spots (n=69) and 2) the group of pigs without liver milk spots (n=51). Student t-test was used to examine the effect of liver milk spots on the hematological, carcass and meat quality parameters. Data were described by descriptive statistical parameters as the

mean value and standard error of the mean. The distribution of pork quality classes in relation to the liver milk spots was determined by Fisher's exact test. A value of $P<0.05$ was considered significant.

Results and Discussion

From a total of 120 examined pig livers, milk spots were detected in 57.50%. The prevalence of livers affected by milk spots was higher to that found in comparable studies (*Dalmau et al., 2009; Rocha et al., 2016; Dalmau et al., 2016*). In addition, the results of our investigation found that the percentage of milk spots in pig livers exceeded the alarm threshold of 23% set for this health criterion by the *Welfare Quality® protocol (2009)*. Pathological lesions detected at the slaughter line, such as liver milk spots, are often related to suboptimal production systems (*Harley et al., 2012*) and indicate a serious health and welfare problem on the farm of origin (*Welfare Quality® protocol, 2009*). According to *Pyz-Lukasik and Prost (1999),* in mild cases (from 1 to 7 lesions) milk spots may be removed from the liver and the remaining organ and meat may be approved for human consumption. On the other hand, in the case of more than 8 milk spots occurring, the livers should be condemned (*Pyz-Lukasik and Prost, 1999*). However, other researchers (*Cugmas et al., 2013; Fausto et al., 2015*) suggest that the livers with milk spots, regardless of their number, should be deemed unfit for human consumption. Hence, meat industry suffers indirect economic losses through increased trimmings and disposal of livers, a byproduct that adds value to the supply chain (*Harley et al., 2012; Fausto et al., 2015*). Even though the losses due to milk spots are very variable because of different slaughterhouse costs and the fluctuating market prices of livers, the damage can be assessed at €0.26 per kg to destroy the livers and €0.87 per liver lost (*Kanora, 2009*). Moreover, the financial loss to the US pig industry due to increased fees to gain ratio was estimated to US$ 155 million annually (*Stewart and Hale, 1988*). In addition, *Stewart (2001)* estimated the financial loss of US$ 17.5 million as a result of liver condemnation at slaughter line as well as an additional economic loss of US$ 60.1 million for extra feed to finish pigs for slaughter.

The effects of liver milk spots on hematological parameters in slaughter pigs are shown in Table 1. Pigs with liver milk spots showed increased values of middle-sized cells (monocytes, eosinophils and basophils), neutrophils and the percentage of middle-sized cells compared to the pigs without pathological lesions in the livers ($P<0.05$). These results correspond to the findings of *Wieczorek et al. (2006),* who observed leukocytosis and eosinophilia in fattening pigs caused by the presence of *Ascaris suum* infection. Moreover, *Kalai et al. (2012)* point out that parasitic infections lead to necrosis and subsequently to neutrophilia and eosinophilia. In addition, pigs with liver milk spots had a significantly lower number of red blood

cells, hemoglobin concentration, hematocrit and MCV (P<0.05, Table 1). Similar results were reported by *Makinde et al. (1996)*, who found that liver milk spots, alone or in combination with pneumonia, induced changes in hematological values, such as reduced red blood cell count, hemoglobin concentration, hematocrit and MCV. Furthermore, *Zanga et al. (2003)* reported that the presence of *Ascaris suum* infection in fattening pigs decreased the red blood cell count, hemoglobin concentrations, hematocrit, MCV, MCH and MCHC. Therefore, hematological alterations in slaughtered pigs associated with liver milk spots are presumably connected with a recent uptake of infective *Ascaris suum* eggs.

Table 1. Mean values (±standard error of the mean) of hematological parameters according to liver milk spots (n=120).

Parameter	Milk spots	No milk spots	Significance
Number of pigs	69	51	-
White blood cells (10^9/L)	21.94±0.74	20.4±0.71	ns
Lymphocytes (10^9/L)	13.92±0.49	14.16±0.69	ns
Middle-sized cells (10^9/L)	0.27±0.04	0.12±0.01	*
Neutrophils (10^9/L)	7.77±0.58	6.10±0.38	*
Lymphocytes (%)	64.90±1.75	67.95±2.03	ns
Middle-sized cells (%)	1.31±0.22	0.59±0.01	*
Neutrophils (%)	33.80±1.71	30.09±1.65	ns
Red blood cells (10^{12}/L)	7.22±0.14	7.95±0.13	*
Hemoglobin (g/L)	132.80±2.07	146.40±2.12	*
Hematocrit (%)	39.37±0.43	40.76±0.57	*
MCV (fl)	49.28±0.38	51.10±0.49	*
MCH (pg)	17.96±0.14	18.04±0.12	ns
MCHC (g/L)	359.60±1.10	360.50±1.33	ns
Platelet count (10^9/L)	244.00±15.05	248.40±15.81	ns

Legend: **MCV** – Mean corpuscular volume; **MCH** – Mean corpuscular hemoglobin, **MCHC** – Mean corpuscular hemoglobin concentration.
***** – P<0.05; **ns** – no significance (P>0.05).

The effects of liver milk spots on carcass and meat quality parameters and pork quality classes are depicted in Table 2. Pigs showing liver milk spots had significantly lower slaughter, hot and cold carcass weights, and dressing percentage compared to the pigs free of milk spot lesions (P<0.05), which is consistent with the findings of *Hale et al. (1985)* and *Theodoropulos et al. (2004)*. It has been reported that the occurrence of a subclinical form of *Ascaris suum* infection in fattening pigs leads to a decrease in daily weight gain of about 80 g and increase in feed consumption of 230 g on 1 kg of body weight gain (*Knecht et al., 2012*), resulting in later date of slaughter weight attainment of 10–15 days (*Knecht et al., 2011*). Considering that milk spots can appear as early as 3 days post-infection, start to resolve after about 2–3 weeks (*Vlaminck et al., 2015*), and disappear in the course of 3–6 weeks post-infection (*Boes et al., 2010*), it can be assumed that the

reduction in slaughter weight occurred during the last few weeks of fattening. In addition, pigs showing liver milk spots had a significantly higher fat thickness, but had a lower meatiness than pigs without pathological lesions in the livers (P<0.05, Table 2). *Knecht et al. (2011)* examined the influence of gastrointestinal parasites, including *Ascaris suum*, on the percentage of meat, and demonstrated that pigs free from parasites had a significantly higher meatiness than infected animals (53.68% vs. 52.12%). Moreover, the same researchers reported that meat obtained from fattening pigs with gastrointestinal parasites, such as *Ascaris suum*, was of a lower class compared to the pigs free from parasites. Parasitic infection, even in subclinical form (i.e. no apparent clinical signs), decreases feed intake and assimilation (*Jankowska-Mąkosa and Knecht, 2015*), and negatively affects digestion and the intestinal absorption of nutrients (*Hale et al., 1985; Kanora, 2009*). Hence, when animals are infected with parasites, they ingest fewer nutrients than what is necessary for the maximum expression of their genetic potential for protein deposition (*Kipper et al., 2011*). In addition, instead of utilizing nutrients to increase in body mass, parasitic infection leads to a reduction in the muscle and fat tissue synthesis and increases their degradation rate (*Kipper et al., 2011*). This results in a repartition of nutrients from the productive processes, like muscle deposition and bone formation, for the processes that have a greater need - the plasmatic protein synthesis, gastrointestinal repair and mucus replacement, which induces a reduction in body weight and significantly downgrades carcass quality (*Kipper et al., 2011; Knecht et al., 2011, 2012*).

Table 2. Mean values (±standard error of the mean) of carcass and meat quality parameters according to liver milk spots (n=120).

Parameter	Milk spots	No milk spots	Significance
Number of pigs	69	51	-
Carcass quality			
Slaughter weight (kg)	113.50±0.92	115.90±0.45	*
Hot carcass weight (kg)	92.55±0.78	95.23±0.47	*
Cold carcass weight (kg)	89.48±0.77	92.95±0.46	*
Dressing percentage (%)	81.55±0.18	82.15±0.24	*
FTB (mm)	22.03±1.02	13.90±0.56	*
FTS (mm)	49.70±2.53	21.84±1.05	*
Meatiness (%)	36.58±0.69	44.10±0.30	*
Meat quality parameters			
pH_{45}	6.32±0.02	6.19±0.02	*
T_{45} (°C)	39.48±0.12	39.49±0.13	ns
Pork quality classes (%)			
PSE	18.84	15.69	ns
Normal	55.07	76.47	*
DFD	26.09	7.84	*

Legend: **FTB –** fat carcass thickness on the back; **FTS –** fat carcass thickness at the sacrum; **pH_{45}** – meat pH values measured 45 minutes postmortem; **T_{45} –** Meat temperature measured 45 minutes postmortem. **DFD meat** – pH_{45}> 6.4; **Normal meat**– pH_{45} between 6.0 and 6.4: **PSE meat** – pH_{45}< 6.

*** –** P<0.05; **ns –** no significance (P>0.05).

Pigs with liver milk spots had significantly higher pH_{45} value and incidence of DFD meat than unaffected pigs ($P<0.05$). This can be attributed to the fact that pigs during period of sickness need a higher amount of energy which leads to a reduction in glycogen and adenosine-triphosphate reservoirs in muscles after slaughter, resulting in a lower production of lactic acid and higher pH value of meat which increasing tendency towards DFD meat (*Dailidavičienė et al., 2*008). Furthermore, pigs without liver milk spots had a significantly higher percentage of normal meat quality ($P<0.05$), while there were no differences between two groups of pigs for the incidence of PSE meat ($P>0.05$) (Table 2). The impact of *Ascaris suum* infection and liver milk spots on pork quality has not been well studied. Only one published article is available in the literature about the association between *Ascaris suum* infection and meat quality parameters in pigs (*Theodoropulos et al., 2004*). However, *Theodoropulos et al. (2004)* did not examine the effect of *Ascaris suum* infection on the incidence of PSE and DFD meat. Based on *Theodoropoulos et al. (2004)* results, the meat obtained from slaughtered pigs with liver milk spots had increased moisture values and was more red and yellow than from pigs free of milk spot lesions. Therefore, it may be argued that pigs with liver milk spots produce lower meat quality.

Conclusion

The study showed a high prevalence of liver milk spots in slaughtered pigs, indicating a serious health and welfare problem on the farm of origin. Liver milk spots caused significant changes in several hematological parameters, including middle-sized cells (monocytes, eosinophils and basophils), neutrophils, red blood cell count, hemoglobin concentration, hematocrit and MCV. Furthermore, the presence of milk spots in pig livers significantly downgraded carcass quality, so that slaughter weight, hot and cold carcass weights, dressing percentage as well as the percentage of meat became significantly reduced. In addition, the occurrence of milk spots in pig livers caused a significant deterioration in meat quality. It can, therefore, be concluded that scoring of liver milk spots at the slaughter line has potential to serve not only as an indirect measure of pig health and welfare, but also for the carcass and pork quality.

Hematološki parametri, kvalitet trupa i mesa zaklanih svinja sa i bez mlečnih pega na jetri

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Rezime

Cilj istraživanja bio je da se utvrdi uticaj prisustva mlečnih pega na hematološke parametre, kvalitet trupa i mesa svinja. Ispitivanja su obavljena na 120 svinja, starosti šest meseci, žive mase oko 115 kg. Prisustvo mlečnih pega na jetri svinja je ocenjivano na liniji klanja na osnovu *Welfare Quality® (2009)* protokola. Pored toga, analizirana je i kompletna krvna slika. U cilju utvrđivanja kvaliteta trupa, analizirani su sledeći parametri: živa masa, masa toplog i hladnog trupa, randman, debljina leđne slanine i mesnatost. Od parametara kvaliteta mesa određivani su pH vrednost i temperatura 45 minuta *post-mortem*. Meso svinja je razvrastavano u klase kvaliteta (bledo meko vodenasto-BMV; meso normalnog kvaliteta; tamno, čvrsto i suvo meso-TČS) na osnovu pH vrednosti merene 45 minuta *post-mortem (Adzitey i Nurul, 2011)*. Analizom hematoloških parametara utvrđeno je da su svinje sa mlečnim pegama na jetri imale statistički značajno veće vrednosti monocita, eozinofila, bazofila i neutrofila, dok su vrednosti za broj eritrocita, koncentraciju hemoglobina, hematokrit i MCV bile statistički značajno niže u odnosu na svinje bez promena na jetri. Svinje sa mlečnim pegama na jetri imale su statistički značajno nižu živu masu, masu toplog i hladnog trupa, randman i mesnatost u odnosu na svinje bez mlečnih pega na jetri. Ista grupa svinja je imala statistički značajno višu pH vrednost kao i učestalost pojave TČS mesa. Stoga se može zaključiti da ispitivanje prisustva mlečnih pega na jetri na liniji klanja može da bude značajan pokazatelj ne samo dobrobiti svinja na farmi, već i kvaliteta trupa i mesa svinja.

Ključne reči: TČS meso, mlečne pege, svinje, klanica

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INFLUENCE OF THE COBB 500 HYBRID PARENT AGE AND EGG STORAGE PERIOD ON INCUBATION PARAMETERS

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Abstract: Main goal of this research was to determine the influence of Cobb 500 hybrid broiler parent age (BPA) and egg storage (ES) period, the impact of egg maturity on egg fertilization and chick hatching, as well as on embryonal mortality of chicks during incubation period. There were three phases of production cycle, three different ages of broiler parents 25, 41 and 58 weeks (BPA25, BPA41, BPA 58). The eggs there were differentiated according to storage time: eggs stored up to 7 days and eggs stored over 7 days (ES<7; ES>7). Using the random sample method, 1.050 eggs were chosen (total number of chosen eggs was 6.300), with the aim to determine above mentioned reproductive parameters, one day old chick weight and relative share of chick weight in total egg weight were determined. Age of broiler parents had the highest influence on egg fertility as the highest number of fertilized eggs was recorded during the middle of production cycle (BPA41 = 97.05%), then at the beginning of the cycle (BPA25 = 96.09%), and lowest number of fertilized eggs was during the last phase of the cycle (BPA58 = 93.00%). The storage period of the eggs did not have any influence on egg fertility. However, the age of broiler parents and storage period had significant influence on hatching, therefore it influenced embryonal mortality during incubation period. Without considering the storage period, the lowest embryo mortality was detected with eggs that originated from BPA41 – 13.05%, eggs that originated from BPA58 had significantly higher embryo mortality rate 15.87%, and the highest mortality rate was noted with eggs that originated from BPA25 16.93%. However, extended storage period for the eggs or egg maturity (ES<7 and ES>7) had influence on total embryonal mortality rate in all three phases of the production cycle. Moreover, broiler parent age had statistically significant influence on increase of egg weight (P<0.001) and hatched chick weight

(P<0.001), while the relative share of chick weight in total egg weight was decreased, therefore storage period in all three phases of production cycle had negative influence on chick percent, with increase of storage time of the egg, relative share of chick weight in total egg weight decreased, especially during start BPA25 and end BPA58 phase of the production cycle (P<0.001).

Key words*:* storage, parents, eggs, embryo, mortality, chicken.

Introduction

In optimal production conditions, incubation stations insert eggs after 3-5 days of storage time and by doing so they minimize the negative effects of egg storing on hatchability and quality of one day old chicks. The highest hatching percent compared to number of fertilized eggs (embryo mortality is minimal) and good quality vital chicks are achieved. However, incubation stations in some situations have to extend storage period of the eggs, which happens due to availability of breeding eggs, station capacity, market demand and price for one day old broiler chicks.

Numerous researches, such as - *Reis and Soares (1993), Gustin (1994), Reis et al. (1997), Suarez et al. (1997), Tona et al. (2004), Miclea and Zahan (2006), Elibol and Brake (2006), Petek and Dikmen (2006), Schmidt et al. (2009), Al-Bashan and Al-Harbi (2010), Abudabos A. (2010), Mitrović et.al. (2012), Alsobayel et al. (2013), Kopecky (2015), Malik et al. (2015), Jaiswal et al. (2016), Araujo et al. (2016), Iqbal et al. (2016)* have determined that the age of broiler parents of different genotypes and egg maturity (storage time) influence the incubation results therefore influence egg fertility and embryo mortality during incubation period.

Above mentioned authors have also determined that extended storage period of eggs extends incubation period, decreases the hatching percent, decreases the chick quality after hatching which further has negative influence on growth rate, mortality, food conversion of broiler chicks during the fattening period. Similarily with the age of broiler parents' the egg weight increases, while the percent of fertilization and hatchability decrease, especially during the end of production cycle. However, even though negative effects of extending the storage period in certain measure are known, it has not been fully researched how the age of broiler parents, and especially the egg maturity (storage time) influence embryo development during incubation period, number and percent of hatched high quality one day old chicks.

Therefore, main goal of this paper was to determine the influence of specific factors on incubation results, especially the age of broiler parents (BPA) and the storage time (ES). The egg fertility, hatchability (compared to number of

incubated, and to number of fertilized eggs) as well as embryo mortality during incubation, hatched chick weight and relative chick weight share in total egg weight were determined during different age of broiler parents 25, 41 and 58 weeks BPA25, BPA41 and BPA58, as well as for eggs stored up to 7 days ES<7, and eggs stored over 7 days ES>7.

Material and methods

Experimental part of this research was conducted at chicken farm and incubation station Agreks d.o.o. Donji Zabari, Republika Srpska – B and H. Among other thing,s this farm is engaged in breeding and rearing of Cobb 500 broiler parents, production of breeding eggs and one day old chicks.

With the goal to determine influence of the age of broiler parents in different phases of production cycle (beginning, middle and end) at the flock age of 25 (BPA25), 41 (BPA41), and 58 (BPA58) weeks, and storage period (egg maturity) up to 7 days (ES<7), and over 7 days (ES>7) on the fertility and hatchability, embryo mortality and chick weight, research was conducted on total number of 6.300 eggs. Using the random sample method, six groups (treatments) of eggs were chosen and during each phase 1050 eggs stored up to 7 days were incubated and 1050 eggs stored fpr over 7 days (1.050 x 2 = 2.100 x 3 = 6.300 eggs). All eggs were kept in storage room at temperature ranging from 15° C to 18° C at relative air humidity ranging from 75% to 85%.

Special attention was given to fertilized eggs and eggs from which chicks hatched, to number of embryo that died during the incubation period and to one day old chick weight.

During all production phases, at all ages of broiler parents (BPA25, BPA41, BPA58), eggs were individually measured (and numbered) twice, specially eggs from ES<7 group (stored up to 7 days) and eggs from ES>7 group (stored between 8-14days). That means that in all phases 1.050 eggs were incubated that were up to 7 days old and 1.050 eggs that were over 7 days old. During transfer of eggs from laying section of incubator to hatchery (18^{th} day), they were placed in specially built compartments so that each hatched chick could be identified and determined from which egg it originated.

Basic data calculation was done by using computer programme *Stat. Soft. Inc.* (2003) STATISTICA (data analysis software system) version 6, usual variation statistic methods were used (descriptive statistics). For the most of monitored parameters the following was calculated: arithmetic mean ($\bar{x}$), arithmetic mean error ($S_{\bar{x}}$), standard deviation (S) and variation coefficient (C.V.).

Difference of significance testing between researched incubation parameters was conducted by applying appropriate variance models (two-factorial experiment plan – 3 ages of broiler parents x 2 periods of egg storing; $Y_{ijk} = \mu +$

$(BPA)_i + (ES)_j + (BPAxES)_{ij} + e_{ijk}$) using equal and different number of repeptitions per treatment.

Relative share of chick weight in total egg weight (percent of the chick/PC) was determined using the formula: P.C. = [(chick weight/egg weight) x 100].

Results and discussion

The effects of parent flock age (BPA25, BPA41 and BPA58 weeks), the phase of production cycle (starting, middle and ending) and time spent in storage to and over 7 days (ES<7 and ES>7) in incubation station on incubation parameters are shown in table 1.

Table 1. Egg fertility, chich hatchability, and embryo mortality (%)

Parentflock age (BPA)	Egg maturity (ES)	Fertilized eggs	Hatched chicks[A]	Hatched chicks[B]	Embryo mortality
BPA25	<7	96.95	81.43	83.99	16.01
	>7	95.24	78.29	82.20	17.80
	Total	96.09	79.86	83.10	16.90
BPA41	<7	97.43	87.62	89.93	10.07
	>7	96.67	81.14	83.94	16.06
	Total	97.05	84.38	86.95	13.03
BPA58	<7	93.05	79.43	85.36	14.64
	>7	92.95	76.76	82.58	17.42
	Total	93.00	78.09	83.97	16.03

Data from table 1 shows that the age of broiler parents influenced the fertility of eggs, both those up to and over 7 days. The highest fertility compared to number of incubated eggs was during the middle of production cycle (BPA41 = 97.05%), followed by the start phase (BPA25 = 96.09%), and lowest at the end phase (BPA58 = 93.00%) of reproductive cycle. However, age of broiler parents and storage period had significant influence on percent of chick hatching, and especially on the embryo mortality rate during incubation period. The highest chick hatchability compared to number of incubated eggs (number of fertilized eggs) was in BPA41 (84.38% and 86.95%), followed by BPA25 (79.86% and 83.10%), and lowest was in BPA58 (78.09% and 83.97%). Moreover, extending of egg storage period influenced the decrease of hatching percent of chicks in all three phases of production cycle.

From above mentioned it can be seen that the age of broiler parents influenced egg fertility and hatchability, and that egg maturity (storage period) influenced hatchability percent and hatched chicks quality (vitality). Similar research is conducted by *Tona et al. (2004), Petek and Dikmen (2006)* for broiler parents of different age and during different storage period of brooding eggs. *Tona*

et al. (2004) has found that 7 day old eggs of Cobb broiler parents that were 35 weeks old have higher hatchability percent by aproximatelly 4% (88.36% - 84.65%) than parent flock that was 45 weeks old. For both age groups of broiler parents, incubated fresh eggs had statistically significantly lower hatchability percent than eggs that were stored for 7 days. *Petek and Dikmen (2006)* have found that by extending storing period for eggs originating from same age broiler parents (37weeks) percent of hatchability significantly decreases compared to percent of fertilized eggs. Therefore chick hatchability compared to number of fertilized eggs stored for five days is 97.78%, and from eggs stored over five days only 61.82%. Similar results are obtained by *Reis and Soares (1993), Schmidt et al. (2009).* Above mentioned authors state that regardless of the parent flock age, the percent of hatchability significantly decreases if storage period is extended. *Schmidt et al. (2009)* have determined that hatching percent compared to number of fertilized eggs (two days old) is 93.83% and that it decreases to only 74.13% for eggs stored for 14 days. *Jaiswal et al. (2016)* have found that age of broiler parents influences the egg weight, and egg weight influences the hatchability of the chicks, therefore they have determined that light weight eggs have the lowest hatchability percent compared to number of incubated eggs 66.0%, medium weight eggs have hatchability percent of 74.4% and the highest hatchability percent is for heaviest eggs 80.2%. They have used same age broiler parents. For broiler Barents of Cobb hybrid that are 26 and 44 weeks old, *Abudabos (2010)* has determined that chick hatchability, compared to number of incubated eggs, is 85.2% and 70.4% and compared to number of fertilized eggs 92.3% and 82.8%, respectively, which is contrary to our results *Malik et al. (2015)* have also determined for 64 week old Cobb 500 broiler parents, the highest fertility of eggs and the highest hatchability with light weight eggs and the lowest for heaviest eggs.

In general, the results show that embryo mortality during incubation period is influenced by the age of broiler parents as well as the duration of egg storing period. The lowest embryo mortality 13.05% without considering the parent age is determined for the eggs from broiler parents 41 week old (BPA41), significantly higher at the end of production cycle (BPA58) 16.03%, and the highest at the beginning of production cycle (BPA25) 16.90%. Moreover, in all three production phases, extending of egg storage period over 7 days influenced increase of embryo mortality during incubation period. For the eggs originating from 41 day old broiler parents, the greatest difference between storage periods was determined (5.99%), total embryo mortality for the eggs that were stored up to 7 days was 10.07%, and for the eggs that were stored over 7 days it was 16.06%. For the eggs that were laid at the beginning and at the end of production cycle, storage period had less influence on embryo mortality, differences were lower, but in those production phases total embryo mortality was higher.

Reis and Soares (1993), Gustin (1994), Reis et al. (1997), Suarez et al. (1997), Elibol and Brake (2006), Miclea and Zahan (2006), Schmidt et al. (2009),

Al-Bashan and Al-Harbi (2010), Mitrović et al. (2012), Kopecky (2015) and *Jaiswal et al. (2016)* also disscused this problem of determining the influence of storage period on incubation results for the different age broiler parent eggs, and especially on embryo mortality.

Reis and Soares (1993) have also determined that with the age of Cobb 500 broiler parents embryo mortality increases during incubation period, however it is significantly lower result compared to our research. Therefore total embryo mortality for eggs from 33 week old parents was the lowest (2.46%), slightly higher for 43 week old flock (4.84%) and the highest at the end of production cycle 7.19%. Similarily, *Reis et al. (1997)* have established the embryo mortality of 7.9% when incubating eggs from 32 and 34 week old parents and 8.5% for eggs from 48 and 50 weeks old parents. *Suarez et al. (1997)* have determined the highest embryo mortality for the youngest parent flock of 29 weeks and it was 10.2%, then for the oldest parents 52 weeks 8.8%, and the lowest for 41 week old broiler parents 5.8%. Compared to our results, above mentioned authors have obtained lower or similar total embryo mortality during incubation of the eggs that were stored up to seven days and are from parents of different age. Unlike other authors, *Mitrović et al. (2012), Iqbol et al. (2016)* have obtained values most similar to our results in regard to embryo mortality rate.

Elibol and Brake (2006) have incubated eggs from broiler parents of different age (37, 41, 59 and 63 weeks) and tried to determine early, medium and late, as well as total embryo mortality. Contrary to our research, above mentioned authors have determined the lowest (8.33%) embryo mortality for the youngest flock (37 weeks), followed by the 41 week old flock (9.50%), and significantly higher embryo mortality rate for the flock that was 59 and 63 weeks old (12.28% and 12.64%, respectively). However, total embryo mortality rate is quite similar to our results (table 1), especially if we compare our results with *Kopecky et al. (2015)* who have determined the lowest embryo mortality for medium weight eggs 9.71%, for the lightest eggs 11.92% and for the heaviest eggs 16.74%. *Jaiswal et al. (2016)* have determined the highest embryo mortality for the lightest eggs 15.3%, and the lowest for the heaviest eggs 6.13% which is in a way contradictory to our results.

Data from the next table (table 2.) shows that before inserting of the eggs in to the incubator, at the starting phase of the cycle BPA25 the lowest average weight of fertilized eggs was determined (54.77 g and 53.84 g), and the highest (67.25 g and 66.31 g) at the ending phase BPA58 without considering the storage time of the eggs.

Table 2. Average values and variability of fertilized eggs (g)

Production phase/BPA	Egg maturity (ES) (days)	n	$\bar{x}$	S $\bar{x}$	S	C.V.
Start/BPA25	<7	1018	54.77	0.12	3.69	6.74
	>7	1000	53.84	0.12	3.78	7.02
	Total	*2018*	*54.31*	*0.08*	*3.76*	*6.92*
Middle/BPA41	<7	1023	63.19	0.13	4.12	6.52
	>7	1015	62.55	0.13	4.09	6.54
	Total	*2038*	*62.87*	*0.09*	*4.11*	*6.54*
End/BPA58	<7	977	67.25	0.14	4.36	6.48
	>7	976	66.31	0.15	4.65	7.01
	Total	*1953*	*66.78*	*0.10*	*4.53*	*6.78*

The fact that age of broiler parents influences the egg weight, that with the age of different parent genotypes during production cycle egg weight increases was confirmed by research of many authors *Viera et al. (2005), Enting et al. (2007), Schmidt et al. (2009), Đermanović et al. (2010), Mitrović et al. (2011), Abudabos (2010), Alsobayel et al. (2013), Araujo et al. (2016), Igbal et al. (2016).*

Average weight of all eggs (AWE) stored up to 7 days was 61.70 g, WHE – 61.61 g. CW – 42.52 g andCP – 68.99% (table 3.). Extending of egg storage time influenced the decrease in average egg weight (AWE and WHE) and in weight of day old chicks (CW and CP). Highest variation coefficient (over 11, actually 12%) was determined for CW coming from eggs stored up to and over seven days, and lowest (below 5%) with chick weight percent in total egg weight (CP).

Table 3. Desriptive statistic parameters for egg and one day old chick traits depending on storage time

Traits	n		$\bar{x}$		S $\bar{x}$		S		C.V.	
	<7	>7	<7	>7	<7	>7	<7	>7	<7	>7
AWE[A]	3150	3150	61.70	60.88	0.12	0.12	6.79	6.91	11.01	11.35
WHE[B]	2609	2480	61.61	60.74	0.13	0.14	6.73	6.79	10.92	11.18
CW[C]	2609	2480	42.52	41.46	0.10	0.11	5.08	5.27	11.95	12.71
CP[D]	2609	2480	68.99	68.19	0.05	0.06	2.83	3.20	4.10	4.69

AWE[A] – weight of all eggs, g; WHE[B] – weight of eggs that hatched, g: CW[C] – chick weight, g; CP[D] – Chick percent.

Average weight of all eggs stored to and over seven days, eggs that hatched and weight of one day old chicks originating from young parents BPA25 was statistically significantly lower (P<0.001) compared to eggs from BPA41 and BPA58 is shown in the table 4. Differences between BPA41 and BPA 58 for all eggs (-3.88 g), from the eggs that hatched (-3.84 g) and day old chick weights (-1.46 g) were also statistically significant (P<0.001). Relative chick weight share in

the total egg weight (CW) was highest in BPA41 (69.66%), and lowest in BPA58 (67.81%), all differences were confirmend at the level P<0.001. From the data it can be concluded that with the age of broiler parents egg and one day old chick weight increased, while chick weight share in total egg weight mostly decreased.

Table 4. Average egg and chick trait difference significance depending on parent age BPA and egg maturity ES

Traits	Parent age weeks	$\bar{x}$	d
AWE[A]	BPA25 – BPA41	54.30 – 62.84	-8.54***
	BPA25 – BPA58	54.30 – 66.72	-12.42***
	BPA41 – BPA58	62.84 – 66.72	-3.88***
WHE[B]	BPA25 – BPA41	54.16 – 62.80	-8.64***
	BPA25 – BPA58	54.16 – 66.64	-12.48***
	BPA41 – BPA58	62.80 – 66.64	-3.84***
CW[C]	BPA25 – BPA41	36.99 – 43.76	-6.77***
	BPA25 – BPA58	36.99 – 45.22	-8.23***
	BPA41 – BPA58	43.76 – 45.22	-1.46***
CP[D]	BPA25 – BPA41	68.27 – 69.66	-1.39***
	BPA25 – BPA58	68.27 – 67.81	0.46***
	BPA41 – BPA58	69.66 – 67.81	1.85***
AWE[A]	ES<7– ES>7	61.70 – 60.88	0.82***
WHE[B]	ES<7– ES>7	61.61 – 60.74	0.87***
CW[C]	ES<7 – ES>7	42.52 – 41.46	1.06***
CP[D]	ES<7 – ES>7	68.99 – 68.19	0.80***

AWE[A] – weight of all eggs, g; WHE[B] – weight of eggs that hatched, g: CW[C] – chick weight, g; CP[D] – Chick percent. ns – P>0,05; ***P<0,001.

Data from the table 4 shows that storage period for the eggs has statistically significantly (P<0,001) influenced egg and day old chick traits, with extending the egg storage period in all three cycle phases incubated egg weight that hatched decreased as well as day old chick weight and relative chick weight share in total egg weight.

Similar trend of increase, actually decrease of hatched egg weight, depending on parent flock age (BPA) and storage period (ES) was determined for day old chick weight (table 5.)

Table 5. Average values and variability of chick weight and chick share in total egg weight

Production cycle phase/BPA	Egg maturity (ES) (days)	n	$\bar{x}$	S $\bar{x}$	S	C.V.
Day old chicks weight (g)						
Start/BPA25	<7	855	37.61	0.11	3.29	8.75
	>7	822	36.34	0.11	3.26	8.96
	Total	*1677*	*36.99*	*0.08*	*3.34*	*9.02*
Middle/BPA41	<7	920	44.02	0.12	3.53	8.01
	>7	852	43.48	0.12	3.61	8.30
	Total	*1772*	*43.76*	*0.08*	*3.57*	*8.17*
End/BPA58	<7	834	45.89	0.14	4.18	9.11
	>7	806	44.53	0.16	4.51	10.13
	Total	*1640*	*45.22*	*0.11*	*4.40*	*9.72*
Relative chick share in total egg weight (%)						
Start/BPA25	<7	855	68.87	0.09	2.77	4.02
	>7	822	67.64	0.10	2.87	4.25
	Total	*1677*	*68.27*	*0.07*	*2.89*	*4.23*
Middle/BPA41	<7	920	69.73	0.08	2.59	3.72
	>7	852	69.58	0.09	2.57	3.69
	Total	*1772*	*69.66*	*0.06*	*2.58*	*3.71*
End/BPA58	<7	834	68.30	0.10	2.95	4.32
	>7	806	67.30	0.13	3.60	5.35
	Total	*1640*	*67.81*	*0.08*	*3.32*	*4.90*

Average weight of day old chicks hatched from eggs that were stored up to seven days was between 37.61 g (BPA25) and 45.89 g (BPA58), and chicks hatched from eggs that were stored over seven days was between 36.34 g (BPA25) and 44.53 g (BPA58). That means that weight of hatched chicks increased with the age of laying hens, while extending of storage period of eggs influenced the decrease of body weight of day old chicks (table 5.). From the data in the table 5 it is further visible that highest relative chick share in total egg weight share, not considering the storage period of the eggs was highest during the middle of production cycle (BPA41 – 69.66%), and lowest in the ending phase (BPA58 – 67.81%). However, highest relative chick share in total egg weight (69.73%) was with young flock BPA41 and that with eggs that were stored up to seven days, lowest relative chick share in total egg weight (67.30%) was at the end of production cycle BPA58 and that with eggs that were stored for over seven days. Moreover, extending of storage period in all three phases of production cycle influenced the decrease of relative chick share in total egg weight.

Similar average one day chick weight in certain phase of production cycle was determined by *Vieira et al. (2005), Enting et al. (2007),* slightly lower was determined by *Abudabos (2010), Miclea and Zahan (2006),* and *Schmidt et al.(2009)* determined slightly higher body weight.

Data from table 5 show that relative chich share in total egg weight (CW) was variable and it pointed out specific trend of decrease connected with age of broiler parents for eggs that were stored up to seven days (BPA25 = 68.87% andBPA58 = 68.30%), while for the eggs stored over seven days highest chick percent was determined for the eggs originating from BPA41 (CP = 69.58%), and lowest was for the eggs that were produced during the ending phase of production cycle (BPA58 = 67.30%). Moreover, in all three phases of production cycle relative chick share (CP) in the egg weight (EW) was higher for the eggs that were sored up to seven days (ES<7), compared to eggs that were kept over seven days (ES>7).

If we look at it from broader point of view, relative chick share in total egg weight, regardless of the phase of production cycle and storage time fits the results of other researchers who dwelled on this matter. It shows that with the age of broiler parents egg and day old chick weight increases but relative chick share in total egg weight, as rule, decreases *(Abudabos, 2010; Alsobayjel et al., 2013; Iqbal et al., 2016).*

Similar, even better (higher) percent of chick in egg weight (around 70% and more) was determined by *Schmidt et al. (2009), Miclea and Zahan (2006), Abudabos (2010),* and lower, actually significantly lower relative chick share in total egg weight was determined by *Vieira et al. (2005), Enting et al. (2007).*

Statistic significance of determined differences for average day old chick weights and relative chick share in total egg weight depending on broiler parent age, storage time is shown in tables 6 and 7.

Table 6. Significance of average chick weights (CW) depending on parent age (BPA) and egg maturity (ES)

Parent age – egg maturity	$\bar{x}$	d
BPA25ES<7 – BPA41ES<7	37.61 – 44.02	-6.41***
BPA25ES<7 – BPA58ES<7	37.61 – 45.89	-8.28***
BPA25ES<7 – BPA25ES>7	37.61 – 36.34	1.27***
BPA25ES<7 – BPA41ES>7	37.61 – 43.48	-5.87***
BPA25ES<7 – BPA58ES>7	37.61 – 44.53	-6.92***
BPA25ES>7 – BPA41ES<7	36.34 – 44.02	-7.68***
BPA25ES>7 – BPA58ES<7	36.34 – 45.89	-9.55***
BPA25ES>7 – BPA41ES>7	36.34 – 43.48	-7.14***
BPA25ES>7 – BPA58ES>7	36.34 – 44.53	-8.19***
BPA41ES<7 – BPA58ES<7	44.02 – 45.89	-1.87***
BPA41ES<7 – BPA41ES>7	44.02 – 43.48	0.54*
BPA41ES<7 – BPA58ES>7	44.02 – 44.53	-0.51^{ns}
BPA41ES>7– BPA58ES<7	43.48 – 45.89	-2.41***
BPA41ES>7– BPA58ES<7	43.48 – 44.53	-1.05***
BPA58ES>7– BPA58ES<7	44.53 – 45.89	-1.36***

^{ns}P>0.05; *P<0.05; ***P<0.001.

Highest difference in average one day old chick body weight (-9.55 g) was determined with BPA25ES>7 and BPA58ES<7, and lowest (-0.51 g) with BPA41ES<7 and BPA58ES>7. Determined differences in average chick weights as results of parent age and storage time were statistically confirmed at the level P<0.001, except for the difference between BPA41ES<7 and BPA41ES>7 which was statistically significant but at the level P<0.05, while difference – 0.51 g (BPA41ES<7 – BPA58ES>7) was not statistically significant at the level P>0.05 (table 6).

Table 7. Significance for average chick relative share in total egg weight (CW) depending on parent age (BPA) and egg maturity (ES)

Starost roditelja – starost jaja	$\bar{x}$	d
BPA25ES<7 – BPA41ES<7	68.87 – 69.73	-0.86***
BPA25ES<7 – BPA58ES<7	68.87 – 68.30	0.57***
BPA25ES<7 – BPA25ES>7	68.87 – 67.64	1.23***
BPA25ES<7 – BPA41ES>7	68.87 – 69.58	-0.71***
BPA25ES<7 – BPA58ES>7	68.87 – 67.30	1.57***
BPA25ES>7 – BPA41ES<7	67.64 – 69.73	-2.09***
BPA25ES>7– BPA58ES<7	67.64 – 68.30	-0.66***
BPA25ES>7– BPA41ES>7	67.64 – 69.58	-1.94***
BPA25ES>7– BPA58ES>7	67.64 – 67.30	0.34^{ns}
BPA41ES<7– BPA58ES<7	69.73 – 68.30	1.43***
BPA41ES<7– BPA41ES>7	69.73 – 69.58	0.15^{ns}
BPA41ES<7– BPA58ES>7	69.73 – 67.30	2.43***
BPA41ES>7– BPA58ES<7	69.58 – 68.30	1.28***
BPA41ES>7– BPA58ES>7	69.58 – 67.30	2.28***
BPA58ES<7– BPA58ES<7	67.30 – 68.30	-1.00***

^{ns}P>0.05; *P<0.05; **P<0.01; ***P<0.001.

Broiler parents age influence (BPA) and storage period (ES) had slightly different effect on relative chick share in total egg weight, that it had on fertilized eggs that hatched and one day old chicks (table 7). Determined differences 0.34% (BPA25ES>7 – BPA58ES>7) and 0,15% (BPA41ES<7 – BPA41ES>7) were not statistically confirmed (P>0,05), while other differences regarding the chick relative share in egg weight between researched groups were statistically significant and confirmed at the P<0.001 level.

Conclusion

If we observe the production phases (usage) it can be said that broiler parents have best production, reproduction results during the middle of production cycle. Cobb 500 broiler parents are during the middle of production cycle BPA41,

compared to start phase BPA25 and end phase BPA58 of hatching eggs achieved best production and reproductive results. In that period 5,08 eggs, 4,74 fertilized eggs and 4,40 day old chicks per laying hen were produced, while average food consumption per hen was 181,00 g. Average egg weight that were stored up to 7 days was within optimal limits and it counted 63,11 g, egg fertility was 97,43%, chick hatchability was 87,62% (compared to incubated eggs), or 89,93% (compared to fertilized eggs), day old chick body weight was at satisfying level 44,02 g and relative chick share in total egg weight was 69,73%. Moreover, age of broiler parents influenced egg weight increase and hatched chick weight increase, while chick percent in total egg weight decreased, storage period had in all three phases of production cycle negative influence on chick percent, with increase of storage time relative chick share in total egg weight decreased, especially in starting BPA25 and ending BPA58 phase of production cycle.

Uticaj starosti brojlerskih roditelja hibrida Cobb 500 i perioda skladištenja jaja na inkubacione pokazatelje

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Rezime

Osnovni cilj rada bio je ispitivanje uticaja starosti brojlerskih roditelja (SR) Hibrida Cobb 500 i perioda skladištenja jaja, odnosno starosti jaja (SJ) na oplođenost jaja i leženost pilića, kao i na embrionalni mortalitet pilića u toku inkubacionog perioda. U tri faze proizvodnog ciklusa, odnoso različite starosti brojlerskih roditelja (SR25ned., SR41ned. i SR58ned.) i kod jaja skladištenih do 7 i preko 7 dana (SJ<7 i SJ>7), metodom slučajnog uzorka, odabrano je po 1.050 jaja (ukupno 6.300 jaja), u cilju utvrđivanja pomenutih reproduktivnih pokazatelja, težine pilića starih jedan dan i relativnog udela pileta u težini jajeta.Starost brojlerskih roditelja je uticala na oplođenost jaja jer je najviše fertilnih jaja bilo sredinom proizvodnog ciklusa (SR41 = 97,05%), zatim početkom (SR25 = 96,09%), a najmanji u završnoj fazi (SR58 = 93.00%) gajenja jata. Period skladištenja jaja nije imao uticaja na to koliko je jaja oplođeno, dok je starost brojlerskih roditelja i period skladištenja jaja bitno uticao na procenat izvodljivosti pilića, a samim tim i na embrionalni mortalitet u toku inkubacionog perioda. Najmanji ukupan embrionalni mortalitet (13,05%), bez obzira na period skladištenja, utvrđen je kod jaja poreklom SR41, znatno veći 15,87% kod SR58 i najveći 16,93% kod SR25. Zatim, produžavanje perioda skladištenja jaja, odnosno

starost jaja (SJ<7 i SJ>7) je uticalo na povećanje ukupnog embrionalnog mortaliteta u sve tri faze proizvodnog ciklusa.

Pored toga, starost brojlerskih roditelja je statistički značajno uticala na povećanje mase jaja (P<0,001) i izleženih pilića (P<0,001), dok se procenat pileta u masi jajeta smanjivao, a period skladištenja jaja, kod sve tri faze proizvodnog ciklusa, negativno je uticao na procenat pileta, tj. sa produžavanjem perioda skladištenja jaja, relativni udeo pileta u masi jajeta se smanjivao, posebno u početnoj (SR25) i završnoj fazi (SR58) proizvodnog ciklusa (P<0,001).

Ključne reči: skladištenje, roditelji, jaja, embrion, mortalitet, pilići

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CONSUMER CRITERIA FOR PURCHASING EGGS AND THE QUALITY OF EGGS IN THE MARKETS OF THE CITY OF BELGRADE

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Original scientific paper

Abstract: In order to examine the consumers' attitude towards eggs, 239 respondents in the area of the City of Belgrade were surveyed, and the assessment of the quality of eggs on the Belgrade market was done by examining the quality of eggs in super/hypermarkets. In the survey, consumers expressed their views about the place of purchase of eggs, the criteria for purchasing and the significance of certain quality traits/properties. In the egg quality test eggs of class A were used, and it was done on egg samples from 5 super/hypermarkets, from a total of 10 egg manufacturers. Based on the results of the study, it was found that most egg consumers buy in super/hypermarkets (39.62%), that the brand/manufacturer was not important for 30.37% of the respondents, that they prefered to buy larger eggs (SS, S and A classes). The quality of eggs is very important for 73.28% of subjects, and the colour of the yolk prefered by the respondents was extremely yellow (62.76%). The supply of table eggs on the market was different (from 1 to 5 manufacturers) per retail store. The quality of eggs, observed for all super/hypermarkets and all manufacturers, expressed in Haugh units, decreased with the shelf life of eggs. The quality of eggs from category 1 to 10 days, in all super/hypermarkets, observed for all manufacturers, was within the studied class A, with egg weight ranging from 60.9 to 64.1g, egg shell colour from 3.6 to 4.2, egg cleaniness 4.4 to 5.0, the colour of the yolk (Roche) from 10.9 to 13.2, and the number of Haugh units from 73.2 to 91.7. The results of the research indicate that consumers in the City of Belgrade are placing importance on the quality of table eggs, that there are differences in the supply and freshness of the eggs between the markets, and that there are differences in the quality within the same market, regardless of the manufacturer.

Key words: eggs, consumers, poll/survey, market, quality of eggs, Belgrade

Introduction

The habits and attitudes of consumers in different areas have been gaining importance over the last decades, whereby the consumer can no longer be considered a passive observer, but someone with an important role in creating an environment in terms of the production, market, environment, etc. Consumer attitudes are of importance from several aspects, and while the knowledge of attitudes is recognized by some authors as the basis for successful marketing (*Jovović and Femić, 2006*), on the other hand, some authors point to a strong relationship between consumer perception, quality and food safety (*Savović et al. al., 2012*).

In the last two decades, poultry production has faced a variety of challenges in terms of changes in legislation and numerous requirements in the field of food safety, ecology, production technology - production systems, animal welfare, production sustainability, as well as socio-economic changes, which has opened many issues relating to consumer attitudes. Also, it can be observed that with the tendency to appreciate consumer attitudes, the number of research in this field, mainly polls or surveys, is focused on consumer preferences of table eggs (*Fearne and Lavelle*, *1996*; *Mizrak et al.,2012*,; *Huang, 2013*; *Kralik et al.,2014; Tolimir et al., 2016; Zelić et al., 2016).*

When it comes to the quality of the eggs, while the manufacturers primarily give attention to the egg weight and quality of the egg shell, as a prerequisite for good price and marketing, consumers also show interest in quality, but with special attention to the egg weight, the colour of the egg shell and of the yolk, the quality of the egg white and the absence of meat and bloody stains (*Tolimir et al., 2008*). *Škrbić et al. (2006)* indicate an increase in consumer interest in the safety and quality of eggs, and according to research by *Hernandez et al. (2005),* consumer safety and egg freshness are the most important factors for them, and in relation to the sensory qualities of the quality of the eggs, the strength of the egg shell, the consistency of the egg white and the colour of the yolk are properties specially valued by consumers in a number of European countries (France, Germany, Italy, Great Britain, Spain , Poland and Greece).

Since the initial quality of the eggs is at its highest in the moment of laying, and from that moment on the internal egg quality begins to decline, egg handling and management in terms of storage conditions and storage are very important *(Jin et al., 2011).* In this sense, from the aspect of the quality, later procedures and handling of eggs within the sales points, i.e. the conditions in the facility, as well as the time from the moment of supply of eggs to the retail facility to the sale of eggs, are very important. The study of the quality of eggs in retail facilities is subject of research by many authors, mainly in order to determine the quality of eggs (shelf life of eggs, egg weight, albumen height, Haugh units, colour of yolk, number of broken eggs) which come from different manufacturers and different production

systems - conventional or alternative or eggs from functional food programs *(Bell et al., 2001; Burley and Johnson, 2013; Patterson et al., 2001).*

The aim of this study is to determine the habits of consumers in the City of Belgrade when purchasing eggs and their attitudes, that is, the criteria for purchasing eggs and their preferences. At the same time, the aim of the study is to enable the examination of the supply of eggs on the Belgrade market in super/hypermarkets, through the representation of various manufacturers within the market, and to contribute to the assessment of the quality of eggs in this market segment, observed through the shelf life of eggs sold to consumers, and the manufacturers that are present within a single retail facility and at the level of all retail facilities.

Material and Methods

Survey was conducted in 2016, on the territory of the city of Belgrade. A total of 239 consumers of table eggs took part in the survey, randomly selected, and taking into account that they represented different categories (sex, age, education). Respondents filled out surveys without the presence of interviewers, for the purpose of data objectivity. The structured survey questionnaire consisted of: 1) data on the respondent obtained by circling the offered responses related to the sex (male, female); 2) the questions of a closed type, according to the principle of the nine-step Likert scale - where **1** was: "It does not matter at all", to **9** "Very important to me" - applied to the question of how important the brand/manufacturer is when purchasing eggs and how much important freshness of eggs is; 3) the question „Where do You purchase eggs most frequently“ - offered answers: in the market, in the mini market, in the super/hyper market and other (do not buy eggs/you have your own production, you are buying directly from the manufacturer); 4) the question „Which size or class of eggs you prefer to buy“ - offered answers: SS (70g and above), S (65-70g), A (60-65g), B (55-60g), C (50-55g), D (45-50g), E (less than 45g), and „I don't care about the class of eggs“, and 5) the question „Which colour of the egg youlk You prefer“ – offered answers: light yellow, medium yellow, very yellow (yellow-orange) and „The yolk colour does not matter to me“. Only fully filled questionnaires were processed statistically.

The quality of eggs was tested in October 2017, referring to eggs exclusively of A class, and it was performed on egg samples from 5 super/hypermarkets in the City of Belgrade (A, B, C, D, E) for a total of 10 egg manufacturers that were present in these retail facilities (indicated by numbers 1 to 10). Sampling of eggs for egg quality analysis was performed always on the same day, in three repetitions in all retail facilities. In each of the markets, the supply of eggs was recorded - the number of suppliers, i.e. the manufacturers, and the best before date (date

indicating the deadline for sale), based on which the egg shelf life was determined on the day of sampling. For each egg manufacturer, the sample consisted of 10 eggs (one pack), each of which was tested individually for the properties of external and internal egg quality. Analysis of the quality of all eggs was done in one day, the first day after purchase, whereby eggs from the moment of purchase in the markets until the next day, when they were analyzed, were stored in the cooling cabinets, i.e. in the same conditions as in the retail store. In order to determine the impact of egg shelf life on egg quality at the level of all retail facilities, the egg samples of all manufacturers were classified into three categories - from 1 to 10, 11 to 20, and 21 to 30 days shelf life. For each egg group, the following quality properties were determined: egg weight, albumen height and HU. In the category of eggs from 1 to 10 days, for eggs obtained from all retail facilities and all manufacturers, the following egg quality properties were determined: egg weight (measured on a technical scale of 0.01g), egg shell colour (visually estimated from 1 to 5), cleaniness of the egg shell visually estimated from 1 – the lowest to 5 – the highest score), colour of the egg yolk (visually estimated with Roche Yolk Color), the albumen height (measured by tripod micrometer) and Hough units (determined by American Yolk Color calculator) and egg shell thickness (determined by using the micrometer).

Respondents' responses were processed using the standard method of analysis in the Microsoft Excel program. Statistical processing of the obtained data for the egg quality was done in the Statistics 8 program, by the variance analysis StatSoft. Inc. (www.statsoft.com).

Results and Discussion

Table 1 gives an overview of the results related to the consumers' habits in the purchase of eggs, i.e. answers to the questions: "Where do you most often purchase the eggs from?", "How important to you is the brand/manufacturer when purchasing eggs?" and "You prefer the eggs of which size/class, when purchasing eggs?".

Based on the results of the study (Table 1), it can be concluded that most of the surveyed egg consumers buy in the super/hypermarket (39.62%), that for the majority of consumers the brand/manufacturer of eggs is not important in terms of egg choice are (30.37%) and that consumers in the area of Belgrade prefer large eggs, of SS, S and A categories (a total of 69.82%), with the highest number of respondents choosing the class A (28.38%).

Regarding the purchase of eggs, the results of this study may be associated with the research of *Kralik et al.(2014),* who also have found by survey research that 38.78% of respondents choose a supermarket as a place of purchasing of eggs. The results obtained in the present study, that consumers prefer large eggs as a selection criterion when buying are in line with the research by *Zelić et al. (2016).*

However, when compared with the results of the same authors, the data on the importance of a particular brand of eggs are different, as in the research conducted in the Tuzla region it was established that 56.16% of the respondents have chosen a particular brand.

Table 1. Habits of Belgrade consumers in the purchase of eggs

Categories of respondents	Answers to the question: "Where do you most often purchase the eggs from?"									
Answers										
	%	Green market		Mini-market		Super/ hypermarket		Other		
Sex										
Male	27.78	26.79		10.71		44.64		17.85		
Female	72.22	22.44		10.90		37.82		25.85		
Collectively	100	23.58		10.85		39.62		26.85		
Answers to the question: "How important to you is the brand/manufacturer when purchasing eggs?"										
Score range										
	%	1	2	3	4	5	6	7	8	9
Sex										
Male	27.78	32.20	8.47	10.17	8.47	10.17	8.47	5.08	3.39	13.56
Female	72.22	29.68	10.32	9.68	7.10	9.68	3.87	9.68	5.16	14.84
Collectively	100	30.37	9.81	9.81	7.48	9.81	5.14	8.41	4.67	14.49
	Answers to the question: "You prefer the eggs of which size/class, when purchasing eggs?".									
	%	SS	S	A	B	C	D	E	Not important	
Sex										
Male	27.78	21.67	26.67	26.67	6.67	0.00	0.00	0.00	18.33	
Female	72.22	11.11	27.78	29.01	11.73	0.62	1.23	0.00	18.52	
Collectively	100	13.96	27.48	28.38	10.36	0.45	0.90	0.00	18.47	

* 1 – It is not at all important to me; 9 - It's very important to me

Table 2 gives an overview of the results related to the consumers' attitude towards the quality of the eggs, i.e. the answers to the following questions: "How important is the freshness of eggs?" and "Which colour of egg yolk do you prefer?"

Table 2. Importance of the egg quality for consumers

Categories of respondents		Answers to the question: "How important is the freshness of eggs?"*								
		Score range								
	%	1	2	3	4	5	6	7	8	9
Sex										
Male	27.78	4.76	1.59	0.00	1.59	1.59	3.17	3.17	12.70	71.43
Female	72.22	5.92	1.78	1.18	1.18	2.37	2.96	1.78	8.88	73.96
Collectively	100	5.60	1.72	0.86	1.29	2.16	3.02	2.16	9.91	73.28
Answers to the question: "Which colour of egg yolk do you prefer?"**										
		Score range								
	%	1		2		3			4	
Sex										
Male	27.78	3.08		23.08		61.54			12.31	
Female	72.22	1.15		29.31		63.22			6.32	
Collectively	100	1.67		27.62		62.76			7.95	

* 1 – It is not at all important to me; 9 - It's very important to me

** 1 - light yellow; 2 - medium yellow; 3 - extremely yellow (yellow orange); 4 - I do not care about the colour of the yolk

The results of the study of the importance of egg freshness (Table 2), from the angle of the consumer, indicate that Belgrade consumers pay great attention to egg freshness, since 83.19% of respondents voted for the highest score (8 and 9). The result of this research is in agreement with the general tendency of increasing consumers' criteria for food quality and safety *(Savović et al., 2012),* as well as the results of *Hernandez (2006)* according to which the freshness of eggs is rated as the most important parameter by consumers in Spain. One of the essential characteristics of the egg quality is the colour of the yolk (Table 2), which consumers pay great attention to, and in most EU countries, the more intense (darker) yellow colour is appreciated *(Parrott et al., 2013; Hernandez et al., 2005)*. The obtained results of this research, according to which the majority of consumers (62.76%) prefer the extremely yellow colour of the yolk, are in agreement with the research of the mentioned authors.

Part of the study concerned the market research in terms of supply in Belgrade super/hyper markets, for which, based on the results of the survey, it can be concluded, are the places where Belgrade consumers are most often purchasing eggs, regardless of whether they are female (37.82%) or male (44.64%). In terms of supply, it was noticed that the number of manufacturers present in the retail facilities was different and that it was at least 1 (in the C, D, E retail facilities), while in the retail facility B it was 2, and in the A there were 5 manufacturers.

The results of the monitoring of egg weight in retail facilities and quality expressed by Haugh units (Figures 1 and 2) related to all the eggs that were offered

in the markets, with the analysis including all manufacturers (10) and eggs of all shelf lives (classified in three groups - from 1 to 10, from 11 to 20, and from 21 to 30 days). The weight of eggs ranged from 60.00 g to 63.05 g, which for manufacturers and all shelf life groups, was in accordance with the class in which the eggs were categorized. Class A eggs were subject to analysis, given the survey found that the majority of consumers in the choice of egg class preferred this class, which was 29.01% for females and 26.67% for males.

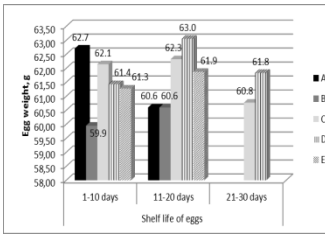

Figure 1

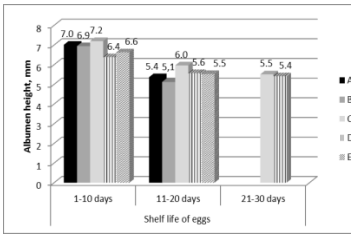

Figure 2

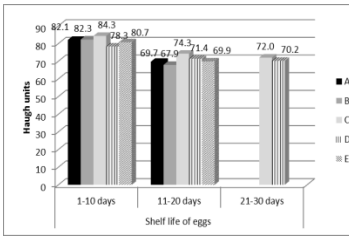

Figure 3

Figure 1. Egg weight in super/hypermarkets
Figure 2. Albumen height depending on shelf life of eggs and hypermarket
Figure 3. Haugh units depending on shelf life of eggs and hypermarket

The analysis of data for Haugh units showed that with the increase in shelf life of eggs, the number of Haugh units decreased, i.e. the quality of eggs declined, indicating that three markets that offered only eggs from the first two groups (up to 20 days) had better offer for consumers compared to two markets where the shelf life of eggs ranged from 1 to 30 days. The number of Haugh units decreases with egg shelf life is also confirmed in the study *by Jin et al., (2011),* which can be linked to research results suggesting that the egg has the best quality immediately after laying and that it is further reduced in dependence from further manipulation (*Pavlovski et al., 1996).*

The results of egg quality analysis in Belgrade hypermarkets are given in Table 3.

Table 3. Quality of eggs in Belgrade hypermarkets depending on the manufacturer

Market											
	A						B		C	D	E
Manufacturer											
Trait		1	2	3	4	5	6	7	8	9	10
Egg weight, g	$\bar{x}$	60.9^{c}	63.9^{ab}	62.4^{abc}	64.1^{a}	62.1^{abc}	61.7^{bc}	61.8^{bc}	61.3^{c}	62.1^{abc}	62.0^{abc}
	SD	1.7	1.8	0.8	1.9	1.2	1.4	1.4	1.7	1.8	1.6
Egg shell colour, points	$\bar{x}$	4.0	3.6	3.9	4.2	4.1	4.2	4.1	4.0	3.9	4.0
	SD	0.5	0.7	0.3	0.4	0.6	0.4	0.6	0.5	0.6	0.00
Egg shell cleanliness, point	$\bar{x}$	5.0	5.0	5.0	5.0	4.9	5.00	4.4	5.0	5.0	5.0
	SD	0.0	0.0	0.0	0.0	0.3	0.0	0.9	0.0	0.0	0.0
Egg yolk colour, (Roche)	$\bar{x}$	12.9^{a}	12.9^{a}	12.5^{abc}	10.9^{e}	12.7^{ab}	12.0^{bcd}	11.6^{de}	13.2^{a}	12.8^{cd}	12.8^{ab}
	SD	0.3	0.3	0.5	1.0	0.5	0.5	0.7	0.9	0.4	0.6
Albumen height, mm	$\bar{x}$	6.5^{bcd}	8.0^{ab}	7.4^{ab}	5.9^{cd}	7.2^{abc}	8.5^{a}	5.7^{d}	6.6^{bcd}	7.2^{abc}	6.8^{bcd}
	SD	1.1	0.8	0.8	0.7	1.0	0.9	1.1	1.3	1.2	0.8
Haugh Unit (HU)	$\bar{x}$	79.5^{bcd}	88.0^{ab}	85.0^{ab}	74.1^{cd}	83.8^{abc}	91.7^{a}	73.2^{d}	80.7^{bcd}	83.0^{abc}	81.6^{bcd}
	SD	8.4	4.3	4.2	5.5	6.4	4.9	9.4	7.0	8.5	5.4
Shell thickness, 0.01mm	$\bar{x}$	40.5	41.5	40.3	38.7	41.2	39.6	41.8	40.2	38.4	41.9
	SD	2.1	2.1	2.3	4.2	2.3	2.1	3.0	2.6	2.0	2.5

* a-d average values in each row without common superscript are significantly different at p <0.01

Based on the fact that consumers appreciate the freshness of eggs, and that it is important to both female (73.96%) and male (71.43%) consumers in this study, the study of the quality of eggs was carried out only in the group of eggs of shelf life 1 to 10 days. The established values of the parameters, in all markets (5), and for all manufacturers (10), for egg weight ranged from 60.9 to 64.1g, shell colour from 3.6 to 4.2, egg cleanliness from 4.4 to 5, yellow colour (Roche) of 10.9 to 13.2 and the number of Haugh units from 73.2 to 91.7. The results of the study indicate that the quality of the eggs differed significantly, observed within single retail facility/market and at the level of all retail facilities/markets, for the properties of egg weight, colour of yolk, albumen height and Haugh units. The statistically significant differences between the manufacturers can be associated with the research of *Škrbić et al. (2006)* indicating the variability of individual quality characteristics of table eggs depending on the manufacturer. Given that the quality of the eggs is influenced by a large number of different factors prior to laying - genetics, production system, nutrition, chicken health, chicken age, and after the laying of eggs - conditions for storage, packaging, transport and sales points, as well as the shelf life of eggs *(Pavlovski et al., 2007; Tolimir et al., 2008; Jin et al., 2011),* the quality of eggs in the retail facilities could be viewed as a result of the collective impact of all of these factors.

Pavlovski et al. (2007) state that for consumers an optimal egg weight is between 53g and 73g, while eggs of good freshness are those with 75 or more Haugh units, which could classify eggs obtained from 8 of the total of 10 analyzed manufacturers be considered as eggs of good quality. Observed according to the number of Haugh units, the quality of eggs of all manufacturers was satisfactory, i.e. the eggs showed values ranging from 73,2 to 91,7 Haugh units). The obtained values for the clenliness of the shell indicate that the clenliness of eggs in Belgrade retail facilities was satisfactory, as well as the strength of the shell, which should be about 0.375 mm *(Pavlovski and Vitorović, 1996*). The established colour of the yolk, irrespective of the variation between the manufacturers, indicated that the eggs in the markets, were in line with the preferences of consumers in our area, considering that according to the survey, 27.62% preferred the medium yellow and 62.76% extremely yellow color of the youlk. Consumers preference to the more intense colour of the yolk, is also established in the study of *Pavlovski and Mašić (1994)*, according to whom the majority of consumers (56.5%) prefer the yellow colour of the yolk (up to 9 points on Roche scale), and 27% of consumers over 40 years of age prefer a dark yellow color (over 9 points on Roche scale).

Also, by analyzing the data (Table 3), it can be concluded that the number of brands within a single super/hypermarket ranged from 1, which was established in three retail facilities/markets (C, D, E), to 5 brands/manufacturers in one market (A), which indicates a different supply in this segment of the Belgrade market. Considering that according to the obtained results, the quality of the eggs of different producers, within and between the markets, varies considerably, and the survey survey indicates that a significant number of consumers in the City of Belgrade are not important producers (30.37%), while only 14.49% of manufacturers consider it very important when buying eggs, future trials should focus on additional parameters that are important for choice (price, packaging, etc.).

Considering that according to the obtained results, the quality of the eggs of different manufacturers, within single retail facility and between the retail facilities, varied considerably, and that the survey showed that for considerable number of consumers in the City of Belgrade the brand/manufacturer (30.37%) was not important, while only 14.49% considered the manufacturer as very important criterion when buying eggs, future studies should focus on additional parameters that are important for choice (price, packaging, etc.).

Conclusion

The first part of the study presents the results of the survey of the habits of egg consumers in the City of Belgrade (239 respondents - 27.78% female and 72.22% male) in terms of place of purchase, the criteria they have for choosing

when purchasing eggs - the importance of the brand/manufacturer and class of eggs, as well as what qualities of quality consumers prefer - the colour of yolk and freshness eggs. In the second part of the study, results are given regarding the supply of eggs on the Belgrade market, in super/hypermarkets and the quality of eggs of different manufacturers observed within single retail facility and between the retail facilities.

According to the survey, the majority of consumers in the City of Belgrade are purchasing eggs in super/hypermarkets (39.62%), and when purchasing eggs, the manufacturer is not important at all for 30.37% of respondents. At the same time, the freshness of eggs is very important for consumers (73.28%), they prefer to buy larger eggs (class SS, S and A are prefered by 69.82%) and prefer eggs of extremely yellow colour (62.76%).

In regard to the egg supply, differences were registered between the super/hypermarkets (the number of brands/manufacturers within the single retail facility/market varied from 1 to 5), as well as the differences in the freshness of eggs within a single market, or between individual markets. The results of egg quality in super/hypermarkets indicate that the quality of eggs, expressed in Haugh units, declines with the shelf life of eggs. The quality of eggs within the single retail facility/market differed statistically significantly, depending on the manufacturer, and significant differences were determined by comparing the manufacturers at the level of all retail facilities/markets for the properties of egg weight, colour of the yolk and Haugh units. The analysis of eggs displayed in retail facilities from 1 to 10 days, in all markets (5) and for all manufacturers (10), and the established values of egg quality parameters indicated that the quality of eggs in the Belgrade markets was satisfactory.

Kriterijumi potrošača pri kupovini konzumnih jaja i kvalitet jaja u marketima na području grada Beograda

Nataša Tolimir, Marijana Maslovarić, Zdenka Škrbić, Miloš Lukić, Borislav Rajković, Robert Radišić

Rezime

U cilju ispitivanja stavova potrošača jaja anketirano je 239 ispitanika na području Grada Beogradu, a ocena kvaliteta jaja na beogradskom tržištu obavljena je ispitivanjem kvaliteta jaja u super/hipermarketima. Kroz anketno ispitivanje potrošači su se izjasnili o mestu kupovine jaja, o kriterijumima pri kupovini i o značaju pojedinih osobina kvaliteta. Ispitivanje kvaliteta jaja odnosilo se na jaja

klase A, a obavljeno je na uzorcima jaja iz 5 super/hipermarketa, za ukupno 10 proizvođača jaja. Na osnovu rezultata ispitivanja konstatovano je da većina potrošača jaja kupuje u super/hipermarketima (39,62%), da pri kupovini nije bitna robna marka/proizvođača za 30,37% ispitanika, da najradije kupuju jaja veće mase (SS, S i A klase). Kvalitet jaja veoma je važan za 73,28% ispitanika, a boja žumanca koju preferiraju je iz kategorije izrazito žuta (62,76%). Ponuda konzumnih jaja bila je različita (od 1 do 5 proizvođača) po marketu. Kvalitet jaja, posmatrano za sve super/hipermarkete i sve proizvođače iskazan kroz Hogove jedinice opadao je sa starošću jaja. Kvalitet jaja iz kategorije 1 do 10 dana, u svim super/hipermarketima, posmatrano za sve proizvođače, bio je u okviru ispitivane klase A, pri čemu se masa jaja kretala od 60,9 do 64,1g, boja ljuske od 3,6 do 4,2; čistoća jaja od 4,4 do 5,0; boja žumanca (Roche) od 10,9 do 13,.2, a broj Hogovih jedinica od 73,2 do 91,7. Rezultati istraživanja upućuju na zaključak da potrošači u Gradu Beogradu poklanjaju pažnju značaju kvaliteta konzumnih jaja, da između marketa postoje razlike u pogledu ponude i svežine jaja, kao i da unutar jednog marketa i posmatrano na nivou svih marketa postoje razlike u kvalitetu, u zavisnosti od proizvođača.

Ključne reči: jaja, potrošač, anketa, market, kvalitet jaja, Beograd

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COMPARATIVE EXAMINATION OF THE MEAT QUALITY OF THE FEMALE CATTLE OF SIMMENTAL BREED AND CROSSES WITH CHAROLAIS BREED

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Abstract: The paper presents the results of a comparative examination of the beef quality obtained from females of two genotype groups: domestic Simmental breed (A) and its crosses with Charolais breed. The sample included a total of 20 heads, 10 in each group. Cattle were slaughtered at the same age with uniform body weights. After slaughtering, warm carcass sides were individually weighed, with and without kidney fat. After cooling, the left carcass sides were cut into the basic parts according to the Rulebook and the three-rib cut was cut off from the back part (9-10-11 rib). The content of tissues in the three-rib cut was statistically different among the groups, the content of muscle tissue was significantly higher ($p < 0.05$) in cattle of the group (A) and the content of fat tissue was statistically ($p < 0.05$) significantly higher in the group (B). The chemical composition of *M. longissimus dorsi* did not differ statistically between groups. The technological quality of the meat was evaluated through the tenderness of the *M. longissimus dorsi* which was statistically significantly better ($p < 0.05$) in the cattle of the group (B) and the content of total pigments statistically ($p < 0.05$) significantly higher in the cattle of group (B). The sensory traits of *M. longissimus dorsi* did not differ statistically significantly between groups.

Key words: Simmental breed, *M. longissimus dorsi*, instrumental colour, instrumental texture, sensory properties

Introduction

Meat is the indispensable component of the highest quality of the right and well-balanced human diet (*Biesalski, 2005*). The definition of meat quality is very complex and can be presented through nutritive, technological and sensory quality of meat.

Nutritional aspect of meat quality can be referred to the content of proteins and fats. Beef is characterized by exceptional nutritional value compared to other types of meat (*Petrović et al., 2002*). Numerous factors (breed, sex, age, diet, production method) affect the variation in the chemical composition of beef. Literary data on meat fat content vary and show great variability associated with production and genetic factors. The fat content in meat ranges from 1-20% (*Žlender and Gašparlin, 2005*). Although fats are considered to be an unfavorable meat component, fat and fatty acids are factors that determine the nutritional quality of the meat and significantly affect the sensory properties of the meat (*Lefaucheur, 2010*). The fat content of the muscle tissue contributes to the succulence, taste, texture and preferable sensory properties (*Šević et al., 2017*).

The technological quality of meat represents its suitability for different processing methods, and comprises technological and physical-chemical properties, pH value, colour intensity, firmness and uniformity of the meat structure (*Mancini and Hunt, 2005* and *Dalmau i sar., 2009*).

Sensory quality of the meat includes a range of properties (colour, marbling, tenderness, succulence, odour, taste) and has great impact on consumer satisfaction (*Dransfield et al., 2003*). In order to assess the quality of meat, a good knowledge of these meat properties is needed. The quality of meat is affected by the characteristics of the animal's muscles and *post mortem* biochemical reactions (*Ouali, 1990; Dransfield et al., 2003*).

The numerous biological, physiological and technological factors influence the yield and quality of beef meat. Consumer demands are changing in the direction towards better meat quality with less fat content. In order to achieve this, adequate nutrition of cattle is necessary, also important is the choice of breed for fattening, the system keeping and housing of fattening cattle and pre-slaughter body weight of animals (*Dokmanović et al., 2014*). The entire process of meat production, from genetics and selection, management of animals to processing and storage of meat, can affect the characteristics of meat. Therefore, the task of each segment of the production chain is to adapt production processes in order to ensure the production of desirable, quality meat (*Karolyi, 2006*).

Materials and Methods

The trial was carried out at the experimental farm of the Institute for Animal Husbandry (Belgrade, Serbia). Two groups of female cattle were formed: group A (n = 10) Simmental breed and group B (n = 10) crosses of F1 generation of this breed with the Charolais breed. Both groups of cattle were fed at will with a

combined diet consisting of whole maize plant silage according to the nutrition table depending on the weight group. Final pre-slaughter weights were uniform between groups. One day before slaughter, animals did not receive food, but they had free access to water. Animals were measured just before slaughter and then slaughtered according to standard commercial procedures. Slaughtering and primary processing of carcasses, cutting off the carcass sides and dissection of the three-rib cut were carried out in the experimental slaughterhouse, and the chemical composition, technological and sensory properties determined in the laboratory of the Institute for Animal Husbandry. The three-rib cut (9-10-11 rib) was separated from the left chilled carcass side by a cut along the cranial edge of the 9th and 11th ribs and the cut parallel to the spinal column. The weighing scale of accuracy of 0.001 kg, was used to measure the weight of muscle (especially measured M. *longissimus dorsi*), fat and connective tissue and bones. The chemical composition of the M. *longissimus dorsi* sample was determined (water content - method of sample drying at 103 ± 2 ºC (SRPS ISO 1442, 1998), fat content by the Soxhlet extraction method (SRPS ISO 1444, 1998), the amount of mineral matter (ash) by the method of sample burning at temp. 550 ± 25 ºC (SRPS ISO 936, 1999) and protein content by the Kjeldahl method (SRPS ISO 937, 1992).

Following technological properties of the *M. longissimus dors*i sample were determined: the cooking loss, based on the difference in weight of meat pieces (size: 3 x 4 x 1.5 cm and weight about 70 g) before and after cooking in distilled water (where the meat to water ratio was 1: 2) in a closed glass vessel (at 100 ° C for 10 min) and expressed in percentages relative to the weight of the sample before cooking (Official Gazette of SFRY, No. 2/85, 12/85 and 24/86); the roasting loss, based on the difference in the weight of the pieces of meat before and after roasting. The cut of *M. longissimus dorsi*, which was transversely cut into a muscle fiber parabolic, weighing 150 ± 1 g, was rolled into aluminum foil and roasted for 25 minutes at 250 ° C. The meat was removed from the foil immediately after roasting and measured/weighed. Softness (tenderness) of meat was determined using the consistometer according to Volodkevich (1938) by cutting a piece of meat transversely to the direction of muscle fibers. The pH value of meat, 24 hours post mortem, was measured using pH-meter with combined probing electorde Hanna HI 83141 (Hanna Instruments, USA). Before measuring the pH in the meat, the calibration of the pH meter was carried out using a buffer of known pH value (pH = 5).Determination of total pigments was carried out using the Horsney method (*Bunning and Hamm, 1970*) wherein the total pigment content was expressed in mg/kg. The *M. longissimus dorsi* cross-section was determined at the cross-section of the *M. longissimus dorsi* in the area of the 11th rib by marking on the paus paper and then measuring using the planimeter. Instrumental colour measurement was done with the Chroma Meter CR-400 (Minolta, Japan), which was previously calibrated against a standard white surface (illumination D65, viewing angle 2 ° and 8 mm probe) using fresh meat samples (24 hours *post-*

mortem). Meat samples were cut and left for 30 minutes in air to stabilize the colour. The values of the colour are presented in the CIE L*a*b* system (*CIE, 1976*), where the measure L* indicates the lightness of the meat, a * the relative share of the colour red and b * relative share of the colour yellow. For each sample of meat, three readings were made and their mean value was used for statistical data processing. Sensory assessment of odour, taste, tenderness and succulence of meat was carried on a piece of meat after determining the cooking loss. Seven semi-trained assessors were included in sensory evaluation. For each evaluated parameter, a quantitative-descriptive scale of 5 points was used: odour and taste : 1 - very bad, 2 - bad, 3 - nor bad or good, 4 -good, 5 - very good; tenderness: 1 - very firm, 2 –firm, 3 – nor firm or soft, 4 - soft, 5 - very soft; succulence: 1 - very dry, 2 - dry, 3 – nor dry or succulent, 4 - succulent, 5 - very succulent.

The obtained data were processed by analysis of variance in one-way ANOVA program SPSS Statistics 20, and all results are displayed as the mean value ± standard deviation. The statistical significance of the difference between mean values was determined by t-test.

Results and Discussion

Table 1 shows the proportion of individual tissues in the three-rib cut. The share of *M. longissimus dorsi* did not differ between groups. The share of other muscle tissue in the group (A) statistically significantly ($p<0.05$) differed from the group (B). The statistically significant ($p<0.05$) difference was found in the content of fatty tissue that was higher in group (B) and was 23.92% compared to cattle in group (A) - 18.89%.

In the research of *Petričević et al. (2015)* the share of *M. longissimus dorsi* is 29.9%, the remaining muscle tissue is 32.06%, fat tissue 19.93%, connective tissue 0.84% and bone 16.96% determined in the females of Simmental breed.

Table 1. The share of tissues in three-rib cut

Item	A	B	t-test
Three-rib cut (%)			
M. longissimus dorsi	34.26 ± 5.63	34.53 ± 4.00	ns
Other muscle tissue	30.43 ± 2.89	23.30 ± 3.80	*
Fat tissue	18.89 ± 5.40	23.92 ± 4.14	*
Connective tissue	0.71 ± 0.42	0.93 ± 0.08	ns
Bones	15.54 ± 6.61	16.67 ± 3.78	ns

ns – not significant

* significant at the level of ($p<0.05$)

The chemical composition of *M. longissimus dorsi* is shown in Table 2. There was no statistically significant difference in the chemical composition of *M.*

longissimus dorsi. *Petričević et al. (2015)* report the following values: the water content 73.54%, fat content 3.33%, ash 1.07% and protein 22.04% for females of domestic Simmental breed. In the paper by *Filipčík et al. (2009)*, the protein content is 21.13% for the female crosses of the Simmental and Charolais breed. *Śmiecińska et al. (2006)* state that the fat content ranges from 2.73 to 2.94%, protein 21.47 to 21.64%, ash 1.11 to 1.13% and water 74.00 to 74.90%, in female crosses of the Simmental and Charolais breed. In their research, *Bures and Bartoň (2012)* confirm the value of the protein content of 21.20% for the female crosses of the Simmental breed and Charolais, while the value of the dry matter is 26.60%.

Table 2. The chemical composition of M. *longissimus dorsi*

Item	A	B	t-test
Water, (%)	73.08 ± 1.58	73.11 ± 1.29	ns
Fat, (%)	2.80 ± 1.84	2.95 ± 1.42	ns
Ash, (%)	1.03 ± 0.09	1.08 ± 0.03	ns
Protein, (%)	23.07 ± 0.49	22.86 ± 0.32	ns

ns – not significant

Table 3 shows the technological properties of *M. longissimus dorsi* significant difference (p $p<0.05$) was found in meat softness that was more favourable in group (B). The content of total pigments was statistically significantly ($p<0.05$) higher in group (B) and amounted to 198.57 mg/kg compared to the group (A) - 161.02 mg/kg.

The colour of meat plays an important role in the purchase of meat and can be influenced by numerous factors before and after slaughter (*Mancini and Hunt, 2005*). The colour of the meat surface is inversely related to the iron content that increases with the age of the animal (*Chambaz et al., 2003*). Changes in the meat brightness can be explained by changes of the final pH and intramuscular fat content (*Priolo et al., 2001*). An increase in the pH value 48 hours *post mortem* leads to a change in the colour of beef (*Węglarz, 2010*). In the paper by *Filipčík et al. (2009)* pH value of 5.50, value L * 38.67, a * 12.52 and b * 11.05 are reported. The cooking loss is 28.50% for the female crosses of the Simmental breed and Charolais. In the research of *Petričević et al. (2015)*, the value of cooking loss is 26.64%, roasting loss 38.81%, meat colour (L *) 38.44%, softness 9.77% and pH24 5.52 for female cattle of domestic Simmental breed. *Śmiecińska et al. (2006)* state the pH value from 5.40 to 5.41 for female crosses of the Simmental breed and Charolais. *Bureš and Bartoň (2012*) cite the value of 58.6 cm^2 for the surface of the *M. longissimus dorsi* cross section, while the pH24 is 5.46 for the female crosses of Simmental and Charolais breed. The value of L* is 42.20, a* 13.70 and b* 13.00 for female crosses of the Simmental breed and Charolais (*Bures and Bartoň, 2012*).

Table 3. The technological properties of *M. longissimus dorsi*

Item	A	B	t-test
Cooking loss, %	40.60 ± 1,72	40.36 ± 0.21	ns
Roasting loss, %	40.07 ± 2.66	38.56 ± 0.23	ns
Softness/tenderness	10.93 ± 2.34	8.51 ± 0.72	*
pH_{24}	5.50 ± 0.07	5.56 ± 0.01	ns
Total pigments (mg/kg)	161.02 ± 52.19	198.57 ± 18.32	*
Cross-section surface *M. longissimus dorsi* (cm^2)	77.49 ± 9.78	76.07 ± 6.37	ns
Colour			
L*	36.42 ± 0.51	37.08 ± 0.19	ns
a*	19.54 ± 0.52	20.23 ± 0.35	ns
b*	5.86 ± 1.36	6.08 ± 0.07	ns

ns – not significant
* significant at the level of ($p<0.05$)

The sensory properties of cooked and roasted meat are shown in Table 4. Based on the results of the sensory evaluation of *M. longissimus dorsi*, the statistical significance of the parameters tested was not observed. The sensory evaluation (odour, taste, softness, succulence) of cooked and roasted meat was practically the same in both groups of cattle (average aggregate estimate), group (A): cooked meat - 4.61 and roasted meat - 4.92; group (B): cooked meat - 4.72 and roasted meat - 4.92. Better sensory estimates were determined in animals of the group (B) in all parameters.

Table 4. The sensory properties of *M. longissimus dorsi*

Item	A	B	t-test
Cooked			
Odour	4.88 ± 0.25	5.00 ± 0.00	ns
Taste	4.93 ± 0.19	5.00 ± 0.00	ns
Tenderness	4.37 ± 0.75	5.00 ± 0.00	ns
Succulence	4.25 ± 0.87	4.67 ± 0.58	ns
Roasted meat			
Odour	4.88 ± 0.25	5.00 ± 0.00	ns
Taste	4.75 ± 0.50	5.00 ± 0.00	ns
Tenderness	4.62 ± 0.75	5.00 ± 0.00	ns
Succulence	4.62 ± 0.75	4.67 ± 0.58	ns

ns – not significant

The tenderness (texture) and succulence of cooked or roasted meat, and to a certain extent aroma and taste, as reported by *Petričević et al. (2015),* are important parameters of meat quality. *Glitsch, (2000)* also states that the tenderness and taste are the most important attributes that determine the quality of food in Europe. Differences in the sensory properties of the meat can occur as a result of the different content of intramuscular fat in the meat (*Christensen et al., 2011).*

Based on the results of the sensory evaluation of the cooked meat, *Petričević et al. (2015)* state that the tenderness of the meat in female cattle is statistically significantly better compared to the males of the Simmental breed.

Conclusion

Based on the results of the study, it can be concluded:

- Simmental cattle of group (A) achieved statistically significant ($p<0.05$) greater share of remaining muscle tissue and significantly ($p<0.05$) lower share of fat tissue in the three-rib cut;
- Crosses of Simmental breed and Charolais - group (B) had statistically significant ($p<0.05$) better meat tenderness and significantly ($p<0.05$) greater content of total pigments;

Based on presented results, it can be concluded that the female beef of the domestic Simmental breed and crosses of Charolais with Simmental breed have approximately the same quality of meat except in regard to the tissue of the three-rib cut, which was better in the Simmental breed, and in regard to the meat tenderness which was better in crosses of Charolais with Simmental breed.

Uporedno ispitivanje kvaliteta mesa ženske junadi simentalske rase i meleza šarolea sa simentalskom rasom

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Rezime

U radu su prikazani rezultati uporednog ispitivanja kvaliteta mesa junadi ženskog pola dve genotipske grupe: domaće simentalske rase (A) i njenih meleza sa šarole rasom. Uzorkom je obuhvaćeno ukupno 20 grla, po 10 u svakoj grupi. Junad su zaklana u istom uzrastu sa ujednačenim telesnim težinama. Nakon klanja izvršeno je pojedinačno merenje toplih polutki sa i bez bubrežnog loja. Posle hlađenja leva polutka je rasecana u osnovne delove prema pravilniku i iz leđnog dela izdvojen je trorebarni isečak (9-10-11 rebro). Udeo tkiva u trorebarnom isečku se statistički razlikovao među grupama i to u udelu mišićnog tkiva koji je bio

statistički ($p<0.05$) značajno veći kod junadi grupe (A) i udelu masnog tkiva koji je bio statistički ($p<0.05$) značajno veći u grupi (B). Hemijski sastav *M. longissimus dorsi* se nije statistički razlikovao između grupa. Što se tiče tehnološkog kvaliteta, mekoća *M. longissimus dorsi* je statistički ($p<0.05$) značajno bila bolja kod junadi grupe (B) i sadržaj ukupnih pigmenata je statistički ($p<0.01$) značajno bio veći kod junadi grupe (B). Senzorne karakteristike *M. longissimus dorsi* nisu se statistički značajno između grupa.

Ključne reči: simentalska rasa, M. *longissimus dorsi*, instrumentalna boja, instrumentalna tekstura, senzorne osobine

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TECHNOLOGICAL PROCESS OF ADDED VALUE CHEESE MAKING ON REGISTERED AGRICULTURAL HOUSEHOLDS IN VOJVODINA

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Abstract. The technological process of cheese making is a process of transforming milk as a raw material into cheese and value adding. Small producers at registered agricultural households (RAHs) use milk of good quality that they produced. Also, they produce cheese by applying skills and experience as an indispensable part of quality, in contrast to big dairy plants where milk originating from a large number of producers is used, and furthermore the production automation is applied. RAHs produce many traditional cow cheeses, and more recently, goat and sheep cheese production is growing. Lisnati cheese (rolled cheese), "podliveni" cheese with or without spices, cream cheese, brined cheese "kriška", smoked goat cheese, various types of semi-hard and hard cheeses are products with added value, due to the good milk quality, as well as the specific technological process. Cheese yield contribute that profit is higher than in case when milk is sold to dairy plant. The aim of this study was to monitor the technological processes of the most important cheeses that are produced on RAHs in Vojvodina. Study is shown that quality should be constantly improved in order to achieve sustainability of these products on the market. When considering the assortment and quality of cheeses on RAHs in Vojvodina, it can be said that many of them have the characteristics of branding products. The impact of adding value to cheeses and other dairy products is manifested through higher primary milk production, employment and the livelihoods of people in the countryside, as well as the economic prosperity of small family farms in general.

Key words: traditional technology, cheese, registered agricultural households (RAHs), added value

Introduction

In the production on small registered agricultural households (RAHs) in Vojvodina, human and cultural resources are used in addition to natural resources, which are recognized as a factor of added value. The number of consumers that are looking for a healthier, fresh and unique product, where production and sales are close and in line with its requirements, is constantly increasing. It is important to point out that consumers in EU recognize quality of cheeses that are produced on small agricultural households and sold at an open market (*Havranek et al., 2012*). EU agricultural policy stimulates a certain form of extensive agricultural production that contributes to the protection of the environment, biodiversity and rural areas (*Samardžija et al., 2006*).

The production of value added dairy products provides a better profit than in case when milk is sold to dairy plant. It is also assumed that the trends of increased consumption of value added products will continue. This means that the investment in value added will be paid off more in the long run, but it is necessary to constantly monitor the market demands and that the product is always safe (*Živkov, 2013*). Small producers have a great possibility to develop and improve their own production through dairy products with a geographical origin (*Dozet et al., 2004; Popović-Vranješ et al., 2011*), as well as those with a particular specificity (*Đorđević et al., 2014*). Many of the cheeses that are produced on RAHs in Vojvodina meet the requirements for recognition of the origin (lisnati cheese, podliveni cheese, sremski cheese). Moreover, most RAHs provide conditions for hygienic production, storage and distribution as well as permanent control and education. The production based on the principles of good hygienic practice that guarantees the safety and quality of the product is essential. A support to producers is also provided by scientific and professional staff from the Faculty of Agriculture, Novi Sad (*Popović-Vranješ et al., 2015*).

Material and methods

The paper describes the technology, by surveying several cheese producers from the area of Vojvodina. For the last several years Provincial Secretariat for Agriculture, Water Management and Forestry of AP Vojvodina have supported small cheese producers in terms of plant adaptation, purchase of equipment, design and education. The activities were realized by the Faculty of Agriculture Novi Sad, in the period from 2013 to 2016. Moreover, cheese analyses were made at the Laboratory for quality control of feed and animal products, at the Faculty of Agriculture in Novi Sad. The composition of cheese was analyzed by applying standard methods. Weight loss after drying (*AOAC 926.08-1927*) was used for dry matter determination. Protein analysis was done by Kjeldahl-Van Slyke method for

total N determination (*AOAC 2001.14*). Fat content was determinated using butyrometric method (*AOAC 933.05*). NaCl content was calculated from sodium content which was analyzed by flame photometry (Sherwood, type M410) using a method described elsewhere (*Kirk and Sawyer, 1991*).

Results and Discussion

Lisnati cheese

Lisnati cheese is characterized by its spun paste structure and belongs to the group of *pasta filata* cheeses. According to the literature data (*Vujičić et al., 1998),* lisnati cheese has been produced in Vojvodina since the late 1970s. Considering kashkaval cheese, it is possible that the production of *pasta filata* (spun paste) probably existed before. On RPGs in Vojvodina, lisnati cheese is especially appreciated and highly sought after by consumers today. It is a cheese that is well sold, has a good price (800-900 RSD/kg) and it is also interesting from the aspect of production profitability. For the production of lisnati cheese, it is important that the one who directly produces cheese (cheese maker) has the skills required for its making. The best quality of the cheese, in terms of mixing and curd stretching, is achieved if the acidity of the mixture (sweet and sour milk) is 16-17 ºSH while the pH value of the mixture is from 5.5 to 5.6 (*Popović-Vranješ, 2015*). Based on the technology recordings at some manufacturers, it can be noticed that there are differences which may reflect the quality, among other things. After stretching, the paste is shaped into a 1-3 kg roll, 10 cm in diameter and 20-30 cm in length. The stratification and the spun structure of the paste is clearly visible on the cross-section. It can be produced with or without both, spices and ham. Producers on RAHs, work in their modest conditions and with smaller amounts of milk, so the method of production mainly resembles the traditional old technology of the Balkan kashkaval cheese. Similar cheese is produced in Montenegro (*Mirnecki et al., 2012).* Lisnati cheese is produced from raw cow, sheep or mixed milk, by letting 1/3 of milk to spontaneously ferment. The fermentation lasts for up to 24 h, resulting in a slightly sour and gentle curd. On the next day, the obtained curd is mixed with 2/3 fresh milk wherein the rennet is added, stirred well and heated at 36-37 ºC. In the heated milk, rennet is added in such amount that milk is coagulated in about half an hour. After that, the resulting curd is cut and mixed in a warm whey until is shaped in a lump. Then the whey is decanted, the lump is divided into several parts and cooked (steamed) in water heated at 65-70 ºC. Finally, the cheese mass is stretched into a spun paste.

Based on the composition, this cheese belongs to a group of full-fat cheeses, since the content of milk fat in dry matter is 52.03%. According to the moisture content in fat-free matter (71.65%), it can be categorized as soft cheese.

Lisnati cheese is a real specialty that is produced with various additives (spices, ham, etc.). It is also sold under the name "rolled" cheese. It has a characteristic of soft, elastic and smooth structure, mildly acidic (pH 5.3) and porcelain white color.

Table 1. The composition of different cow cheeses collected on RAHs in Vojvodina

Cheese type	"lisnati" cheese	Kashkaval	Podliveni	Podliveni with paprika	White brined cheese "kriška"	Cottage	Semi-hard
RAH*	Kikinda (S.T.)	Rusko Selo	Obrovac (J.L.)	Obrovac (J.L.)	Obrovac (J.L.)	Vrbas (M.I.)	Čurug (P.R.)
Fat (%)	23.52	25.60	16.00	17.00	21.80	3.67	24.10
Protein (%)	12.54	28.50	24.61	24.30	23.41	10.76	29.09
DM (%)	45.20	55.87	38.00	41.00	48.67	17.46	59.61
FDM (%)	52.03	44.03	43.00	40.00	45.00	20.87	39.42
MFFM (%)	71.65	58.52	67.85	67.46	65.64	85.62	52.79
NaCl, %	1.50	2.00	1.00	1.00	1.60	1.50	1.80

DM – dry matter; FDM – fat content in dry matter; MFFM – moisture content in fat free matter
* RAH locations, RAH owner's initials in brackets

Kashkaval cheese

The production of kashkaval cheese in Vojvodina is based on traditional technology that is used for kashkaval cheese production on Stara Planina and Pirot area. This technology is mainly based on Balkan production technique from raw cow or sheep, or mixed cow and goat milk. The entire process of cheese making has remained specific and promising for small producers to date (*Mančić, 1994*). Immediately after milking, the milk is warmly pre-coagulated, and its acidity should be 8-9 ºSH. If the milk is fresh, it is necessary to wait for acidity (biological ripening) to increase. When the acidity is increased, the liquid rennet is added in ratio 40-45 ml per 100 l. After the coagulation, when the curd looks nice, glasslike, when it ruptures when cut by knife, and when nice, clear whey starts to separate, the curd should be cut into cubes about 2 cm in diameter. Afterwards, the curd is mixed (crumbled) until the corn size grains are obtained. In the next stage, the curd is left to stand for about ten minutes to settle ("sediment") and harden, and then the whey is separated and prepressed. After 1.5-2 h of prepressing, a cheese lump is obtained which is subjected to further processing. The curd is then cut with a knife into a 20x15x10 cm (weighing 2-3 kg) pieces and placed in a 20-cm thick layer on the cheese table until reaching a pH of 5.1-5.2 (baskia ripening). In a separate vessel or cheese boiler the water is heated to 70-80 °C and about 7% NaCl is added. Then, the baskia is cut into a perforated bowl (a stainless-steel basket) and "cooking (steaming)" is performed, whereby the cut mass is transformed into a rubbery, stretchy paste, with a gleaming look. A cheese is placed on a cheese table and a skillful master kneads it as the dough for bread. In the next stage, the cheese

is molded by placing it in a mold. Some producers, after putting cheese into the mold, narrowly puncture it using a 1-2 mm thick needle. A total of 20-30 punctures are made. Cheese is dried in molds for 24 hours. During this period, the cheese is flipped and dried. When the cheese is dry, it is transferred into the cooling chamber. After cooling, the cheese is packaged and can be sold. If it is desired to ripe the cheese, then it is placed in the chamber for ripening, where the temperature is 15-18 ºC, and the humidity is 75-80%. During the ripening process, the cheese is nurtured and flipped. Kashkaval cheese is pale-yellow in color, paste is monolithic, partly spun and elastic.

Regarding kashkaval cheese that is sold almost fresh (Figure 1C), milk fat in dry matter was 44.03%, which is a little below the minimum for full-fat cheese (55%) prescribed by Serbian regulation (2014) and Institute for standardization of Serbia (*SRPS E.C2.010:1997*). The water content in fat-free matter corresponded to semi-hard cheesed (55.87%). Considering its composition, it is very close to the standard for Pirot cheese (*Ostojić et al., 2012*).

Production of this cheese is present in a large number of RPGs in Vojvodina. This is because the technology is simple, it is mostly made from raw milk, thermal processing of curd ensures product safety, and it is sought on the market especially for the production of pizza. Finally, if not sold, it can be left to ripe, giving it a distinctive taste and smell of mature cheese made from the steamed cheese paste.

Podliveni cheese

Podliveni cheese is a variant of Serbian brined cheese with small modifications of technology, which is produced in some households from raw full-fat and or partially skimmed milk (cream is removed) (*Popović-Vranješ, 2015*). In the production of fresh milk (36 °C) on the household, 10 l of milk is poured into a deep pan. Four tablespoons of rennet are poured in 1 dl of water and stirring is performed for 5 min. Then, the curdled milk is left for about 40 minutes. During that time, the mass coagulates and looks like fermented milk. The following step is the curd cutting to large cubes which are then transferred into the cheese cloth and put into the press. The pressing lasts for 3-4 hours. For 1 kg of cheese, about 5 l of whole milk is utilized. In small dairy plants, which own duplicators, “podliveni” cheese is produced from full fat pasteurized milk. Pasteurization is done in a duplicate at 62-72 °C, where the milk is kept for 20 min. After pasteurization, the milk is cooled to 32 °C, liquid rennet is added and stirred. Then, the milk is allowed to settle, so the coagulation is performed in about 30 minutes. Normally, it is waited until the curd is separated from the container. The curd is cut into 15x15 cm cubes and it is allowed to settle for 15-20 minutes to isolate the whey. The curd is slowly released into the press and left for 30-60 minutes to self-press. Then it is progressively loaded for 1-2 hours, by slowly increasing the pressure to 1.5-2 bar. After pressing, the curd is cut, salted a little, and then the whey is released (not

immersed in whey). The produced cheese can be put up for sale on the next day (pH 5.2 - 5.7). Podliveni cheese from skimmed milk is produced on households so that the milk is cooked in deep pans (10 l volume) and the cream is removed (Figure 1D, 1E). The remaining milk is then heated to boiling point. In the heated milk, 1 liter of fermented milk, 4-5 tablespoons of vinegar and NaCl are added. The mass is left to cool to 50 °C and then transferred into the cheese cloth and put in a small press (Figure 1D, 1E). Before pressing into the curd, peppers and parsley leaves can be added. In the press, cheese stands for 50 minutes with a gradual increase in pressure. Finally, the cheese is allowed to stand in the press for another 15 minutes without being "tightened" (no additional pressure). After the pressing is completed, cheese is extracted from the press and left for 1-2 hours to stand at room temperature. Then, the cheese is placed in the refrigerator to cool. After that, it is cut into slices and can be packaged and sold. It is a soft, semi-fat cheese.

If required, podliveni cheese with cream cheese consistency can also be produced from skimmed milk, with some modifications. Milk is thermally treated at 62-72 °C for 20 min. After that, it is cooled to 32 °C, and calcium chloride, rennet and sugar (2 tablespoons/10 l) is added. Then, 0.2 l sour cream (containing 20% fat) and a small amount of NaCl (3 tablespoons) are added to 10 l of milk. Finally, the previously explained procedure for whole milk cheese making is used.

Serbian white brined cheese “kriška”

This type of cheese is made quite often in Vojvodina and it can be a replacement for white soft cheese, although in most cases it is near semi-hard cheeses. Fresh white cheese called Serbian white cheese or “cheese slice” (Serbian: *kriška*)" is obtained by rennet coagulation of heat-treated milk. It is characterized by soft consistency, pleasant mild to lactic acid taste and white color. Before consumption it is usually stored in whey (*Živković, 1971*). On RAHs, fresh cow milk is heated at 18-20 ºC during summer and at 25-30 ºC in winter. The rennet is added into the pre-heated milk in the amount needed for the milk to curdle in 4-6 hours. In some households more rennet is added, so the curd is formed in an hour. The curd is cut crisscross into 4 pieces using a clean knife, and then into cubes. The green and clear whey that comes out of the curd is a sign that the milk is well coagulated. The curd is then placed in a cheese cloth and left to strain. Occasionally, it is shaken for the whey to come out. Then, the cheese cloth containing the curd is removed and placed on a cheese table. A plank and a stone are placed over the cheesecloth, or the pressure is applied using a vessel with water. This is the second stage of pressing. The pressure should be about 1-2 kg per 1 kg of curd. The cutting and pressing lasts 6-12-24 hours and continues until the cheese curd is well strained. The straining in winter can be done in the kitchen, and during summer months in the basement or other cooler room, so the cheese does not get over-fermented or spoiled. After the straining, the cheese curd is removed from the cheesecloth and cut into slices. Each slice is then salted on all sides and

when NaCl is absorbed, slices are put in ripening vessels (usually plastic bins). Each row of cheese slices is salted, and the container is filled up to the top. After a couple of days, the plank is placed on the cheese and pressed. When the cheese settles, the container is filled with more cheese. Cheese ripening lasts 2-3 weeks, and well-produced cheese can be kept for months. The production of this cheese itself is not difficult. It requires fresh cow milk, skillful staff and hygiene during cheese making. About 100 liters of milk yield approximately 10 kg of cheese. The specificity of the production of this type of cheese in brine is the use of raw milk as well as certain stages of the technological process. The curd processing and straining are directed to obtaining a cheese high in moisture and a soft curd. Salting is done using dry salt and in brine. At the beginning of ripening (2-3 days), when the intense development of lactic acid occurs, there is no pressing. Later, the ripening is done under pressure in salted whey or salted water (brine). After ripening, the cheese is packed and soaked in brine, and it goes on the market (pH 5.12, acidity 26.80 °SH). Kriška cheese of a good quality must have moderately sour-salty taste with a hint of unripen wall nuts. In the beginning, this taste is strongly pronounced, while with the ripening it slightly decreases. On the cross-section, the cheese has no or only few round holes. Technical holes are visible but in a small number, since it is a cheese with very small pressing. Mature cheese must melt in the mouth, although it is firm under the fingers (*Živković, 1971*). Based on the laboratory tests, this is a semi-hard (moisture in fat-free matter was 65.64%) and full-fat cheese (45% fat in dry matter).

Cottage cheese

In most households, cottage cheese is made from completely or partially skimmed milk. The production of cottage cheese with yogurt culture is a very interesting product and a relatively new technology that has found great application in smaller dairy plants (*Popović-Vranješ, 2015)*. It is a cottage cheese that is widely used in the bakery industry and is quite demanded and profitable product. The skimmed milk (0.5-1%) is highly pasteurized (92 °C for 10 minutes), cooled to 45 °C, yogurt culture is added and allowed to ferment to 30 °SH (pH about 4.6). In this way, yoghurt is practically the first to produce. Then, gentle mixing and slight warming up to 65 °C for 15 minutes is involved. If the cheese is made from full-fat milk, the curd is heated to a higher temperature (65-70 °C for 15 minutes). When the required temperature is reached, the mixer and heat source are switched off, and it is waited for the curd to form in 20-30 minutes.

Next, the mass is slightly cooled to 55-60 °C and released into cheesecloth to strain. If the cheese is made from the skimmed milk, the straining takes about 2-3 hours, and if full-fat milk is employed this is 4-5 hours at room temperature. After straining, cheese is placed in the container, then mixed and salted (just under 1% NaCl). After that, the cheese is transferred into the refrigerator and cooled to 4-8 °C. Finally, it is packed in 10 kg plastic containers or 500 g plastic bags. Cheese

yield from skimmed milk is from 3.5 to 4.0 l/kg, while from full-fat milk this is 3.1-3.3 l/kg. Based on the water content, consistency and paste structure, this cheese belongs to a group of fresh cheeses *(Serbian regulation, 2014)*. Considering the water content in fat-free matter of cheese (more than 80%) this product can be categorized as soft cheese. The taste is sourly (pH 4.40-4.58, 35.45-36.22 °SH) and salty, consistency is creamy, and no whey is separated. It is practical for use in the production of pies and various cheese pastries.

Semi-hard cheese

Semi-hard cheese is mainly made by producers who have production equipment and a chamber for ripening. Cheese is made of pasteurized milk, which is cooled to 31-32 °C, with the addition of calcium chloride (0.02%) and mesophilic culture. After biological ripening of milk, rennet is added and left for 40-60 minutes to coagulate. Afterwards, the curd cutting, and mixing is performed. Next, the curd is heated at 36-38 °C and slowly mixed to the size of the pea grain (20 min), followed by molding and pressing. Salting in brine (containing 18% NaCl) for 1 day is applied when the pressed cheese pH is 5.3-5.4. After salting, the cheese is dried in the room where it is salted and then transferred to the chamber for ripening (relative humidity 75-80%, temperature 14-16 °C). During the ripening, cheese is cultivated (flipped and coated with salty water or Plasticoat®). Some manufacturers place cheese in semi-permeable foil, vacuum it and leave it to ripe. After ripening is done (2-3 weeks) cheese is packed and sold. Based on the content of fat in dry matter (39.42%) it can be categorized as a full-fat cheese, and on the basis of the moisture content in fat-free matter (52.79%) this cheese belongs to a group of semi-hard cheeses (*Popović-Vranješ et al., 2004*).

Figure 1. Cow cheeses form RAHs in Vojvodina. **(A)** Lisnati cheese, (T.S) Kikinda, **(B)** Lisnati cheese, (T.S) Kikinda, **(C)** Kashkaval cheese, (M.R.) Rusko selo **(D)** Podliveni cheese, (L.J.) Obrovac **(E)** Podliveni cheese, (L.J.) Obrovac, **(F)** Kriška cheese, (L.J.) Obrovac, **(G)** Cottage cheese, (M. I.) Vrbas, **(H)** Semi-hard cheese, (P.R.) Čurug

Goat milk cheeses

At this moment in Vojvodina, the entire amount of goat milk is processed in the traditional way and is done mostly on RAHs of goat breeders. There are some differences in the technology on individual farms that are usually linked to the cheese assortment. In the goat cheese assortment, which is getting larger every day, there are mostly white kriška cheeses that are sold in brine or vacuum, semi-hard or hard cheeses in vegetable oil with olives and various spices, various varieties of fresh cheeses in the form of cream cheeses with and without various spices. Semi-hard cheeses are also produced quite often, while hard cheeses are produced somewhat less. Some RAHs also produce smoked cheese. There are producers who produce kashkaval and rolled cheese made from goat milk.

Goat brined cheese "kriška"

The production of goat brined cheese "kriška" is done from raw or pasteurized milk, depending on the producer. If the production includes raw milk, then the milk is used immediately after the milking while it is still warm. The rennet is added, mixed and left for an hour. When a nice curd is obtained, the whey is drained. A small amount of warm water is added, the mixture is mixed, and then waited for whey and water mixture to separate. The mass is transferred into the

mold and the whey is drained by self-pressing. The cheese is cut into slices, packed and sold. Some manufacturers place cheese in jars, add vegetable oil and various spices. Manufacturers who have appropriate equipment, produce kriška cheese from pasteurized goat milk. Into the milk heated at 36-37 °C, calcium chloride and mesophilic culture are added, and the milk is kept a bit to biologically ripe. Then the rennet is added, and the milk is left to coagulate (about 1h). Further, crisscross cutting, cutting into cubes, short mixing without reheating, and curd transferring into the pre-press is performed. Pressing takes 1-1.5 h (low pressure 1-1.5 bar) on the pre-press. Then, the cheese slices are arranged into containers and dry salting (1.5% NaCl) is done. The pH is 6.5 and the height of slices is 3-4 cm. The cheese is quite soft and gentle when it is arranged. Arranged and salted in such manner, the cheese is pressurized for 12 hours. The next day, cheese pH is 5.2-5.4. Some producers immediately cut the cheese, vacuum it and put it up for sale, while others leave slices to ripe a bit longer. The ripening is performed in brine (4% NaCl) at 15-16 °C for about 10 days. At that moment, the cheese pH is 4.2, it is sufficiently solid and compact, and slightly sour. As such, it is sold in bins, or washed in water, dried and then packed in vacuum bags. According to the laboratory tests, the moisture content in fat-free matter was ranged from 70.75 to 85.06%, which categorizes it as soft cheese, and based on the fat content in dry matter (45.59-45.83%), it is a full-fat cheese (*Serbian regulation, 2014*).

Table 2. The composition of goat cheeses collected on RAHs in Vojvodina

Cheese type	Kashkaval	Brined cheese "Kriška"	Hard cheese	Semi-hard cheese	Semi hard cheese with spices	Soft brined cheese "kriška"
RAH*	Karađorđevo (M.Ć.)	Karađorđevo (M.Ć.)	Kanjiža (E.K.)	Srpski Krstur (J.B.)	Srpski Krstur (J.B.)	Srbobran (K.D.)
Fat (%)	22.40	25.00	27.50	26.60	24.99	20.06
Protein (%)	24.00	23.36	23.00	24.08	25.12	19.45
DM (%)	58.35	46.86	60.08	58.74	60.31	44.00
FDM (%)	45.39	53.35	45.83	45.22	41.44	45.59
MFFM (%)	65.27	70.75	55.24	56.28	50.92	85.06
NaCl, %	2.00	2.10	2.00	2.87	2.00	1.50

DM – dry matter; FDM – fat content in dry matter; MFFM – moisture content in fat free matter
* RAH locations, RAH owner's initials in brackets

Semi-hard and hard goat cheese

Semi-hard goat cheese is produced in the same way as semi-hard cheese from cow milk, except the curd is processed at a lower temperature (36-37 °C). The curd is slightly gentler and placed in molds. At the beginning, more frequent flipping in the molds is performed. Later, the frequency decreases. The molded cheese is placed in brine (containing 17-18% NaCl) for 24 hours, and then dried. After this, the cheese ripens at 14-16 °C, relative humidity of 75-80%, and

ventilation of 4 air changes/24h). The ripening can be in foil and without foil, and takes 2-3 weeks. During the ripening process, the cheese is flipped. In case of cheese ripening process without foil, in addition to flipping, cheese nourishing is performed. Some producers place the cheese into nettings and exposed it to a smoke for a few days. Then, the cheese is vacuumed and sold.

Hard goat cheese is produced from pasteurized milk (72 °C) cooled to 32 °C, then calcium chloride, mesophilic and/or thermophilic cultures are added. When the milk is ripened, the rennet is added. Once the cheese curd is firm enough it is cut, and the cheese grain is processed at 38-40 °C. Further, the cheese grain is transferred to the pre-press or directly into the molds. The pressure per kilogram of cheese is initially lower, and later it reaches up to 3 kg/kg of cheese. During a 4-hour pressing, the cheese should be flipped twice so that microbiological processes could be developed as smoothly as possible. Pressed cheese is salted in brine for 24-48 hours, depending on the size of the cheese. Brine is maintained at 11-14 °C and an acidity of l8-20 °SH. The NaCl concentration should be 20% or 19 °Be. After salting is done, cheese is placed to ripen at 14-18 °C and relative humidity of 75-85%. In ripening room daily care is done, and includes cheese rubbing using cloth pre-dipped in NaCl solution, cheese flipping, manual cheese coating, repeatedly. Cheese ripening takes 60 days in total. After ripening is finished, cheese is packed and placed on the market. Table 2 shows the composition of goat cheeses on individual RAHs. In figure 2, goat cheeses from individual farms are shown.

Figure 2. Goat cheeses form RAHs in Vojvodina. **(A)** Kashkaval cheese, (M.Ć.) Karađorđevo, **(B)** Smoked cheese and cheese with spices (J.B.) Srpski Krstur, **(C)** Kriška cheese, (K.D.) Srbobran **(D)** Hard cheese, (E. K.) Kanjiža

Bearing in mind the requirements of the *Serbian regulation (2014)*, the composition of goat cheeses, with small deviations, was in accordance with the regulation. In terms of sensory properties, taste, smell, appearance and cross section, cheese is highly rated.

Considering the assortment and quality of cheeses from RAHs in Vojvodina, it can be said that many of them have the characteristics of branding products. Branding creates a picture of the added value for consumers. The food brand promises consumers food with added value (healthy, local, organic) (*Đorđević et al., 2014*).

Conclusion

This paper describes the technology of making important dairy products with added value on RAHs in Vojvodina to bring the production technology to a level that ensures high and standardized quality, and safe product in order to fulfill all the necessary conditions for acquiring the label originality and geographical origin. This can be achieved by encouraging the cooperation among individual

producers, scientific institutions and competent ministries. Many of the described products are missing or insufficient on the market. By investing in premises and equipment on RAHs, conditions in the technical and technological area have been created for the production of the described products. Correspondingly, this facilitates production, and increases the quality and quantity.

In the future, there is a need for branding certain cheeses from Vojvodina that are produced on RAHs. Formation of added value as a result of consumer skills and territorial strategies is becoming increasingly important. Adding value to products strengthens the territorial capacity of the area, improves the image of the territory and increases employment.

Tehnološki proces izrade sireva sa dodatnom vrednošću na registrovanim poljoprivrednim gazdinstvima u Vojvodini

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Rezime

Tehnološkim procesom izrade sira se vrši transformacija mleka kao sirovine u sir i dodavanje vrednosti. Mali proizvođači na registrovanim poljoprivrednim gazdinstvima (RPG) koriste svoje vlastito mleko koje je kvalitetno, proizvode sir primenom veština i iskustvo kao neizostavan deo kvaliteta, za razliku od mlekara gde se koristi zbirno mleko velikog broja proizvođača i koristi se automatizacija proizvodnje. Mali proizvođači na RPG proizvode veliki broj tradicionalnih sireva od kravljeg, a u novije vreme sve više i od kozjeg i znatno manje od ovčijeg. Lisnati sir (rolovani sir), podliveni sir sa i bez začina, sir-kajmak, sir kriška, kačkavalj od kravljeg i kozjeg mleka, dimljeni kozji sir, razne vrste polutvrdih i tvrdih sireva, su proizvodi sa dodatom vrednosti, kako zbog kvaliteta mleka, tako i specifičnog tehnološkog procesa. Randman sireva je takav da se prodajom mleka kroz sir znatno povećava ukupna zarada. Na osnovu praćenja tehnološkog procesa proizvodnje sireva sa dodatom vrednosti na RPG u Vojvodini, konstatovano je povećanje kvaliteta koji se za duži opstanak na tržištu, stalno mora unapređivati. Kada se posmatra asortiman i kvalitet sireva sa RPG u Vojvodini, vidi se da mnogi od njih poseduju karakteristike proizvoda za brendiranje. Uticaj dodavanja vrednosti sirevima i ostalim mlečnim proizvodima, pokazuje se kroz veću primarnu proizvodnju mleka, zaposlenosti i egzistenciju ljudi na selu, u celini ekonomski prosperitet malih porodičnih gazdinstava.

Ključne reči: tradicionalna tehnologija, sir, RPG, dodata vrednost

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DETECTION OF SUBCLINICAL MASTITIS IN DAIRY COWS USING CALIFORNIA AND DRAMINSKI MASTITIS TEST

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Abstract: Control of udder health is an essential element in the process of safe milk production. Thus through the mastitis control program dairy farms regularly conduct measures of detection and prevention of udder diseases. Subclinical mastitis is an important disease of dairy cows causing economic losses and physical and chemical changes in milk. The aim of this research was to evaluate the usefulness of the California and the Draminski mastitis test to detect the subclinical mastitis in dairy cows. The efficacy of indirect mastitis tests for diagnosis of the subclinical mastitis was determined by comparing results of mastitis tests with bacteriological findings. The experiment was conducted on two dairy farms (farm A and farm B) Holstein - Friesian breed. A total of 245 quarter milk samples were examined, 95 quarter milk samples with the California mastitis test from farm A and 150 quarter milk samples with the Draminski mastitis test from farm B. A quarter milk samples for bacteriological analysis were taken aseptically during the morning milking in sterile test tubes. On farm A, bacteria growth has not been detected in 46.32% (44/95) quarter milk samples, while on farm B negative bacteriological findings have been found in 50.67% samples (76/150). In present study, sensitivity of the California mastitis test (78.57%) is higher than sensitivity of the Draminski mastitis test (74.32%). The specificity of the California mastitis test and the Draminski mastitis test is 82.05% and 30.26%, respectively. Efficacy of the California mastitis test in detection of the subclinical mastitis in dairy cows is better than that of the Draminski mastitis test, since accuracy of the California mastitis test has been higher.

Key words: California mastitis test, Draminski mastitis test, subclinical mastitis, cow

Introduction

In modern livestock industry, mastitis is one of the most important diseases of dairy cows which cause a huge production loss. Mastitis is an inflammation of the mammary gland that leads to physical and chemical changes in milk and affects on the production of dairy cows (*Khan and Khan, 2006; Boboš et al., 2013*). Early detection of mastitis is important for dairy farmers to reduce economic losses which are associated with reduction in yield, increased treatment costs and discarded milk (*Bhutto et al., 2012*). Mastitis most common occurs in one of two forms - a clinical or a subclinical infection. Detection of clinical mastitis is easy, because of the visible changes in the affected mammary gland and its secretion, while diagnosis of subclinical is problematic since cow shows no physical symptoms. The milk can appears normal during subclinical mastitis, but more common can notice the increase somatic cell count and concentration of certain ions, Na^+, K^+, and Cl^-.

Various screening methods are used for diagnosis of subclinical mastitis during lactation, based on physical and chemical changes of milk (*Sharma et al., 2010*). They have differences with respect to accuracy (sensitivity and specificity) and cost (*Pyorala, 2003; Fosgate et al., 2013*). According to the International Dairy Federation (IDF) recommendations, detection of mastitis is based on the somatic cell count and microbiological status of the udder quarter. However, somatic cell count increased in the first week postcalving and may remain high up to the first month of lactation (*Atakan, 2008*) and again increased towards the end of lactation as normal physiological condition (*Sharma and Pandey, 2011*). The definitive diagnosis of mastitis requires the isolation of pathogenic bacteria, but this is an expensive method which requires time. Besides that, this method does not provide a measure of the degree of inflammation associated with the infection. The California mastitis test, first described and used by Schalm and Noorlander in 1957, is a simple, quick, inexpensive and rapid test that accurately predict the somatic cell count in milk (*Bhutto et al., 2012*). The California mastitis test is based upon the amount of cellular nuclear protein present in the milk samples. The number of somatic cells in milk increases as the inflammatory process develops in udder tissue. Electrical conductivity/resistance of milk has been used as indicator of mastitis since four decades, and it has a positive correlation with somatic cell count. Electrical conductivity is determined by the concentration of anions and cations in milk. As a results of the damage to the udder tissue during mastitis, concentration of lactose and potassium decrease, and concentration of sodium and chloride increase. Hand-held meters, such as Draminski mastitis test, have been promoted as a screening tool for subclinical mastitis in some countries (*Fosgate et al., 2013*). However, data on the diagnostic value of this method is contradictory. Some authors point to a good correlation between electrical conductivity and

bacteriological tests (*Nielen et al., 1992*), while others consider this method to be insufficiently sensitive (*Pyörälä, 2003*).

The aim of this research was to evaluate and compare the usefulness of the California and the Draminski mastitis test to correctly detect the subclinical mastitis in dairy cows.

Material and Methods

The experiment was conducted on two dairy farms (farm A and farm B) Holstein - Friesian breed in Autonomous Province of Vojvodina, Republic of Serbia. General condition and udder health status were evaluated by clinical examination of animals. Udders were examined visually and by palpating for the presence of any udder changes (redness, swelling, pain, heat). Also, milk samples from each quarters were examined for the presence of flakes and clots. Animals with visible signs of mastitis were not included in the study. Immediately after clinical examination and before milk sampling for bacteriological analysis, milk testing from each udder quarter was performed using California mastitis test and Draminski mastitis test for detection of subclinical mastitis. A total of 245 quarter milk samples were examined, 95 quarter milk samples with the California mastitis test from farm A and 150 quarter milk samples with the Draminski mastitis test from farm B.

California mastitis test was carried out according to the method described by *Schalm and Noorlander (1957)*, at cowside by mixing gently an equal volume of milk with reagent (2 mL). Milk colour changes or formation of a viscular gel are readable within 1-2 minutes. Based on the reactions, the results were graded as negative (-), trace (T), weak positive (+), distinct positive (++) and strong positive reaction (+++).

Draminski mastitis test mesaures electrical resistance of milk. Concentration of sodium and chloride ions increases in milk from infected quarters which lead to decreased milk electrical resistance. The results of the Draminski mastitis test were interpreted according to the manufacturer's instructions (above 300 units - high quality and healthy milk; the incidence of subclinical mastitis is very low; between 300 and 250 units - progressively increasing incidence of subclinical infection as the readings decrease; below 250 - indication of a rapid increase in the severity of infection as subclinical mastitis progresses to clinical states).

A quarter milk samples for bacteriological analysis were taken aseptically during the morning milking in sterile test tubes. Before sampling, teats were washed and desinfected with 70% alcohol. Each sample was marked with cow's identification number and teat from which sample was collected (fore - left, fore -

right, rear - left, rear - right), and submitted to the laboratory for microbiological examination at refrigerator temperature.

From each samples, 0,1 mL of milk was plated on Columbia blood agar base with 5% defibrinated ovine blood, MacConkey agar and Sabouraud dextrose agar. Plated were incubated for 24h to 48h (bacteria) and 5 days (yeasts. mould) at 37°C under aerobic conditions. Bacterial colonies were determined 24, 48 and 72 hours after incubation. The isolates were identified according to their cultural characteristics (shape, size and structure) and physiological features (Gram straining, pigment formation, catalase test, CAMP test, coagulase test).

Characteristics of the California mastitis test and the Draminski mastitis test were determined using the milk bacteriological culture as a gold standard control. Percent sensitivity, specificity and accuracy were calculated by the formulae of *Sharma et al. (2010).*

Sensitivity = TN / TP + FN x 100

Specificity = TN / FP + TN x 100

Accuracy = TP +TN / TP + FP + FN + TN x 100

TP - true positive; FP - false positive; TN - true negative; FN - false negative

Results and discussion

The study included 245 quarter milk samples from dairy cows without clinical signs. After incubation of milk samples the bacterial colonies were determined. On farm A, the California mastitis test was used for detection of subclinical mastitis, and a total of 95 quarter milk samples were examined. Results of the California mastitis test revealed that out of 95 quarter milk samples, the number of samples showing true positive, true negative, false positive and false negative were 44 (86.27%), 32 (72.73%), 7 (13.73%), 12 (27.27%) respectively. These results correspond with the findings of *Sharma et al. (2010)* and *Badiuzzaman et al. (2015).* In our research, the California mastitis test has high percentage sensitivity and specificity (Table 1). *Sargeant et al. (2001)* concluded that the California mastitis test could be used in dairy herd monitoring program as a screening test to detect cows with intramammary infection. On the other side, *Rice (1981)* amounts as disadvantages of the California mastitis test false positive reaction during early and late lactation period.

Table 1. Percentage accuracy of the California mastitis test used for the detection of subclinical mastitis on farm A taking cultural test as standard

Test	California mastitis test		Cultural isolation	
	N	%	N	%
Positive samples	51	53.68	51	53.68
True positive	44	86.27	51	100
False positive	7	13.73	/	/
True negative	32	72.73	44	100
False negative	12	27.27	/	/
Total samples examined	95		95	
Sensitivity (%)	78.57		100	
Specificity (%)	82.05		100	
Accuracy (%)	80		100	

The Draminski mastitis test was used for determination of milk electrical conductivity on farm B. Results of electrical conductivity with Draminski mastitis test revealed that out of 150 quarter milk samples, 72% (108/150) quarter milk samples showing positive and 28% (42/150) quarter milk samples negative results (Table 2). These results correspond with the findings of *Galfi et al. (2015). Chahar (2007)* and *Langer et al. (2014)* have reported lower percentage of true positive cases detected by Draminski mastitis test (38% and 7.6% respectively). *Langer et al. (2014)* indicated that detection of subclinical mastitis with hand-held electrical conductivity meter was very low. *Henningsson et al. (2005)* indicated that milk electrical conductivity is determined by type and concentration of ions, interactive influence of the ions and components contributing to milk viscosity (protein, fat, lactose). During subclinical mastitis, concentration of sodium and chloride ions increases which leads to increased electrical conductivity in milk (*Kitchen, 1981*). Research of *Norberg et al. (2004)* pointed that cows with subclinical mastitis may not always show an increased electrical conductivity of milk from the infected quarter, but the variation in electrical conductivity of milk from infected udder quarter may be larger than variation in electrical conductivity of milk from healthy quarters, while *Sheldrake el al. (1983)* indicated that higher values of electrical conductivity of milk in infected quarters can be noticed only in that quarter. *Morsi et al (2000)* indicated that milk chlorine percentage alone cannot judge the presence of mastitis as it usually give high results in colostrums or at late stage of lactation. Many factors have influence on the measurement of milk electrical conductivity such as breed, lactation stage, age of cow, oestrus, milk temperature, pH and fat concentration in milk (*Biggadike et al., 2000*).

Table 2. Percentage accuracy of the Draminski mastitis test used for the detection of subclinical mastitis on farm B taking cultural test as standard

Test	Draminski mastitis test		Cultural isolation	
	N	%	N	%
Positive samples	108	72	76	50.67
True positive	55	50.93	76	100
False positive	53	49.07	/	/
True negative	23	54.76	74	100
False negative	19	45.24	/	/
Total samples examined	150		150	
Sensitivity (%)	74.32		100	
Specificity (%)	30.26		100	
Accuracy (%)	52		100	

Dingwell et al. (2003) and *Midleton et al. (2004)* indicated in their research that an ideal screening method for detection of subclinical mastitis would have maximum sensitivity to minimize the proportion of false negative results, and also a reasonable degree of specificity to reduce the number of false positive results. Our results suggest that the California mastitis test and the Draminski mastitis test had good sensitivity as predictors of subclinical mastitis. The California mastitis had good specificity, too, but the Draminski mastitis had low specificity, what is its main weakness. Low specificity of the test leads to incorrectly identification a high percentage of udder quarter as infected. Many authors mentioned that the California mastitis test is the most sensitive and specific indirect test for detection subclinical mastitis in dairy cows (*Iqbal et al., 2006; Joshi and Gokhale, 2006*). *Galfi (2016)* pointed that the California mastitis test has higher sensitivity and specificity than Draminski mastitis test and it is better method in detection of subclinical mastitis.

Conclusion

Indirect diagnostic methods, such as the California mastitis test and the Draminski mastitis test, can be used by dairy farmers to identify infected udder quarters and help them to avoid economic losses of mastitis. Our research indicated that the efficacy of the California mastitis test is better than that of the Draminski mastitis test, since accuracy of the California mastitis test has been higher. The California mastitis test represent valuable diagnostic methods in detection of subclinical mastitis in dairy cows, while Draminski mastitis test should not be used as the sole method.

Detekcija subkliničkog mastitisa kod visokomlečnih krava primenom Kalifornija i Draminski mastitis testa

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Rezime

Kontrola zdravlja vimena krava je neophodan element u procesu proizvodnje zdravstveno bezbednog mleka, te se na farmama visokomlečnih krava, kroz program kontrole mastitisa, redovno sprovode mere otkrivanja i prevencije bolesti vimena. Subklinički mastitis je važno oboljenje visokomlečnih krava koje izaziva ekonomske gubitke i promene u fizičkim i hemijskim osobinama mleka. Cilj ovog istraživanja je da se proceni mogućnost primene Kalifornia i draminski mastitis testa za otkrivanje subkliničkih mastitisa. Efikasnost ovih testova je potvrđivana poređenjem rezultata testa sa bakteriološkim nalazima. Eksperiment je sproveden na dve farme krava (farma A i farma B) Holštajn frizijske rase. Ukupno je pregledano 245 pojedinačnih uzoraka mleka, 95 primenom Kalifornia mastitis testa na farmi A i 150 primenom Draminski mastitis testa, na farmi B. Pojedinačni uzorci mleka za bakteriološku analizu su uzeti aseptično za vreme jutarnje muže u sterilne epruvete. Na farmi A rast bakterija nije dokazan u 46,32% (44/95) uzoraka, a na farmi B u 50,67% (76/150) uzoraka. U sprovedenom istraživanju, senzitivnost Kalifornija mastitis testa (78.57%) je veća nego senzitivnost Draminski mastitis testa (74.32%). Specifičnost Kalifornija mastitis testa i Draminski mastitis testa je 82.05% i 30.26%. Efikasnost Kalifornija mastitis testa u detekciji subkliničkih mastitisa kod visokomlečnih krava je veća nego Draminski mastitis testa, jer je validnost Kalifornija mastitis testa bila veća.

Ključne reči: Kalifornija mastitis test, Draminski mastitis test, subklinički mastitis, krava

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THE RAINFALL USE EFFICIENCY AND SOYBEAN GRAIN YIELD UNDER RAINFED CONDITIONS IN VOJVODINA

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Abstract: Rainfall is one of the most important environmental factors influencing crop production under dry land farming conditions. In the Republic of Serbia, the soybean is produced under rainfed conditions, and therefore online monitoring of the rainfall use efficiency (RUE) is essential for efficient management of production. The research aim was to estimate the effects of amount rainfall during the growing season (RGS) and average monthly rainfall on soybean grain yield (GY) in the Vojvodina during the sixteen year period (2000-2015). Distributions of RGS were not satisfactory and negatively influenced the expression genetic yield potential of cultivars. Rainfall deficits during the growing season limited the soybean plant reproductive growth stages leading to GY loss. The coefficient of variation indicated that RGS and monthly rainfall changed moderately from year to year. Regression equations showed that GY tended to increase with the amount of rainfall. GY had strong positive relationship with RGS and rainfall in May, July and August. Since the amount and distribution of rainfall during growing season are critical determinants of GY, soybean cultivars of shorter vegetation periods should be developed and cultivated so that maximum utilization of rainfall is ensured.

Key words: correlation, regression, grain yield, rainfall, soybean, Vojvodina

Introduction

Soybean is very important legume crop used for livestock and human nutrition and industrial processing. Soybean grain is source of edible oil (grain contains about 20% oil) and largest source of high quality protein for animal feed

(grain contains about 40% protein). Generally, the high production of soybean grain is important strategy for stable and profitable livestock production. It is estimated that 47% of soybean grain produced in the US is used for animal feed and that 98% soybean meal as a byproduct in processing is used to feed cows, pigs and chickens *(Soyatech, 2017)*. In Serbia, soybean is a source of protein for livestock, poultry and fish *(Popović et al., 2015)*, commonly contained in cereals mixtures for farm ruminant and non/ruminants nutrition *(Ranđelović, 2009).* It takes one kilogram of soybean flour to produce 2.3 kg of meat and 12 l of milk *(Todorović and Kondić, 1993)*. In Serbia, grain yield of soybean is dependent on RGS (amount and distribution of rainfall during the growing season) because production is exclusively organized under dry land farming conditions. Information on the amount and distribution of rainfall are very important strategy for soybean productivity, because it is estimated that extreme weather conditions (drought and heat wave) will become even more intensive in the future *(Lalić et al., 2011)*. Generally, in Serbia variation of rainfall regime is typical during summer seasons *(Mandić et al., 2015a, 2017)*. The critical stages for water requirement for soybean are from the beginning of flowering until the end of grain filling when the main yield components are formed. *Bošnjak (2004)* and *Mandić et al. (2015a)* concluded that in Serbia GY of soybeans depends on the amount of rainfall from June to September when soybeans plants are in flowering and pod-filling growth stages. *Randjelović et al. (2010)* and *Mandić et al. (2015b)* reported that GY especially depends on the amount of rainfall in August when soybean plants are in the grain filling stage. Water deficit in flowering stage may enhance the flower abscission *(Hoque et al., 2015)*, while drought during seed filling caused soybean plants to produce smaller seeds, influencing total yield *(Dornbos and Mullen, 1991)*. According to *Fageria et al. (2010)*, soybean can be cultivated between 0° to 55° latitude and to 2000 m altitude. Therefore, Vojvodina district is very suitable for soybean production because of its favorable geographic location, climate and natural characteristics. However, amount and distribution of rainfall are limiting factors for soybean development. *Nenadić et al. (1995)* state that soybean can achieve a satisfactory GY if the level of precipitation during June, July and August is about 300-350 mm.

The aim of this investigation was to estimate the effects of amount of rainfall during the growing season (RGS) and monthly rainfall (MR) on soybean GY in Vojvodina during sixteen years (2000-2015) and to evaluate the degree of association between monthly rainfall variation and grain yield.

Materials and Methods

Vojvodina is located in the north of Serbia (Latitude 45° 0' 0 N; Longitude 20° 0' 0 E). Rainfall data (2000-2015) were retrieved from Meteorological

yearbooks *(Republic Hydrometeorological Service of Serbia)* including seven meteorological stations which have data of monthly rainfall (Table 1). Selected rainfall stations are equally distributed on the region Vojvodina (one station per 3000 km^2). The elevations of the meteorological stations range from 80 m a.s.l. to 102 m a.s.l.

Table 1. List of meteorological stations included in the study

Station	Latitude	Longitude	Altitude (m)
Vršac	45°08'N	21°18'E	84
Zrenjanin	45°22'N	20°25'E	80
Kikinda	45°51'N	20°28'E	81
Palić	46°06'N	19°46'E	102
Rimski Šančevi – Novi Sad	45°20'N	19°51'E	84
Sombor	45°46'N	19°09'E	88
Sremska Mitrovica	45°06'N	19°33'E	82

Data for soybean GY for Vojvodina were extracted from the Statistical Yearbook of the Republic of Serbia from 2000 to 2015.

Descriptive statistics (mean (M), coefficients of variation (CV), maximum and minimum values) were used to summarize data. The formula according to *Oweis (1997)* was used for estimate rainfall use efficiency (RUE): RUE (kg ha^{-1} mm^{-1}) = grain yield / rainfall received during the growing season. The programs 'Excel' and STATISTICA (version 10; StatSoft, Tulsa, Oklahoma, USA) were used in the analysis of data, while the Shapiro-Wilk test was used to assess data normality. The linear regression method and correlation analysis were used for analysis of data at the level of significance $P \leq 0.05$ and $P \leq 0.01$. The Pearson's correlation coefficient was used for determining the strength of the linear relationship.

Results and Discussion

Results showed that RGS, in average for all meteorological stations and years, was 364.5 mm and ranged from 117.4 mm (Kikinda) to 757.0 (Vršac), Table 2. In general, the rainfall data for Vojvodina showed irregular temporal and spatial distribution of rainfall. Earlier studies showed that Vojvodina district received 305 mm of rainfall from April to August during sixteen years *(Mandić et al. 2017)*, and 303.5 mm during sixty five years *(Milošević et al. 2015)*. RGS varied from 38.7% in Sremska Mitrovica to 43.8% in Rimski Šančevi, with an average value of 41.7%. It means that the RGS is moderately variable. Monthly rainfall during June was the highest (76.6 mm) and contributed 21.02% of RGS (364.5 mm), followed by May (19.29%), July (16.38%), September (16.32%), August (14.73%) and April (12.26%). The CV was the highest in August (82.0%), followed by April (78.3%), June (71.4%), September (68.2%), July (67.9%) and May (59.5%). In Vojvodina

rainfall variability is important for explaining soybean yield variability, because soybean is not irrigated. Also, *Ray et al. (2015)* point out the importance of rainfall variability for soybean grain yield in northeastern China. These authors concluded that 36% of the GY variability was explained by rainfall variability. Vojvodina district received the highest amount of rainfall during growing season in June when soybean plants were at the stage of flowering and the formation of the first pods started. Vojvodina region received the lowest amount of rainfall in April, which is the optimal time for sowing of soybean. The high CV recorded in August and April were an indication of lowly dependable rainfall. Also, the CV in other months confirmed the moderate variability of the average monthly rainfall, and generally monthly rainfall are lowly dependable.

Table 2. Descriptive statistics of the rainfall growing seasons (RGS) and monthly rainfall for seven meteorological stations in Vojvodina (mm)

Station	Item	IV	V	VI	VII	VIII	IX	RGS
Vršac	M	52.7	62.2	76.5	76.1	63.7	58.7	389.9
	CV, %	68.4	43.5	66.4	98.0	88.0	79.8	43.0
	Maximum	130.4	120.1	202.9	275.0	184.6	193.4	757.0
	Minimum	1.0	15.2	14.2	0.6	0.4	2.2	193.6
Zrenjanin	M	39.2	60.2	74.0	52.7	48.4	63.4	337.9
	CV, %	71.7	70.5	47.5	72.4	101.8	72.6	41.4
	Maximum	97	162.1	139.6	153.5	155.1	185.3	615.6
	Minimum	2.5	19	32.3	12.5	0.6	10.2	144.4
Kikinda	M	43.6	62.0	68.1	58.7	45.4	55.6	333.4
	CV, %	88.5	70.9	79.8	54.0	71.9	57.9	43.0
	Maximum	121.2	184.2	202.6	115.3	129.8	121.8	683.7
	Minimum	1.3	14	9.4	13.5	3.9	4.6	117.4
Palić	M	42.5	72.3	77.8	57.7	52.2	56.8	359.3
	CV, %	81.3	51.5	80.5	58.1	68.1	63.5	39.2
	Maximum	132.3	158.9	243.3	115.1	135.1	137.4	584.2
	Minimum	2.7	14.1	8	3.7	2.6	7.6	128.1
Rimski Šančevi	M	46.0	84.9	87.9	59.4	56.8	61.5	396.4
	CV, %	93.5	60.8	71.2	61.1	91.1	65.3	43.8
	Maximum	156.0	202.1	237.4	141.1	168.5	160.1	742.0
	Minimum	0.0	21.9	26.7	2.6	1.5	13.1	148.1
Sombor	M	42.0	75.4	78.6	62.5	55.7	61.5	375.7
	CV, %	75.7	66.8	86.1	70.8	70.1	65.3	42.5
	Maximum	109.0	195.4	240.0	195.5	154.7	138.7	694.0
	Minimum	0.5	13.0	9.8	18.3	5.5	15.0	138.3
Sremska Mitrovica	M	46.7	75.3	73.6	50.8	53.8	59.0	359.2
	CV, %	70.1	53.5	67.9	49.4	81.6	72.1	38.7
	Maximum	109.8	187.0	220.4	93.5	156.2	154.9	632.2
	Minimum	0.0	28.3	20.8	10.4	0.1	5.8	135.2
M	M	44.7	70.3	76.6	59.7	53.7	59.5	364.5
	CV, %	78.3	59.5	71.4	67.9	82.0	68.2	41.7
	Maximum	156	202.1	243.3	275	184.6	193.4	757
	Minimum	0	13	8	0.6	0.1	2.2	117.4
% Contribution to RGS		12.26	19.29	21.02	16.38	14.73	16.32	100

In Serbia, soybean needs 450-480 mm of RGS for grain production, by months - in April 10 to 40 mm, May 30 to 60 mm, June 90-110 mm, July 100 to 125 mm, August 100 to 120 mm and September 50 to 80 mm *(Glamočlija, 2004)*. The most water is needed from the beginning of flowering until the end of grain filling, which is time from late June to early September *(Srebrić and Perić, 2014)*, depending on the group of maturity. *Glamočlija (2004)* concludes that in order to achieve high and stable grain yield and above-ground biomass, there should be 250-300 mm of rainfalls during summer months (June-August). In the 3-month period June-August for the period of observed sixteen years, Vojvodina received an average rainfall of 190mm (76.6 mm in June, 59.7 mm in July and 53.7 mm in August) which is lower than maximum water consumption for this period.

During the 16-year period, enough rainfalls for successful soybean production were registered in 2001, 2005, 2010 and 2014 (Table 3). Generally, RGS values in investigated period were lower than optimal amount of rainfall for soybean production. CV of monthly rainfall for all years of research ranged from 56.5% (July) to 72.5% (August), while for RGS it was (39.1%). Increased rainfall variability indicates greater seasonal fluctuations of rainfall in Vojvodina district. Likewise, increased CV indicates that the rainfall is highly variable and less predictable. This amount seems to be sufficient to cultivate of soybean. Given that the established RGS have wide variations in Vojvodina, and that production of soybeans and all crops on arable land depends on rainfall, and minor adaptation strategies, it is realistic to expect a large loss of yield in future. Therefore, the breeders must create drought and heat tolerant genotypes, and farmers should cultivate these cultivars.

Difference between maximum and minimum of rainfall within a month represents monthly range of rainfall. The increase in the monthly range of rainfall is associated with larger maximum of rainfall and smaller minimum of rainfall. Monthly range of rainfall ranged from 100.6 mm (April) to 178 mm (June). Generally, the long-term average monthly rainfall in Vojvodina district shows that most of the months were below average rainfall for soybean growth, with the exception of 2001, 2005, 2010 and 2014.

Table 3. Descriptive statistics for monthly rainfall from 2000 to 2016 (mm), grain yield – GY (t ha^{-1}) and rainfall use efficiency – RUE (kg ha^{-1} mm^{-1})

Year	IV	V	VI	VII	VIII	IX	RGS	GY	RUE
2000	33.0	30.8	22.3	25.7	4.7	27.2	143.6	1.2	8.4
2001	102.8	52.8	200.5	43.5	35.7	154.5	589.8	2.4	4.1
2002	26.5	63.4	46.3	48.2	51.3	48.7	284.4	2.5	8.8
2003	16.0	27.4	30.5	68.7	24.0	68.1	234.6	1.7	7.2
2004	93.5	72.6	73.9	80.8	57.4	45.5	423.6	2.7	6.4
2005	68.2	49.4	91.1	118.4	140.7	62.5	530.3	2.8	5.3
2006	86.3	51.7	91.7	44.7	107.6	17.4	399.5	2.8	7
2007	2.2	94.9	77.0	30.6	57.3	71.2	333.3	2.1	6.3
2008	37.2	33.8	95.1	50.5	27.7	71.7	315.9	2.5	7.9
2009	7.4	49.8	114.5	46.0	44.2	15.0	276.9	2.4	8.7
2010	50.5	142.9	162.2	67.6	104.4	77.7	605.2	3.2	5.3
2011	15.8	54.3	49.7	90.6	10.1	26.9	247.5	2.7	10.9
2012	62.2	59.9	30.7	61.4	3.3	23.5	241.1	1.7	7.1
2013	35.3	103.2	65.9	27.8	44.2	69.2	345.7	2.4	6.9
2014	57.9	149.5	46.3	137.1	71.1	114.1	576.0	3.6	6.3
2015	20.0	88.9	28.6	13.4	75.6	58.7	285.1	2.5	8.8
M	44.7	70.3	76.6	59.7	53.7	59.5	364.5	2.5	7.2
CV	69.9	52.2	64.9	56.5	72.5	61.8	39.1	23.8	23.4
Maximum	102.8	149.5	200.5	137.1	140.7	154.5	605.2	3.6	10.9
Minimum	2.2	27.4	22.3	13.4	3.3	15.0	143.6	1.2	4.1
Range	100.6	122.1	178.2	123.7	137.4	139.5	461.6	2.4	6.8

Legend: RGS - amount of rainfall during the growing season (mm); CV - coefficient of variation (%)

Average GY of soybean over longer period was 2.5 t ha^{-1} and ranged from 1.2 t ha^{-1} (2000) to 3.6 t ha^{-1} (2014). The year 2000 had the lowest RGS and GY. It was a year when the rainfall in the summer months was the lowest (22.3, 25.7 and 4.7 mm). The genetic potential of soybean genotypes grown in Serbia is up to 6 t ha^{-1}, but rarely 50% is achieved. Unstable soybean yields in Serbia are the result of insufficient amount and irregular distribution of rainfall during the growing season. Introduction of irrigation would enable high and stable yields of soybean in variable climate conditions. However, in Serbia only 1% of arable land is irrigated *(World Bank, 2014)*.

The CV of the GY in Vojvodina was 23.8%. This shows that there is no continuous high and stable production of soybean grain per unit area. That may be important as the limiting factor for production of milk, meat and eggs on farms. It should be noted that the CV of GY could be result of the joint effect of the rainfall variability, other climatic factors and non-climatic factors such as soybean cultivars and cropping technologies. It should be noted that the CV of GY could be result of the joint effect of the rainfall variability, other climatic factors and non-climatic factors such as cultivars and cropping technologies. The higher annual variability of soybean GY (39%) is reported by *Milošević et al. (2015)* during the period from 1949 to 2013.

In general, RUE tends to increase when aridity decreases indicating better rainfall utilization. Thus, higher RUE (8.4 kg ha^{-1} mm^{-1}) was found when rainfall was lowest (143.6 mm) compared to RUE (5.3 kg ha^{-1} mm^{-1}) when rainfall was highest (605.2 mm). Here it is assumed that the other conditions are equal.

Regression equations indicate that GY increased with increasing amounts of rainfall (Figure 1). Regression equations shows that for an increase of 1 mm of rainfall in April, May, June, July, August, September and the growing seasons the expected increase grain yield is 5.6, 10.3, 4.3, 9.8, 9.6, 5.2 and 3,1 kg ha^{-1}, respectively. Therefore, the highest increase of GY was could be attributed to the amount of rainfalls in May, July and August. *Dolijanović et al. (2013)* have found that 1 mm of annual rainfall increases the soybean GY from 2.1 to 2.9 kg ha^{-1}. The regression coefficient of determination for April was 9.1%, May 42.1%, June 13.3%, July 32.5%, August 41.5%, September 10.7% and RGS 57.2%, respectively. These percentages explained the variation in GY by rainfall variability. On the other hand, 42.8%, 91.9%, 57.9%, 86.7%, 67.5%, 58.5% and 89.3%, respectively, explained the variation in GY by other genetic and non-genetic factors (technical, other climatic, edaphic and biotic). As said, the highest rainfall in Vojvodina was in June (76.6 mm), but 1 mm of rainfall in June increased GY by only 4.3 kg ha^{-1}. Contrary, 1 mm of rainfall in May increased GY by 10.3 kg ha^{-1}. In May in Vojvodina, soybean plants form new leaves, expand more leaf area and create the optimal sizes of assimilation surfaces. Generally, the water stress reduced stem and leaf cell expansion, why the plants have short stems with less leaf area. Water stress in this period decreases leaf area even for 40% *(Catuchi et al., 2011)* and reduces photosynthetic rates and grain yield *(Neumaier et al., 2000)*. Therefore, a greater development of leaf surface can increase the soybean yield. Rainfalls in July and August contributed to a higher yield increase. In Vojvodina (Serbia), in July and August soybean plants are at the stage of flowering and pods formation. Insufficient rainfall decreases the plant height. Also the plants are with a smaller number of pods, grains per pod, and the grain is small. Rainfall deficit and high temperatures influence the shortening of the vegetation period, so the grain filling stage is shortened. Thus, *Manavalan et al. (2009)* reported that water stress shortened the beginning maturity stage (R7) for 7 days, reducing yields for 44%.

A statistically significant positive correlation was found between GY and total rainfall in May ($r = 0.65$, $p \leq 0.01$), GY and total rainfall in July ($r = 0.57$, $p \leq 0.05$), GY and total rainfall in August ($r = 0.64$, $p \leq 0.01$) and GY and RGS ($r = 0.76$, $p \leq 0.01$). Correlation coefficients between other monthly rainfall amounts and GY were positive, but not significant. Essentially, the amount of rainfall influenced the soybean GY, especially distribution of rainfall within growing season. *Bošnjak (2004)* has found highly significant correlation between GY of soybean and RGS, as well as between GY and total rainfall in summer moths (June, July and August). *Vidić et al. (2009)* have found highly significant

correlation between GY of soybean and amount of rainfall in July and early August. Contrary, *Milošević et al. (2015)* have found that GY did not show significant correlation with rainfall characteristics during long period in Vojvodina (1949-2013). *Teasdale and Cavigelli* (2017) stated that RGS in Beltsville (Maryland, U.S.A.) had the highest correlation with soybean GY.

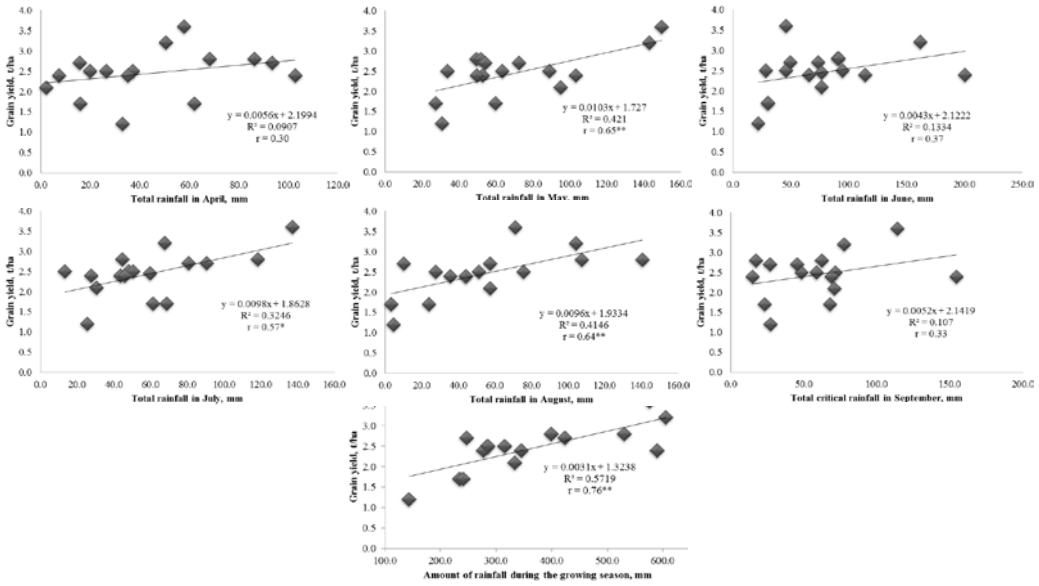

Figure 1. Regression result on the effect of rainfall characteristics on soybean grain yield

Generally, the soybean GY was unstable over the period of 16 years. Rainfall in May, July and August appeared to be the most dominant factor affecting the soybean GY in Vojvodina region. Thus, the higher amount of rainfall during these months would be expected to increase yields. It would be best to irrigate soybean crops from early-July to mid-August, when the most critical stages of grain development occur. Under these conditions soybean could form a large number of pods per plant and large seeds.

Conclusions

In order to achieve a more stable yield, it is necessary to develop cultivars that tolerate water stress. Our study highlights that the variability in soybean production is strongly associated with rainfall during growing season, particularly

in May, July and August. For this reason, farmers should take advantage of all water resources using proper land management, genotypes suitable for this area and proper cropping measures, especially irrigation. Furthermore, as the Vojvodina is major contributor to soybean production in Serbia, our results have significant implications for animal productivity and animal feed security. Our study is important in explaining GY variability. It is necessary overcome the unstable soybean production, ensure stable yield in future, and develop strategies to stabilize food/feed supply and security.

Efikasnost korišćenja padavina i prinos zrna soje u uslovima prirodnog vodnog režima u Vojvodini

Violeta Mandić, Zorica Bijelić, Vesna Krnjaja, Aleksandar Simić, Dragana Ružić Muslić, Vesna Dragičević, Veselin Petričević

Rezime

Padavine su jedan od najvažnijih faktora spoljašnje sredine koji utiče na produkciju useva u uslovima suvog ratarenja. U Republici Srbiji, soja se proizvodi u uslovima prirodnog vodnog režima, pa je praćenje efikasnosti korišćenja padavina (RUE) neophodno za efikasno upravljanje proizvodnjom. Cilj istraživanja bio je da se proceni efekat količine padavina tokom vegetacionog perioda i prosečnih mesečnih padavina na prinos zrna soje u Vojvodini tokom šestnaestogodišnjeg perioda (2000-2015). Distribucija padavina tokom vegetacionog peroda nije bila zadovoljavajuća i nepovoljno je uticala na ekspresiju genetičkog potencijala rodnosti sorti. Deficit padavina tokom vegetacionog perioda soje ograničile su faze reproduktivnog razvoja i dovele do redukcije prinosa. Koeficijenti varijacije pokazuju da se količina padavina tokom vegetacionog perioda i srednja mesečna količina padavina umereno menjaju iz godine u godinu. Regresijske jednačine su pokazale da se prinos zrna povećavao sa količinom padavina. Prinos zrna je u jakoj pozitivnoj korelaciji sa količinom padavina tokom vegetacionog perioda, u maju, julu i avgustu. S obzirom da su količina i raspored padavina u toku vegetacionog perioda kritična determinanta za prinos zrna, treba razvijati i gajiti sorte soje sa kraćim vegetacionim periodom da bi se obezbedila maksimalna iskorišćenost padavina.

Ključne reči: korelacija, regresija, prinos zrna, padavine, soja, Vojvodina

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KEEL BONE DAMAGE IN LAYING HENS REARED IN DIFFERENT PRODUCTION SYSTEMS IN SERBIA

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Communication

Abstract: The European Union in 2012 banned conventional battery cages for the welfare reasons. However, transition to new housing systems uncovered some new problems, such as keel bone damage (KBD), which also could endanger welfare of laying hens. Although KBD is a research topic which attracts a growing attention in the EU, in Serbia it is still rather unknown phenomenon, even among the scientific and professional community. This research is the first attempt to determine the prevalence of KBD in laying hens in housing systems currently existing in Serbia. The results of conducted monitoring show presence of KBD on all observed farms, except the organic one. The occurrence of KBD was at an acceptable level (from the standpoint of hen welfare) in the free-range system, enriched cages without equipment and conventional battery cages (4%, 3% and 1%, respectively), while in the fully equipped enriched cages it was high (39%). One could assume that this high prevalence of KBD in this system is a consequence of a long roosting on a metal perches.

Key words: keel bone damage, laying hens, housing systems, perches

Introduction

New modified housing systems for laying hens have been introduced in the EU countries since 2012, when Directive 1999/74/EC came to force. However, transition to the new systems uncovered some new problems, such as keel bone damage (KBD) in laying hens. The term 'keel bone damage' includes the deviations and fractures of the keel bone which could be painful for the hen and thus could endanger welfare and reduce productivity (*Harlander et al., 2015*). High frequency of KBD in the commercial systems represents one of the greatest challenges which the modern poultry industry faces (*FAWC, 2010, 2013*).

During the last decade, numerous studies, done mostly in the EU countries, have documented fractures and deformation of the keel bone in laying hens, which range between 5% and 97%, depending on the housing system and hen age (*Rodenburg et al., 2008*; W*ilkins et al., 2011; Petrik et al., 2015; Riber and Hinrichsen, 2016; Regmi et al., 2016*). So far, no similar research was conducted in our country. Moreover, this phenomenon is still rather unknown, even among scientific and professional community and there is no sufficient information about KBD not only in Serbia but in all countries from the region, where the Directive 1999/74/EC is still not effective.

The aim of this research was to determine, for the first time, the prevalence of the KBD in laying hens in different housing systems in Serbia and to announce the findings to the scientific and professional community.

Materials and methods

All types of the housing systems currently existing in Serbia were included into this research: fully equipped enriched cages, enriched cages without the equipment, conventional battery cages, organic production and backyard (free range) production. Although many authors *Rodenburg et al. (2008), Sandilands et al. (2009), Kappeli et al. (2011)*, and *Wilkins et al. (2011)* reported the highest prevalence of KBD (more than 80%) in systems equipped with multilevel perches (which is the feature of aviary systems), there is not a single farm with aviary system in Serbia, and therefore these systems could not be included.

Since the other authors reported that the prevalence of keel-bone damage increases with age of hens (*Richards et al. 2012; Petrik et al. 2015*) the examination were done on the flocks which were in the second half of the production cycle (older than 45 weeks of age).

There were 21 farms in total participating in this research. Namely, 3 farms with fully furnished enriched cages, 2 farms with enriched cages without the equipment, 5 farms with conventional cages, 1 organic farm, and 10 small farms, with a free range system.

The most used hybrids on big farms are Hyline brown, Lohmann brown, Tetra SL and on small farms domestic chicken, Partridge colored Italian and autochthonous breeds such as Sombor Crested chicken.

On the large-scale farms (with over 10,000 laying hens), sample of 100 laying hens were randomly selected for palpation assessment, while on the small-scale farms (50 – 300 laying hens) the sample size was 50 laying hens. Within the floor system, laying hens were fenced, while within the cage systems they were taken from the different cages and levels, again based on the system of a random sample.

The prevalence of KBD was assessed by using the technique of palpation according to *Wilkins et al. (2004).* Palpation was performed by running fingers alongside and over the keel bone. It was only determined whether KBD was present (fracture, deformation, deviation – picture 1) or not (completely straight and flat keel bone – picture 2).

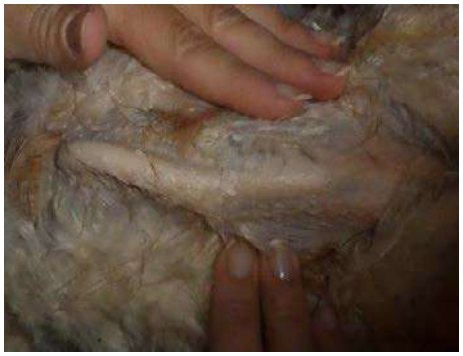


Photo: Vida Rezar
Picture 1. Keel bone damage

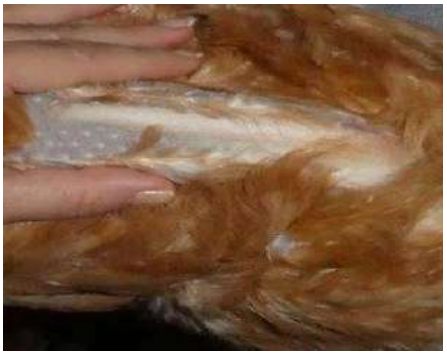
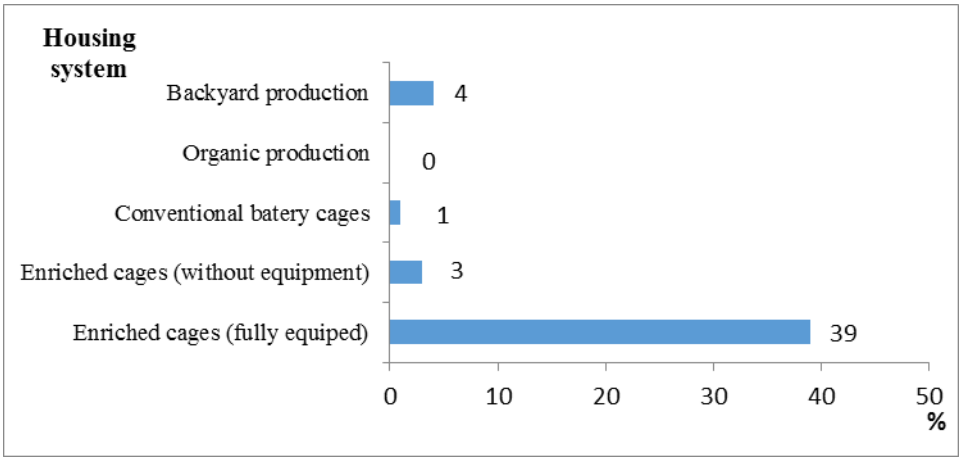


Photo: Mirjana Đukić Stojčić
Picture 2. Keel bone without damage

Results and discussion

The results of the prevalence of KBD are presented in this short communication only in a descriptive way and they are a part of a larger research project which is aimed at enhancing the poultry production in Serbia.

The results of conducted palpation assessment showed that KBD was detected in all production systems, except in the organic one (Graph. 1). The overall range of KBD observed in conventional cages was only 1%, in enriched cages without equipment it was 3% and in the free range system 4%. All these levels are acceptable from the standpoint of animal welfare.



Graph. 1. The prevalence of the KBD in laying hens in different housing systems in Serbia

The highest prevalence of KBD was detected in fully equipped enriched cages. The basic difference between the fully equipped and not-equipped enriched cages is the lack of the perches. The perches in the fully equipped cages were round and made of steel and the hens spend a great deal of time sitting on them. One could assume that this might be the reason of the increased prevalence of KBD in enriched cages compared to the conventional ones.

The assumption that the perches have a key role in the development of KBD in enriched cages was confirmed by other authors too (*Rodenburg et al., 2008; Wilkins et al., 2011*). *Hester et al. (2013)* reported that at the end of the production cycle prevalence of KBD was 9% higher for hens kept in conventional cages with perches compared to the hens kept in cages without metal perches. *Wilkins et al. (2011)* reported a significant increase (10-34%) in KBD when perches were added in the organic mobile houses.

Conclusion

The first monitoring of the prevalence of KBD in laying hens in Serbia was done on the sample which represents all housing system currently existing in poultry production in Serbia. The highest occurrence of KBD was noticed in fully equipped enriched cages. The future research should be focused on the development of effective strategies for reducing occurrence and severity of KBD. Further research on this topic is necessary in our country in order to determine specific risk factors for occurring and strategies for overcoming this problem in enriched cages, especially once the Directive 1999/74/EC is made effective.

Oštećenje grudne kosti kod kokoši nosilja gajenih u različitim sistemima držanja u Srbiji

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Rezime

Evropska unija je, zbog obezbeđenja dobrobiti živine, 2012. godine zabranila držanje nosilja u baterijskim kavezima. Međutim, prelazak na nove sisteme držanja doveo je i do nekih novih problema, kakav je oštećenje grudne kosti (OGK), koje takođe može da ugrozi dobrobit živine. Iako je oštećenje grudne kosti istraživačka tema koja u EU privlači sve veću pažnju, u Srbiji je ovo još uvek relativno nepoznat pojam, čak i u naučnim i stručnim krugovima. Ovo istraživanje predstavlja prvi pokušaj da se u Srbiji utvrdi prisustvo oštećenje grudne kosti kod kokošaka nosilja gajenih u različitim sistemima. Dobijeni rezultati pokazuju da je oštećenje grudne kosti prisutno u svim ispitivanim sistemima držanja, osim u organskom. Relativno nizak procenat oštećenja grudne kosti detektovan je kod kokošaka na ispustu, kokošaka u obogaćenim kavezima bez opreme i u konvencionalnim kavezima (4,3 i 1% respektivno). Najveći procenat oštećenja grudne kosti detektovan je kod kokošaka u obogaćenim kavezima sa kompletnom opremom (39%). Može se pretpostaviti da je ovako visok procenat oštećenja grudne kosti u ovom sistemu držanja posledica dugog sedenja kokošaka na metalnim sedalima.

Ključne reči: oštećenje grudne kosti, kokoške nosilje, sistem držanja, sedala

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Editorial

Link to the retracted article: https://doi.org/10.2298/BAH1603297S

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Milan M. Petrović[1], Stevica Aleksić[1], Milan P. Petrović[1], Milica Petrović[2], Vlada Pantelić[1], Željko Novaković[1], Dragana Ružić-Muslić[1]

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Corresponding author: Milan M.Petrović, **e-mail address**
Review paper

Example 2

EFFECTS OF REARING SYSTEM AND BODY WEIGHT OF REDBRO BROILERS ON THE FREQUENCY AND SEVERITY OF FOOTPAD DERMATITIS

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Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia
Corresponding author: Zdenka Škrbić, **e-mail address**
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