

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

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BOVINE RESPIRATORY DISEASE COMPLEX (BRDC): A REVIEW OF LUNG LESIONS AND REDUCING OF QUALITY OF CARCASSES

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Review paper

Abstract: Bovine respiratory disease complex (BRDC) is the biggest health problem of the cattle industry globally due to the high prevalence and economic consequences which arise due to numerous reasons. Huge economic losses are most often attributed to high morbidity and mortality, reduction of Average Daily Gain (ADG) and food utilization, weight loss, lower quality of carcasses and comprehensive measures of prophylaxis and therapy. BRDC commonly observed throughout the feedlot phase due to the stress factors. Predisposing factors divided didactic on environmental (inclement weather conditions, inadequate humidity and dust), host factors (age, sex, race, genetics, immune status) and stressful management practices (transportation, nutritional stress, metabolic disease, high density of animals, handling, castration, dehorning). In complex etiopathogenesis, in addition to the aforementioned predisposing factors, numerous viral and bacterial agents are involved. Gross lung lesions are most commonly observed in cattle slaughter or autopsies (visible to the naked eye) occur as a result of pneumonia. It is the result of an infection by the bovine respiratory syncytial virus (BRSV), parainfluenza virus type 3 (PI3V), bovine herpes virus type 1 (BoHV1) and bovine viral diarrhea virus (BVDV) alone or in combination with one another, as well the common bacterial pathogens *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Micrococcus spp.* Numerous studies have pointed to the detrimental effects on performance and carcass characteristics.

Key words: Bovine Respiratory Disease Complex (BRDC), cattle, economic loss, lung lesions, carcass quality

Introduction

Bovine respiratory disease complex (BRDC) is among the most prevalent and damaging diseases which adversely affects cattle production globally (Griffin, 1997; Prado et al., 2006; Kurćubić et al., 2018). Total annual costs to beef industry in the U.S. have been calculated to be approximately \$4 billion, including animal and production losses, prevention and treatment costs (Griffin, 1997). The annual loss of the US cattle breeding industry is estimated to be \$ 1 billion. Cost of prevention and treatment is \$ 3 billion a year (Griffin, 2006, Snowden et al., 2007). The average cost of the individual treatment is estimated at \$ 15.60. This cost increases to \$ 92.30 when account is taken of indirect costs, such as reducing ADG and reduced carcasses value (Schneider et al., 2009). BRDC covers 70 to 80% of total morbidity and 40 to 50% of total mortality in North American feedlots (Edwards, 1996; USDA, 1999). In the European Union, production losses (excluding livestock deaths) are about 576 million euros on an annual basis (Barrett, 2000). An estimated 1.9 million animals (Nicholas, 2011) are affected by BRDC each year in the UK cattle industry with costs calculated at around £60 million annually (NADIS, 2007). On slaughter of feedlot cattle is a very common finding of lesions on the lungs, with the prevalence between 29.7 and 77% (Wittum et al., 1996; Bryant et al., 1999; Thompson et al., 2006).

Pathogenesis and etiology of BRDC

The emergence of BRDC is most commonly caused by a primary viral infection. Infection of the respiratory tract of cattle causes pathological changes, which vary depending on the stage of infection. Bovine respiratory syncytial virus (BRSV) gives a major contribution to the emergence of BRDC (Brodersen, 2010; Raaperi et al., 2012). Kurćubić et al. (2013a) confirmed above mentioned findings and determined the genome of the BRSV by Real-Time RT-PCR in all 20 examined samples of discharge from the nasal mucosa of the beef cattle diseased of BRDC. Damage to the upper parts of the respiratory tract and the mucociliary cleansing function make it possible that adhesion of bacteria to virus-infected cells is increased, growth and the formation of colonies. Damages extend to the epithelium of the tracheal mucosa, and then the bacteria open the passage to the deeper parts of the respiratory tract. Viruses damage the function of macrophages and neutrophil leukocytes, which depend on the immune functions of the host and the possibility of phagocytosis. In the final stage, viral causative agents also lead to impaired and depressed humoral immune responses with B cell effector and cellular immune response mediated by T cells (Anderson and Rings, 2008; Rivera-Rivas et al., 2009).

Bacterial pneumonia often occurs after a viral infection or along with it. It often has a similar pathogenesis, which involves the formation of colonies in nasopharynx, the inhalation of aerosolized droplets containing pathogenic bacteria, bronchoalveolar colonization, the immune response of the host, and the elimination

of pathogens and impairment of the immune response of the host (Frank *et al.*, 1996). From the majority of the strong lesions observed on lungs when slaughtering cattle, which are characterized as acute fibrinous pleuropneumonia, *M. haemolytica* type 1 are the most commonly isolated agents. The primary isolate in many cases of bronchopneumonia is *P. multocida* (Welsh *et al.*, 2006). This has been confirmed in five-year study of Kurćubić *et al.* (2000), who examined a total of 1,435 nasal swabs taken from beef cattle with the respiratory symptoms characteristic for BRDC. The most common bacterial isolates were *Pasteurella multocida* and *Pasteurella haemolytica* (62.78 and 40.20%), *Corynebacterium pyogenes* (53.44%), *Staphylococcus albus* (24.87%) and *Streptococcus viridans* (14.07%). Kurćubić *et al.* (2013a, 2013b) have found that the most commonly isolated bacterial pathogens in fattening cattle suffering from BRDC were *P. multocida*, *Aeromonas viridans* and *Corynebacterium bovis*, unlike most of the data from the literature on the highest prevalence of *M. haemolytica*. *M. haemolytica* is associated with numerous virulence factors which inevitably lead to structural changes in the lungs of cattle: leukotoxin, lipopolysaccharide (LPS), capsular components, outer membrane proteins, neuraminidases, and proteases (Ackermann and Brogden, 2000; Jeyaseelan *et al.*, 2002). Lung lesions caused by *M. haemolytica* is cascading, in 4 stages that can not be clearly separated: pre-pneumonia, pulmonary consolidation, localized inflammation and spread, and expansion of pneumonia (Mosier, 2006). In the pre-pneumonic stage, *M. haemolytica* proliferates in the upper part of respiratory tract (initial step of pneumonia), and it appears the colonization of the bronchoalveolar junction. The bacteria begin to produce the virulence factors in sufficient quantities to induce a localized inflammatory response which causes lobular bronchopneumonia, with firm and dark red lesion („liver-like“). As a result of the extensive damage, the entire affected area of pulmonary parenchyma turns from the normal healthy pink appearance to a dark red with a large amount of atelectasis. Subsequent abscessation may occur. Pleural adhesions may develop due to a formation of large amounts of fibrin and fibrinous fluid (Mosier, 2006). The interlobular septa is inflated by yellow, gelatinous edema and fibrin (Kurćubić *et al.*, 2014). Gross findings include consolidated and/or collapsed lung parenchyma, focal or diffuse pleuritis, fibrinous adhesions, thoracic adhesions, abscesses, fibrosis, emphysema, and hemorrhage and are most frequently observed in the (right) cranial ventral lung lobes (Bryant *et al.*, 1999).



Figure 1. Suppurative bronchopneumonia, right lung, calf. Cranioventral parts are consolidated; bronchi are filled with purulent exudate.



Figure 2. Fibrinous pleuropneumonia, right lung, heifer. The pleura is covered with a thick layer of fibrin.

In confirmation of the above results, Figures 1 and 2 show gross lesions in lungs in calves and heifers died with symptoms of BRDC (*Vasković et al., unpublished data*). From the lung tissue shown in Figure 1, *Pasteurella multocida* was isolated, and the presence of the BRSV and BVDV genome was detected.

Mannheimia haemolytica was isolated from the lung tissue shown in Figure 2, and the presence of the BVDV genome was demonstrated. Examination was done in laboratories of the Veterinary Specialist Institute „Kraljevo”, Serbia (*Vasković et al., unpublished data*).

The presence of lung lesions did not greatly influence any of the traits considered within the research of *Schneider et al. (2009)*. They found that the greatest loss of production was in cattle that had active bronchial lymph nodes at slaughter. Such a result was somewhat different than other studies on the effects of lung lesions on performance and carcass traits. Utilizing lung lesions at harvest for BRDC diagnosis improves sensitivity and specificity to 77.4 and 89.7%, respectively (*White and Renter, 2009*). Observation of lungs in cull cows for signs of pulmonary lesions at slaughter may provide useful information for veterinarians and other personnel who control management and health intervention strategies. Pulmonary lesions associated with BRDC were observed in 33.8% of all cattle ($n=1461$). Mild lesions ($\leq 50\%$ consolidation of any lung lobe) were the most common and were found in 23.5% of examined cattle. The high prevalence of lesions characteristic for BRDC and ruminal acidosis points to their significant levels which exist within the dairy cattle population (*Rezác et al., 2014*).

Findings of the lesions on the lungs on the line of slaughtering of cattle

Numerous studies have shown the occurrence of lung lesions at slaughter in cattle. Generally, lung lesion prevalence of 72% with a total respiratory morbidity of 35% in a population of 469 steers was reported by *Wittum et al. (1996)*. Furthermore, 68% of the cattle not treated for BRDC during their lifetime displayed lung lesions at slaughter and 72% of those cattle treated for BRDC

during their lifetime displayed pulmonary lesions at slaughter, significantly associated with a 0.076 kg reduction in ADG.

Gardner et al. (1999) performed monitoring at slaughter of genetically similar Charolais steers (n=204) and concluded that 37% of steers never treated for BRDC during the finishing phase had lung lesions. Only 48% of those cattle treated for BRDC during the finishing phase had pulmonary lesions at slaughter. Cattle without pulmonary lesions at slaughter were observed to have an 11% greater ADG than cattle with lung lesions revealed at slaughter. Different influences of pulmonary lesions and BRD treatment were observed on other performance and carcass traits.

Bryant et al. (1999) observed the prevalence of all types of lung lesions from 33 to 77% in three populations (n=599) of commercially fed cattle.

Thompson et al. (2006) estimated the impact of respiratory disease on growth in 2,036 head of South African feedlot cattle from 2 different feedlots, during early and late finishing periods. Pulmonary lesions are noticeable in 38.5% of cattle never treated against BRDC, 55.4% of cattle treated once against BRDC, and 66.7% of cattle treated against BRDC twice or more.

Schneider et al. (2009) processed the data from 5,976 steers and heifers from Southwest Iowa registered in the Tri County Steer Carcass Futurity database to evaluate the associative effects of BRDC on performance and carcass traits. Observation of pulmonary lesions at slaughter was only collected on 1,665 head, of which, 61.9% had lesions present at slaughter. Among the cattle that have never been treated for BRDC, at 60.6% were observed lung lesions at slaughter with the most common score (26.9%) being mild in nature.

Other authors came in their researches to similar results and observations about the association of pulmonary lesions with treatments against BRDC (*Gardner et al., 1999; Thompson et al., 2006*). A significant decrease in ADG (0.07 ± 0.01 kg), carcass weight (8.16 ± 1.38 kg), and marbling score (0.13 ± 0.04) indicated that it was caused by BRDC, whether cattle were treated against BRDC during a fattening period, or pulmonary lesions were observed in slaughtering cattle. The specific influence of pulmonary lesions at slaughter on carcass quality were not ascertained.

White and Renter (2009) have devised a way of calculating the diagnostic sensitivity and specificity of both customary, clinical scoring and pulmonary lesions at slaughter for diagnosing BRDC in beef cattle. The use of pulmonary lesions at slaughter, improved sensitivity to 77.4% and specificity to 89.7%, and indicate that exploiting pulmonary lesions at slaughter to monitor health and management programs of cattle or as an objective outcome to evaluate the effect of interventions and management techniques is incomparably more complete in relation to independent clinical observation.

Conclusion

Serious and continuous prevention and control of BRDC, which involves long-term planning and recording and evaluating lesions on the lungs and quality of the carcasses, can be a valuable tool for ensuring the sustainability of meat and milk production in cattle breeding. Monitoring and recording of the pulmonary lesions can be of great benefit in the investigations of the pathology of the respiratory tract of cattle in slaughterhouses in Serbia, especially in the light of the abundance of other evidence of the prevalence of BRDC in cattle herds in Serbia.

Kompleks respiratornog oboljenja goveda (BRDC): Pregled lezija pluća i smanjenog kvaliteta trupova

Vladimir S. Kurćubić, Radojica D. Đoković, Zoran Ž. Ilić, Nikola Lj. Vasković, Miloš Ž. Petrović

Rezime

BRDC je najveći zdravstveni problem stočarske industrije na globalnom nivou zbog visoke prevalencije i ekonomskih posledica koje nastaju zbog brojnih razloga. Ogromni ekonomski gubici najčešće se pripisuju visokom morbiditetu i mortalitetu, smanjenju prosečnog dnevnog prirasta (ADG) i iskorišćenju hrane, gubitku težine, nižem kvalitetu trupova i sveobuhvatnim merama profilakse i terapije. BRDC se obično posmatra kroz fazu punjenja tovilišta, zbog faktora stresa. Predisponirajući faktori podeljeni didaktički na ambijentalne (nepovoljnih vremenskih uslova, neadekvatne vlažnosti i prašine), faktora poreklom od domaćina (starost, pol, rasa, genetika, imunološki status) i prakse upravljanja stresom (prevoz, stres povezan sa ishranom, metaboličke bolesti, prenaseljenost životinja, rukovanje, kastracija, obezrožavanje). U kompleksnoj etiopatogenezi, pored navedenih predisponirajućih faktora, uključeni su i brojni virusni i bakterijski agensi. Velike lezije pluća se najčešće primećuju kod klanja goveda ili obdukcije (vidljive golim okom) i javljaju kao posledica upale pluća. To je rezultat infekcije goveđim respiratornim sincicijalnim virusom (BRSV), virusom parainfluence tipa 3 (PI3V), virusom goveđeg herpesa tipa 1 (BoHV1) i virusom goveđe virusne dijareje (BVDV), samih ili u kombinaciji jedan s drugim. Uobičajeno su prisutni bakterijski patogeni *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* i *Micrococcus spp.* Brojne studije ukazuju na štetne efekte BRDC na performanse i karakteristike trupa goveda.

Ključne reči: kompleks respiratornog oboljenja goveda (BRDC), goveda, ekonomski gubici, plućne lezije, kvalitet trupova

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PHENOTYPIC CORRELATION OF TRAITS OF PRODUCTION AND REPRODUCTION OF SIMMENTAL COWS IN DIFFERENT REGIONS OF SERBIA

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Abstract: The main goal of this research was to examine, using modern methods, the variability of phenotypic correlations of production performances and reproductive properties of Simmental cows reared on the farms of individual agricultural producers, in different breeding areas of the Republic of Serbia. The study of phenotypic correlations of milk performance and fertility properties in different regions of Serbia was carried out on a total of 3.056 primi parous Simmental heifers under control, with lactations completed within one year. The examined animals were reared on different individual farms, and mainly in very different conditions of housing and nutrition, depending on the breeding area. The study of phenotypic correlations included the following milk performance traits: milk yield, milk fat content, milk fat yield, yield of 4% corrected milk; and fertility properties: age at first calving and service period.

The results of the study among other things indicate that although the phenotypic correlations between the fertility and milk performance properties show different degrees of variation, they should be taken into account in the final assessment of the breeding value of the animal, so that the breeding selection programs are more comprehensively designed.

Key words: regions, correlations, milk performance, fertility, Simmental breed

Introduction

Phenotypic correlation of milk performance and fertility properties is very important in a comparative selection for several properties, and even more important for indirect selection in conditions where some properties can not be directly improved. At the same time, opportunities to increase the success of

selection are realized by early selection conclusions and decisions (*Pantelić et al., 2007*). In the mentioned study, age at calving was in negative phenotypic correlation with all production indicators: milk yield -0.023, % milk fat -0.005, milk fat -0.023, and production of 4% FCM -0.023. The mutual phenotypic correlation of age at calving and service period was weak positive 0.047. The service period was also in a negative correlation with the milk yield traits, except for the milk fat content 0.001, and the duration of lactation 0.329.

The coefficients of phenotypic correlations between milk performance properties in standard lactation and age at first calving (AFC) and service period (SP) were calculated by *Stojić (1996)*. Correlation coefficient values were: milk yield - AFC 0.034; milk yield - SP 0.095; milk fat % - AFC 0.034; milk fat% - SP - 0.032; milk fat, kg - AFC 0.045; milk fat, kg - SP 0.072; yield of 4% FCM - AFC 0.042; yield of 4% FCM - SP 0.085.

Marković (1999) notes the values of phenotypic correlations between milk performance properties. The results of phenotypic correlations ranged from -0.35 between milk yield and milk fat content up to 0.96 between milk yield and 4% FCM.

According to the results obtained by *Parna and Savelli (1998)*, the phenotypic correlation between the yield of milk and the yield of milk fat and protein in Black and White cows ranges between 0.826 and 0.890,

Gaydarska et al. (2001) have investigated phenotypic and genotypic correlations on a sample of 3,254 cows. The analysis shows a high and positive genetic and phenotypic correlation between milk production and milk fat 0.935 and 0.953. The correlation between the milk yield and the milk fat content was negative -0.155 (genetic) and -0.257 (phenotypic). Weak positive genetic and phenotypic correlations of 0.171 and 0.045, respectively, between the milk fat yield and content were established.

The highly significant influence of the breeding area ($P < 0.01$) on the production of milk and milk fat, milk fat content and yield of 4% FCM is confirmed by *Petrović et al. (2006)* for cows of the first Simmental breed. The authors believe that the results thus obtained indicate the justification of the use of linear methods, i.e. the correction of systematic environmental factors.

In the examination of the phenotypic and genetic correlations of the milk performance properties and the type of bull dams of Holstein Friesian breed, *Pantelić et al. (2012)* have established a negative phenotypic correlation between milk production and milk fat content of -0.28, and highly positive between milk yield and milk fat yield 0.99.

The results of phenotypic correlations in Simmental cows have been established by *Pantelić et al. (2018)*. The mutual positive and complete phenotypic correlation between the yield of milk, milk fat and 4% of FCM have been established in all three lactations with the coefficient of correlation ranging from 0.98 to 0.99. Low and mainly positive correlation was established in yield

indicators and milk fat content (0.13, 0.21, 0.32). Low and generally positive phenotypic correlation has been reported in all three lactations for milk performance and fertility indicators. The coefficient of phenotypic correlations ranged from 0.03 between the milk yield and the duration of service period in the first lactation (Tab.1) to 0.24 between the yield of 4% FCM and the age at first calving.

Material and methods

This study covered 3,056 controlled primi parous Simmental females, with lactations completed within one year. All the primi parous cows were located on the farms of individual agricultural producers in the territory of the Republic of Serbia. The paper examines phenotypic correlations between the following milk performance and fertility properties:

- duration of lactation (days) -DL
- milk yield in standard lactation (kg) -MY
- milk fat content in standard lactation (%) - MFC
- milk fat yield in standard lactation (kg) - MFY
- yield of 4% FCM in standard lactation (kg) - 4% FCM
- age at first calving (days) -AFC
- duration of service period (days) -DSP

The results of the study of phenotypic correlations were obtained using mixed models LSMLMW (Harvey 1990):

$$Y_{ijklm} = \mu + B_i + R_j + G_k + S_l + e_{ijklm}$$

Y_{ijklm} = the manifestation of the trait of m cow, the daughter of the i bull sire, producing in the j region, which calved in k year and l season

- μ = general average
- B_i = random effect of the i bull sire
- R_j = fixed effect of j region
- G_k = fixed effect of k calving year
- S_l = fixed effect of l calving season
- e_{ijklm} = random error

The standard error of phenotypic correlations was calculated using the following formula:

$$SGr_p = \sqrt{\frac{1 - r_p^2}{n - 2}}$$

The symbols have the following meanings:

SGrp-standard error of phenotypic correlations

rp-phenotypic correlations

n-total number of descendants/offspring

The examined animals were reared on different individual farms, and mainly in very different housing and nutrition conditions, depending on the breeding area. The cows were mainly kept in stables in a tied system, on long and medium long boxes with straw. The diet was based on hay and alfalfa haylage, whole maize silage and mainly ready concentrates. Productivity control was carried out according to the principles of the AT4 control of productivity by breeding organizations, which included measuring of the amount of milk daily/once a day, only during the morning or only during the evening milking on the control day (alternative method), but the results must be mathematically corrected to the reference method.

In order to investigate the variability of phenotypic correlations of production performance and reproductive properties across the regions of Serbia, all primi parous cows included in this research were classified into 6 breeding areas. Breeding regions were as follows:

1. Mačva-Kolubara (area of municipalities of Šabac and Valjevo)
2. Braničevo-Podunavlje (area of municipalities Požarevac and Smederevo)
3. Šumadija (municipality of Mladenovac and Kragujevac)
4. Raška-Rasina (area of Kraljevo and Kruševac municipalities)
5. Zlatibor-Moravica (area of municipalities of Užice and Čačak)
6. Zaječar (area of Zaječar municipality)

Because of the specificity of the terrain, i.e. approximately the same configuration, nutrition, as well as the housing conditions, the breeding areas are grouped together, except for the Zaječar region, which is considered as an independent one.

Figure 1. Distribution of the primi parous cows by regions

1	2	3	4	5	6
342	689	737	653	219	416

Results and discussion

In addition to examining heritability to determine optimal methods and selection procedures, it is very important to examine the phenotypic association of properties that are to be promoted through selection. The strength of the correlation of the examined properties is based on the interpretation of Pearson's linear

correlation coefficient: ≥ 0.70 strong connection, $0.30 - 0.69$ mean connection, < 0.30 weak connection, around 0.0 no linear connection (does not exclude the presence of a nonlinear form of correlation).

The coefficients of the phenotypic correlation of milk performance properties, the duration of service period and the age at calving in different regions of Serbia are shown in Tables 1 and 2.

Table 1. Coefficients of phenotypic correlations (r_p) and their errors (Sr_p) between the milk performance and fertility properties in standard lactation in regions 1, 2 and 3.

	REGION 1		REGION 2		REGION 3	
Traits	r_p	Sr_p	r_p	Sr_p	r_p	Sr_p
DL, days						
MY, kg	-0.073	-0.004	-0.046	-0.002	-0.139	-0.005
MFC, %	0.190	0.010	0.082	0.003	0.097	0.004
MFY, kg	-0.026	-0.001	-0.020	-0.001	-0.107	-0.004
4% FCM, kg	-0.046	-0.002	-0.031	-0.001	-0.120	-0.004
DSP, days	0.229	0.012	0.232	0.009	0.197	0.007
ACF, days	0.075	0.004	0.006	0.000	0.004	0.000
MY, kg						
MFC, %	-0.234	-0.013	-0.050	-0.002	0.140	0.005
MFY, kg	0.968	0.052	0.949	0.036	0.976	0.036
4% FCM, kg	0.989	0.053	0.981	0.037	0.991	0.037
DSP, days	-0.170	-0.009	0.066	0.003	-0.012	0.000
ACF, days	0.074	0.004	0.097	0.004	-0.016	-0.001
MFC, %						
MFY, kg	0.016	0.001	0.267	0.010	0.350	0.013
4% FCM, kg	-0.088	-0.005	0.142	0.006	0.269	0.010
DSP, days	0.132	0.007	-0.078	-0.003	0.022	0.001
ACF, days	0.089	0.005	-0.027	-0.001	0.055	0.002
MFY, kg						
4% FCM, kg	0.994	0.054	0.992	0.038	0.996	0.037
DSP, days	-0.139	-0.008	0.038	0.001	-0.007	0.000
ACF, days	0.098	0.005	0.085	0.003	-0.003	0.000
4%FCM, kg						
DSP, days	-0.153	-0.008	0.050	0.002	-0.009	0.000
ACF, days	0.089	0.005	0.091	0.003	-0.008	0.000
DSP, days						
ACF, days	0.076	0.004	0.066	0.003	-0.007	0.000

In the first region, a positive correlation was established between the age at first calving and milk performance traits, and the coefficient of correlation ranged

from 0.074 with milk yield to 0.098 with the milk fat yield. So the correlation was positive and quite weak. Strong and positive phenotypic correlation in this region was determined between the milk yield and milk fat yield 0.968 and 4% FCM 0.989. Service period was in week negative phenotypic correlation with milk yield -0.170 and quantity of milk fat -0.139.

As a general characteristic of all regions, a complete and very strong correlation between milk yield and milk fat yield, and 4% FCM (0.970 and more) was established. Also noticeable is the markedly negative correlation between milk yield and milk fat content, which was weakly positive in Šumadija (0.140), Zaječar (0.153) and Zlatibor-Moravica (0.083) regions.

The phenotypic correlation between the service period and the milk yield varied from the negative ones in Mačva-Kolubara (-0,170), Šumadija (-0,012), Raška-Rasina (-0,047), Zlatibor-Moravica (-0,137) and Zaječar (-0,103) regions to weakly positive in Braničevo-Podunavlje (0.066) region.

The correlation coefficient between age at calving and milk yield, milk fat and 4% FCM ranged from positive values in region 1 (0.044, 0.098, 0.089) to negative values in region 6 (-0.067, -0.089, -0.081). The negative coefficient of phenotypic correlations of these properties was also found in regions 3, 4, 5, 6, and positive in regions 1 and 2.

The presence of information on phenotypic and genetic correlations between milk performance and fertility properties can have multiple relevance in cow selection because it provides the ability to select animals for multiple traits at the same time. This is especially the case with the application of modern methods of mathematical statistics in the assessment of the additive genetic value of bulls and cows. In addition, an early selection of parents based on the first lactation is possible, which significantly shortens the period for the introduction of bulls into the breeding.

A negative phenotypic correlation between milk yield and milk fat content, and a positive between the yield of milk and the quantity of milk fat, and 4% of FCM, have been established in a number of studies: *Moore et al. (1991)*, *Parna and Saveli (1998)*, *Marković (1999)*, *Pantelić et al. (2018)*. Negative phenotypic correlations between the age at calving and milk yield are reported by *Pantelić et al. (2007)*. Contrary to them, *Moore et al. (1991)* and *Stojić (1996)*, provide data on the positive values of the coefficient of phenotypic correlation of these properties. Studying the genetic variability of the persistence of lactation of Simmental cows *Durđević et al. (2002)* conclude that the examination of the effects of known environmental factors is justified because knowledge of their behaviour can contribute to more objective assessment of random effects.

Table 2. Coefficients of phenotypic correlations (r_p) and their errors (Sr_p) between the milk performance and fertility properties in standard lactation in regions 4, 5 and 6.

	REGION 4		REGION 5		REGION 6	
Traits	r_p	Sr_p	r_p	Sr_p	r_p	Sr_p
DL, days						
MY, kg	-0.079	-0.003	-0.030	-0.002	-0.144	-0.007
MFC, %	0.010	0.000	0.010	0.001	-0.025	-0.001
MFY, kg	-0.071	-0.003	-0.028	-0.002	-0.142	-0.007
4% FCM, kg	-0.075	-0.003	-0.029	-0.002	-0.144	-0.007
DSP, days	0.429	0.017	0.385	0.026	0.412	0.020
ACF, days	0.062	0.002	-0.149	-0.010	0.082	0.004
MY, kg						
MFC, %	-0.031	-0.001	0.083	0.006	0.153	0.008
MFY, kg	0.907	0.035	0.980	0.066	0.967	0.047
4% FCM, kg	0.964	0.038	0.993	0.067	0.988	0.048
DSP, days	-0.047	-0.002	-0.137	-0.009	-0.103	-0.005
ACF, days	-0.034	-0.001	-0.017	-0.001	-0.067	-0.003
MFC, %						
MFY, kg	0.383	0.015	0.278	0.019	0.394	0.019
4% FCM, kg	0.229	0.009	0.201	0.014	0.302	0.015
DSP, days	-0.008	0.000	-0.010	-0.001	-0.110	-0.005
ACF, days	0.030	0.001	-0.026	-0.002	-0.117	-0.006
MFY, kg						
4% FCM, kg	0.986	0.039	0.997	0.067	0.995	0.049
DSP, days	-0.045	-0.002	-0.133	-0.009	-0.123	-0.006
ACF, days	-0.013	-0.001	-0.021	-0.001	-0.089	-0.004
4%FCM, kg						
DSP, days	-0.047	-0.002	-0.135	-0.009	-0.116	-0.006
ACF, days	-0.022	-0.001	-0.019	-0.001	-0.081	-0.004
DSP, days						
ACF, days	0.101	0.004	-0.143	-0.010	0.108	0.005

Determination of the degree of correlation of two or more properties depends to a large extent on their manifestation. Knowing genetic and phenotypic correlations between fertility and milk performance properties can help define a breeding goal.

Phenotypic correlations are determined both by genetic and external factors. If the environmental conditions in animals were identical, then the

phenotypic value of the correlations would be equal to the genetic one. However, as there are no identical conditions in practical cattle-breeding, the values between these correlations are also different. If the external environment conditions are more stable, i.e. less variable, the degree of correlation between the phenotypes of the animals will be higher (*Petrović and Pantelić, 2015; Petrović et al. 2018.*).

Conclusion

Phenotypic correlation of properties in cattle breeding refers to the existence of a common positive or negative covariance, which arises as a result of the action of genetic and environmental factors.

The presence of information on phenotypic and genetic correlations between milk performance and fertility properties can have multiple relevance in cow selection because it provides the ability to select animals for multiple traits at the same time.

This is especially the case with the application of modern methods of mathematical statistics in the assessment of the additive genetic value of bulls and cows. In addition, an early selection of parents based on the first lactation is possible, which significantly shortens the period for the introduction of bulls into the breeding.

The effect of the breeding area includes many factors that complement one another, and the most important factor is the nutrition factor as well as the applied management. Although the phenotypic correlation between fertility and milk performance traits shows different degrees of variation in different regions of Serbia, they should be taken into account in the final assessment of the breeding value of the animal, in order to make the selection programs more comprehensively designed.

Ispoljenost fenotipskih korelacija proizvodnih i reproduktivnih osobina krava simentalске rase u različitim regionima Srbije

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Rezime

Osnovni cilj ovih istraživanja bio je da se na imanjima individualnih poljoprivrednih proizvođača primenom savremenih metoda ispita varijabilnost

fenotipskih korelacija proizvodnih osobina u zavisnosti od odgajivačkog područja Republike Srbije, u kojima se grla i odgajaju. Ispitivanje fenotipskih korelacija osobina mlečnosti i plodnosti u različitim regionima Srbije, izvršeno je na ukupno 3.056 kontrolisane prvotelke simentalke rase, sa laktacijama zaključenim u toku jedne godine. Ispitivana grla su gajena na različitim individualnim gazdinstvima, ali se može reći uglavnom u veoma različitim uslovima držanja i ishrane, u zavisnosti od odgajivačkog područja. Ispitivanje fenotipskih korelacija obuhvatilo je sledeće osobine mlečnosti: prinos mleka, sadržaj mlečne masti, prinos mlečne masti, prinos 4% mast korigovanog mleka; zatim osobine plodnosti: uzrast pri prvom telenju i servis period. Rezultati istraživanja između ostalog ukazuju da iako fenotipske korelacije između osobina plodnosti i mlečnosti pokazuje različite stepene variranja, treba ih uzeti u obzir kod konačne ocene priplodne vrednosti grla, kako bi se selekcijski programi što potpunije formirali.

Ključne reči: regioni, korelacije, mlečnost, plodnost, simentalna rasa

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EFFECT OF NON-GENETIC FACTORS ON LONGEVITY TRAITS IN SIMMENTAL COWS

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Original scientific paper

Abstract. The effects of fixed non-genetic factors (farm, season of birth, year of birth, total number of lactations) and a continuous non-genetic factor (age at first conception) on the expression and variability of longevity traits such as age at culling, length of productive life, days in milk and cow efficiency index were investigated in 2548 Simmental cows in three farming areas. Based on the model used for the analysis of the effects of non-genetic factors, including the environment and cow age at first conception, on the expression and variability of longevity traits, the overall means for age at culling, length of productive life, days in milk and cow efficiency index were 2445.21 ± 17.49 days, 1562.55 ± 17.71 days, 1094.17 ± 12.28 days and $58.68 \pm 0.32\%$, respectively. The effect of farming area, year of birth and lactation group on longevity traits was very significant ($P < 0.01$), whereas the effect of season of birth was significant ($P < 0.05$). Age at first conception had a highly significant ($P < 0.01$) effect on age at culling, length of productive life and cow efficiency index, and no significant effect on days in milk ($P > 0.05$). Based on the model used, the coefficients of determination (R^2) were very significant ($P < 0.01$) for all longevity traits, and ranged from 0.898 for age at culling to 0.959 for days in milk.

Key words: longevity, fixed effects, continuous effects, Simmental cows.

Introduction

Research into phenotypic and genetic variability of production traits has an immense practical importance as this variability underlies annual and generational selection effects.

Selection work in dairy herds in the last 50 years has strongly focused on milk performance traits. This direct selection based on milk performance traits has led to deterioration of fertility, longevity and immunity traits, which have a critical role in profitable milk production. As reported by the United States Department of

Agriculture, until 1994, the official selection index used in the selection of dairy cattle included only milk performance traits in calculating the aggregate genotype (Van Raden, 2004).

In the last decade of the 20th century and the first decade of the 21st century, many studies (Allaire and Gibson, 1992; Dekkers, 1993; Smith et al., 2000; Harder et al., 2006; Orpin and Esslemont, 2010) showed a negative effect of a high level of involuntary cullings of animals from the herd on the economic efficiency of milk production on farms. Longevity as a function of animal's constitution and resistance to diseases, along with high productivity and good composition of milk, is an important trait for the economic efficiency of milk production (Pogačar et al., 1998). Consistently with the results of these studies and to solve problems regarding involuntary cullings, a large number of countries have included functional traits in their models for evaluating the breeding value of animals (Sewalem et al., 2008). This approach has led to breeding programmes which have adopted a more balanced approach, with the selection focus moved from milk performance traits to functional traits (Miglior et al., 2005). In terms of milk production, functional traits have today become crucial in defining modern breeding programmes aimed at obtaining productive cost-efficient animals. Their basic effect on the economic efficiency of milk production is not achieved through direct increase in animal productivity, but is evidenced by production cost reduction through animal health, resistance, vitality and ability to last a number of lactations (Bogdanović et al., 2012).

From its earliest beginnings, research on longevity traits has faced difficulties with both their proper interpretation and understanding the processes which affect them. The main problem in the analysis of longevity traits is that the exact value for this group of traits can be obtained only after the animal has been culled, due to which the generational interval is considerably prolonged and the selection effect reduced. In addition, functional traits often have low variability (occurrence of mastitis, calving ease, diseases of some organs, etc.), which significantly complicates their analysis and proper interpretation of results. As reported by Fuerst and Sölkner (1997), many studies have shown that the true productive lifespan cannot be used as an indicator of cow's biological capacity. Therefore, careful attention has been given to functional longevity (yield adjustment) as a better trait to be used in routine genetic evaluation. In Austria, the breeding value of the functional length of productive life has been routinely evaluated since June 1995. These programmes are based on the Cox proportional hazards model.

Longevity and lifetime milk production are significantly affected by a number of non-genetic factors, either discontinuous i.e. fixed (farming area, season

of birth, year of birth, season of calving, year of calving, total number of lactations and their interactions) or continuous i.e. non-categorical factors (age at first conception or age at first calving). In many herds, major reasons for culling include low milk production (30–35%), impaired physiological functions (30–40%) and udder diseases (10–15%). Low production is the main culling reason for cows after the first or second lactation, whereas older cows are commonly culled for infertility.

Cattle breeding practices generally involve the use of linear methods and models combining fixed parameters (year, farm, season, lactation) and random variables (age at first conception or age at first calving, genetic effect of father, genetic effect of an individual animal, etc.), which can be either mutually dependent (related) or independent, depending on the trait analysed, with or without interactions. The model selected is essentially the breeding value of the individual (*Bogdanović et al., 2003*).

The objective of the present research was to contribute to a better understanding of variability of longevity traits in Simmental cattle in Serbia caused by different non-genetic effects. This research can serve as the basis of initial endeavours in including this group of traits in the breeding programme for Simmental cattle in Serbia, as well as in evaluating the breeding value of bulls for these traits.

Material and methods

The effect of non-genetic factors on the phenotypic expression and variability of longevity traits was analysed in 2548 Simmental cows born from 1995 to 2008, which were housed in three farming areas:

1. Zlatiborski Suvati (Mt. Zlatibor Pastures) Dairy Cow Farm on Mt. Zlatibor (n=502), loose housing system,
2. Dairy cow farm at the Dobričevo farm in Čuprija (n=956), tie-stall housing system, and
3. Private dairy cow farms in the region of Kotraž (n=1090), tie-stall housing system.

The distribution of data on cows across classes of major non-genetic effects is presented in Table 1.

Table 1. Distribution of data across classes of major non-genetic effects

Farm	No. of cows	Season of birth	No. of cows	Year of birth	No. of cows	Total no. of lactations	No. of cows
I (Zlatibor)	502	I	589	1995	164	1	396
		II	727	1996	129	2	512
II (Dobričevo)	956	III	570	1997	184	3	466
		IV	662	1998	169	4	385
III (Kotraža)	1090			1999	246	5	314
				2000	250	6	217
2001	192			7	143		
2002	213			8	81		
2003	187			9	23		
2004	167			10	11		
2005	172						
2006	106						
2007	168						
2008	201						

Based on the data obtained from birth records of cows housed in three farming areas, the following longevity traits were analysed:

- age at culling (days),
- length of productive life (days),
- days in milk,
- cow efficiency index (%).

Preparation of data for statistical analysis

Longevity traits were calculated using data obtained from birth records of cows, as follows:

Age at culling – by summing up the length of productive life in days and age at first calving.

Length of productive life – by subtracting age at first calving in days from age at culling.

Days in milk – by summing up the lengths of all full lactations during the cow's lifetime i.e. productive life.

Cow efficiency index (%) as a relative measure of longevity, calculated by dividing the length of productive life by cow's age at culling, and multiplying by 100.

Non-genetic effect of the environment

The effect of the following non-genetic factors on lifetime production traits was analysed:

Farming area (farm): The research was conducted at three farming locations, two in the uplands (dairy cow farm on Mt. Zlatibor, which employed loose housing system, and private farms, which used tie-stall housing systems, in Kotraž) and a farm at Dobričevo in the low lands, which employed tie-stall housing system.

Season of birth:

I – spring season (March, April, May),

II – summer season (June, July, August),

III – autumn season (September, October, November),

IV – winter season (December, January, February).

Year of birth: cows born from 1995 to 2008.

Total number of lactations: 1–10.

Age at first conception.

Evaluation of non-genetic effects of the environment

The effect of non-genetic environment-related factors on longevity traits (arithmetic means (\bar{X}), standard errors of arithmetic means (S_x), significance of the effect of non-genetic factors and coefficients of determination (R^2)) was analysed using the general linear model of the *Statistica 6.0* statistical software. This procedure ensures a simultaneous analysis of a number of different effects, either categorical factors (farming area, total number of lactations, season of birth and year of birth) or continuous factors (age at first conception).

The analysis of the effect of some non-genetic factors on lifetime production traits was performed by the following model:

$$y_{ijkl} = \mu + FR_i + SB_j + YB_k + L_l + b_1(x_l - \bar{x}_l) + e_{ijkl}, \text{ where:}$$

y_{ijkl} – individual of the i -th farming area, j -th season of birth, k -th year of birth and l -th total number of lactations,

μ – general mean value of population when all classes of effects (FR , SB , YB , L) are equally present

FR_i – fixed effect of the i -th farming area (1–3),

SB_j – fixed effect of the j -th season of birth (1–4),

YB_k – fixed effect of the k -th year of birth (1–14),

L_l – fixed effect of the l -th total number of lactations (1–10),

b_1 – linear regression coefficient of the effect of age at first conception, and

e_{ijkl} – other non-determined effects.

Further analysis of longevity traits presents coefficients of determination (R^2) for each trait analysed, which are proportions of residual variance i.e. variance of the model divided by 100.

Results and discussion

The results of the analysis of the effect of non-genetic environment-related factors on longevity traits (cow's age at culling, length of productive life, days in milk and cow efficiency index) are presented in Table 2. Depending on the effect of non-genetic factors (farming area, season of birth, year of birth, total number of lactations and cow's age at first conception), the table presents arithmetic means and standard errors of arithmetic means ($\bar{X} \pm S_x$), the significance of the effect of non-genetic factors and coefficients of determination (R^2) for all longevity traits analysed.

The average age at culling and length of productive life in all tested cows were 2445.21 ± 17.49 and 1562.55 ± 17.71 days, respectively. The total number of days in milk was 1094.17 ± 12.28 , and cow efficiency index was $58.68 \pm 0.32\%$.

Farming area or farm commonly has a significant effect on longevity traits due to housing system, nutrition, animal care, climate, age structure, herd size and other factors associated with farm operation and management practices. The interaction of these factors is characteristic of each farm. Hence, there are differences across herds and farms even when the genetic potential for milk production is similar. The effect of farming area on all longevity traits in this research was very significant ($P < 0.01$). The highest expression of these traits was observed at the farm on Mt. Zlatibor as the result of loose housing system and grazing during the summer season, whereas their lowest values were recorded for cows at the Dobričevo farm, which employed tie-stall housing without grazing. The values of these traits in cows in the Kotraž region were higher than Dobričevo cows, as the result of more relaxed farming conditions, including a small number of animals and more extensive farming, which favoured the longevity traits analysed.

Table 2. Arithmetic means (X), standard errors of arithmetic means (Sx), significance of the effect of non-genetic factors and coefficients of determination (R^2) for longevity traits

Non-genetic effects	n	Age at culling (days) $\bar{X} \pm S\bar{X}$	Length of productive life, (days) $\bar{X} \pm S\bar{X}$	Days in milk $\bar{X} \pm S\bar{X}$	Cow efficiency index, % $\bar{X} \pm S\bar{X}$
Mean - μ	2548	2445.21 \pm 17.49	1562.55 \pm 17.71	1094.17 \pm 12.28	58.68 \pm 0.32
Farming area (A)					
Zlatibor	502	2495.51 \pm 36.10 ^a	1601.12 \pm 36.86 ^a	1246.08 \pm 29.99 ^a	59.99 \pm 0.64 ^a
Dobričevo	956	2373.89 \pm 25.40 ^b	1500.59 \pm 25.93 ^b	1000.49 \pm 18.06 ^c	58.70 \pm 0.49 ^b
Kotražica	1090	2484.59 \pm 29.89 ^a	1599.14 \pm 30.07 ^a	1106.36 \pm 19.13 ^b	58.05 \pm 0.55 ^c
Season of birth (B)					
Spring (1)	589	2433.46 \pm 36.47 ^b	1557.60 \pm 36.82 ^b	1101.11 \pm 25.89 ^b	58.70 \pm 0.68 ^b
Summer (2)	727	2516.28 \pm 34.09 ^a	1627.19 \pm 34.39 ^a	1126.31 \pm 22.50 ^a	59.49 \pm 0.60 ^a
Autumn (3)	570	2345.59 \pm 35.24 ^c	1468.69 \pm 35.90 ^c	1025.15 \pm 24.89 ^c	57.31 \pm 0.69 ^c
Winter (4)	662	2463.37 \pm 33.76 ^b	1576.81 \pm 34.29 ^b	1112.11 \pm 25.05 ^{ab}	58.95 \pm 0.63 ^b
Year of birth (C)					
1995	164	3081.19 \pm 64.50 ^a	2220.41 \pm 64.75 ^a	1593.35 \pm 46.95 ^a	69.67 \pm 0.81 ^a
1996	129	2736.03 \pm 67.75 ^{bc}	1866.03 \pm 68.84 ^{bc}	1365.29 \pm 50.57 ^b	65.23 \pm 1.06 ^b
1997	184	2315.73 \pm 55.90 ^e	1434.43 \pm 56.77 ^f	1048.67 \pm 43.28 ^f	57.50 \pm 1.13 ^c
1998	169	2234.38 \pm 67.95 ^f	1359.22 \pm 67.51 ^g	989.55 \pm 46.23 ^g	54.68 \pm 1.36 ^f
1999	246	2337.93 \pm 52.02 ^e	1452.05 \pm 52.35 ^f	1071.16 \pm 38.29 ^f	57.43 \pm 0.98 ^e
2000	250	2226.55 \pm 56.84 ^f	1346.46 \pm 58.08 ^g	989.04 \pm 41.66 ^g	54.29 \pm 1.11 ^{fg}
2001	192	2683.18 \pm 75.36 ^c	1822.41 \pm 75.10 ^c	1243.71 \pm 48.64 ^c	62.31 \pm 1.19 ^c
2002	213	2559.71 \pm 77.88 ^d	1724.62 \pm 76.47 ^d	1183.60 \pm 49.82 ^d	59.82 \pm 1.29 ^d
2003	187	2770.21 \pm 66.77 ^b	1891.78 \pm 65.94 ^b	1222.17 \pm 43.55 ^c	64.61 \pm 0.91 ^b
2004	167	2544.93 \pm 60.93 ^d	1672.02 \pm 61.51 ^d	1135.57 \pm 41.50 ^e	61.86 \pm 1.06 ^c
2005	172	2516.76 \pm 56.36 ^d	1602.60 \pm 59.92 ^e	1110.46 \pm 43.26 ^e	59.46 \pm 1.18 ^d
2006	106	2220.41 \pm 66.91 ^f	1298.53 \pm 68.44 ^{gh}	864.29 \pm 43.63 ^h	53.88 \pm 1.56 ^f
2007	168	2183.40 \pm 41.74 ^f	1240.21 \pm 43.90 ^h	805.98 \pm 31.17 ⁱ	53.51 \pm 1.13 ^g
2008	201	1980.93 \pm 40.79 ^g	1080.47 \pm 42.37 ⁱ	758.44 \pm 29.18 ⁱ	50.25 \pm 1.15 ^h
Total number of lactations (D)					
1	396	1328.37 \pm 10.66 ^j	413.82 \pm 8.64 ^j	312.15 \pm 3.82 ^j	30.43 \pm 0.40 ^j
2	512	1774.40 \pm 12.86 ⁱ	875.86 \pm 11.66 ⁱ	619.45 \pm 5.23 ⁱ	48.69 \pm 0.29 ^h
3	466	2225.93 \pm 15.44 ^h	1343.13 \pm 14.48 ^h	917.19 \pm 6.65 ^h	59.90 \pm 0.23 ^g
4	385	2668.70 \pm 17.73 ^g	1789.88 \pm 16.67 ^g	1206.49 \pm 8.42 ^g	66.76 \pm 0.22 ^f
5	314	3044.55 \pm 18.98 ^f	2170.70 \pm 17.21 ^f	1515.83 \pm 10.02 ^f	71.13 \pm 0.19 ^e
6	217	3414.48 \pm 23.15 ^e	2563.61 \pm 21.46 ^e	1813.07 \pm 13.90 ^e	74.95 \pm 0.19 ^d
7	143	3788.22 \pm 27.82 ^d	2947.81 \pm 26.42 ^d	2081.24 \pm 17.27 ^d	77.72 \pm 0.21 ^c
8	81	4138.84 \pm 38.22 ^c	3284.18 \pm 35.67 ^c	2361.94 \pm 21.66 ^c	79.28 \pm 0.24 ^b
9	23	4450.74 \pm 70.70 ^b	3621.13 \pm 66.82 ^b	2758.56 \pm 37.29 ^b	81.31 \pm 0.42 ^{ab}
10	11	4987.18 \pm 118.48 ^a	4120.82 \pm 119.47 ^a	3042.64 \pm 5.04 ^a	82.55 \pm 0.73 ^a
Age at first conception (E)					
$b_{xy} \pm S_b$		0.992 \pm 0.002 ^{**}	-0.993 \pm 0.002 ^{**}	0.000 \pm 0.002 ^{ns}	0.009 \pm 0.056 ^{ns}
Anova	df				
A	2	**	**	**	**
B	3	*	*	*	*
C	13	**	**	**	**
D	9	**	**	**	**
E		**	**	ns	**
Coefficient of determination – R^2		0.898 ^{**}	0.901 ^{**}	0.951 ^{**}	0.919 ^{**}

Mean values followed by the same letters across columns are not different ($P > 0.05$) according to LSD test F-test (Anova) and t-test (coefficient of linear regression $-b_{xy}$): N.S. – $P > 0.05$; * – $P < 0.05$; ** – $P < 0.01$;

The effect of season of birth on longevity traits in cows is the result of changes in climate, primarily temperature and precipitation, leading to changes in housing system (barn-based and pasture-based) and cow health as major factors determining the expression of these traits. Longevity traits were significantly affected by season of birth ($P < 0.05$). The highest values were recorded for cows born in the summer season, and the lowest for cows born in autumn. The most possible reason lies in the fact that calves born in the summer season were initially raised in pastures, as opposed to calves born in autumn which were raised in poorer barn-based conditions immediately after calving, which adversely affected their immunity and health in general, as well as the expression of longevity traits.

The effect of year of birth on the expression of longevity traits is evidenced through climate conditions across production years and feed quality and quantity. Moreover, farming technology and health management have been improving over the years, and there has been more or less annual selection success. Year of birth in the present experiment had a very significant ($P < 0.01$) effect on longevity traits (age at culling, length of productive life, days in milk and cow efficiency index). Despite improvement in farming technology and health management, longevity traits across years of birth in this research exhibited a decreasing trend. This was associated with increased selection criteria and intensified production, which directly affected the relationship between planned and unplanned cullings and, hence, the expression of longevity traits and milk production profitability. Most cows are culled from production for undesirable reasons, which can be up to 2.5 times more common than planned culling reasons (Pinedo et al., 2010). Major causes of involuntary culling include reproductive disorders, mastitis, hoof and leg diseases and injuries (Nienartowicz-Zdrojewska et al., 2009; Pinedo et al., 2010; Chiumia, 2011; Ansari-Lari et al., 2012; Stojić et al., 2012).

The effect of total number of lactations on longevity traits was very significant ($P < 0.01$). The increase in lactation numbers directly and indirectly led to an increase in longevity traits. The same finding was reported by Petrović D.M. et al., (2004). In 143 Simmental cows housed at the Zlatiborski Suvati (Zlatibor Pastures) farm, the author used the general linear model procedure and determined a very highly significant ($P < 0.001$) effect of total number of lactations on longevity traits such as age at culling, length of productive life, days in milk, and cow efficiency index. The analysis of the effect of lactation number on culling reasons showed that dominant culling reasons varied with lactation. Dominant reasons for culling of first-calvers are selection issues (primarily low production) and reproduction problems (Seegers et al., 1998; Stojić et al., 2012). First-calvers account for 20–35% of the total number of animals culled per year (Maher et al., 2008; Pinedo et al., 2010; Chiumia, 2011). First-calvers that become pregnant late

for the first time and those that have less expressed standard characteristics are culled earlier due to the higher risk of low production (Dürr, 1997).

Age at first conception had a highly significant effect ($P < 0.01$) on age at culling and length of productive life. The coefficient of linear regression was $b_{xy} = 0.992$ for the first trait, and negative $b_{xy} = -0.993$ for the second trait. Age at first conception had no significant effect ($P > 0.05$) on days in milk and cow efficiency index.

The effect of age at first conception or age at first calving on longevity traits was examined in a number of studies. In their research on the regression effect of non-genetic factors on longevity traits in Simmental cows, Petrović D.M. *et al.*, (2004; 2008), found a highly significant ($P < 0.001$) effect of age at first conception on age at culling and cow efficiency index ($b_{xy} = 1.386$ and $b_{xy} = -0.020$), whereas their effect on length of productive life and days in milk was non-significant ($P > 0.05$). Considerable research has documented that the risk of culling increases (i.e. animals are culled earlier) with increasing age at first calving (M'hamdi *et al.*, 2010; Raguž, 2012; Zavadilová and Štípková, 2013). When evaluating the effect of age at first calving on functional longevity in Hostein cows in Tunisia, M'hamdi *et al.*, (2010) found that culling risk increased linearly with increasing age at first calving. Specifically, age at first calving of 27 months was set as basal age, with the risk set to 1 for this age. In animals that calved at an average age of 21 months, the risk was 0.96, whereas the risk for the calving age of 39 months was significantly higher – 1.43.

Coefficients of determination, which indicate the degree of explanation of variations of longevity traits by the model employed, were very high, above 0.9 i.e. over 90% of variability in longevity traits was induced by the effect of non-genetic environment-related factors and age at first conception. Such high coefficients of determination for longevity traits were mostly due to total number of lactations. The lowest coefficient of determination 0.898 (89.8%) was obtained for age at culling, and the highest 0.951 (95.1%) for days in milk. Somewhat lower coefficients of determination for longevity traits, due to fewer non-genetic factors analysed, were reported by Petrović D.M. *et al.*, (2004). In their study on the effect of lactation number and season of calving as fixed non-genetic factors and age at first conception as a continuous factor on longevity traits in 143 Simmental cows, the coefficients of determination ranged from 0.823 for cow efficiency index to 0.894 for days in milk.

Conclusion

Based on the model for analysing the effect of non-genetic environment-related factors and age at first conception on the expression and variability of longevity traits, the following conclusions are drawn:

The general mean values for age at culling, length of productive life, days in milk and cow efficiency index in 2548 tested cows were 2445.21 ± 17.49 days, 1562.55 ± 17.71 days, 1094.17 ± 12.28 days and $58.68 \pm 0.32\%$, respectively.

The effect of farming area, year of birth and lactation group on longevity traits was very significant ($P < 0.01$), whereas the effect of season of birth was significant ($P < 0.05$).

Age at first conception as a continuous factor had a highly significant ($P < 0.01$) effect on age at culling, length of productive life and cow efficiency index, and no significant effect on days in milk ($P > 0.05$).

The coefficients of determination (R^2), obtained by the model, were very significant ($P < 0.01$) for all longevity traits and ranged from 0.898 for age at culling to 0.959 for days in milk.

Given that non-genetic factors, both fixed and continuous, had a generally highly significant effect on longevity traits, and were largely responsible for their variations, they should necessarily be included in models for evaluating the breeding value of dairy cows i.e. these traits should be corrected for the effect of these non-genetic factors.

Uticaj sistematskih faktora na osobine dugovečnosti kod krava simentalске rase

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Rezime

Uticaj fiksnih (farma, sezona i godina rođenja, ukupan broj laktacija) i kontinuelnih (uzrast pri prvoj oplodnji) sistematskih faktora na ispoljenost i varijabilnost osobina dugovečnosti kao što su uzrast pri izlučenju, dužina produktivnog života, ukupan broj muznih dana i indeks iskorišćavanja krava proučavan je na uzorku od 2548 simentalских krava raspoređenih na tri odgajivačka područja. Na osnovu primenjenog modela za analizu uticaja sistematskih faktora okoline i uzrasta krava pri prvoj oplodnji na ispoljenost i varijabilnost osobina dugovečnosti opšti proseka za uzrast pri izlučenju, dužinu produktivnog života, ukupan

broj muznih dana i indeks iskorišćavanja krava iznosio je $2445,21 \pm 17,49$ dana, $1562,55 \pm 17,71$ dana, $1094,17 \pm 12,28$ dana i $58,68 \pm 0,32\%$. Uticaj odgajivačkog područja, godine rođenja i grupe laktacija na osobine dugovečnosti bio je vrlo značajan ($P < 0.01$), dok je uticaj sezone rođenja krava bio značajan ($P < 0.05$). Uzrast pri prvoj oplodnji visoko značajno ($P < 0.01$) je uticao na uzrast pri izlučenju, dužinu produktivnog života i indeks iskorišćavanja krava, dok na ukupan broj muznih dana nije imao značajan uticaj ($P > 0.05$). Dobijeni koeficijenti determinacije (R^2), na osnovu primenjenog modela, za sve osobine dugovečnosti bili su vrlo značajni ($P < 0.01$) i kretali su se od 0,898 kod uzrasta pri izlučenju do 0,959 kod ukupnog broja muznih dana.

Ključne reči: dugovečnost, fiksni uticaji, kontinuelni uticaji, simentalske krave.

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EFFECTS OF LIGHT INTENSITY IN DIFFERENT STOCKING DENSITIES ON TIBIAL MEASUREMENTS AND INCIDENCE OF LESIONS IN BROILERS

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Original scientific paper

Abstract: The possibility for use of high light intensity in broiler production management to improve the health of broilers' legs was examined in a study aiming to determine the effect of two levels of light intensity in different stocking densities of broiler chickens. The study was carried out on 1200 Ross 308 broilers according to a random block system with 6 treatments (2 x 3 factorial design) and 4 replicates per treatment. The examined light intensity levels of 150 lx (high light intensity-HLI) and 20 lx (low light intensity-LLI) were applied to 3 stocking densities of broilers: 10, 13 and 15 birds/m². Scoring of foot pad (FP) and hock burns (HB) was carried out on the 42nd day, on a sample of 240 broilers. Osteometric and biomechanical measurements on tibia were performed after slaughtering and primary processing, on a sample of 72 broilers. The high level intensity of light exerted no significant effect on the development and firmness of the tibia of broilers grown in the densities of 10, 13 and 15 birds/m². There are indications that in the highest studied stocking density the increased light intensity can improve the individual properties of tibia. The differences in the average assessment of hock burns were concluded to be the result of the stocking density that was observed only in conditions of the increased intensity of light. The average score of foot pad lesions was under the significant main effect of both investigated factors. The differences in the average FP estimation between the light intensity treatments were confirmed in the stocking density of 13 birds/m².

Key words: broiler, light intensity, stocking density, tibia, lesions

Introduction

The recommended conditions regarding the light intensity in broiler production lead to the fact that broilers often spend time in the half-dark, without clearly differentiated periods of light and darkness. Such conditions may influence the establishment of circadian rhythms responsible for the processes of bone mineralization, physical activity of broilers, and eventually, health of the legs.

The light of increased intensity stimulates daily activity, as confirmed by *Blatcherford et al. (2009)* who state that the light intensity of minimum 50 lx has positively influenced daily activity without adverse effects on production performances.

With high level intensity of light, behaviour patterns are more prominent and broilers are more physically active (*Blatcherford et al., 2012; Rault et al., 2017*). Expressed physical activity can be accompanied by weight reduction (*Lien et al., 2008; Blatcherford et al., 2012; Rault et al., 2017*), which can create conditions for the reduction of leg problems. *Škrbić et al. (2011)* have established the presence of indications that higher physical activity in conditions of lower stocking density of broilers, improves the quality of tibia, even with higher final weight of broilers. In support of this are the results of *Kapell et al. (2012)* on the established genetic correlation between the health properties of legs and the broiler weight that is low to moderately negative, which leaves the possibility of improving these properties by breeding methods. The low light intensity does not sufficiently stimulate broiler activity, according to *Rault et al. (2017)*, although there was no clear evidence of adverse effects on the broiler welfare and the strength of the leg. Differences in gait scores were not recorded in *Blatcherford et al. (2009)*, but broilers in treatment with 200 lx had less lesions compared to treatments with 5 and 50 lx. *Deep et al. (2010)* came to similar results in their examination of light intensity levels of 1, 10, 20 and 40 lx that did not show the effect on leg health, estimated on the basis of a gait score, while the incidence of ulcerous foot pad lesions decreased linearly with the increase in light intensity. In the later study by *Blatcherford et al. (2012)* they have confirmed the effect of light intensity on the health of broilers in terms of less incidence of lameness in higher intensity of light. In this regard, it is possible to note certain potential of using increased light intensity in the management of broiler production to improve the health and welfare of broilers, with the necessity of more precise determination of optimal levels in accordance with technological conditions and genotype.

The aim of the study was to determine the effect of two levels of light intensity in different stocking densities of broiler chickens, and thus examine their interactive effect on foot health indicators, that is, the strength of the tibia and the appearance of foot pad and hock burns lesions.

Material and methods

A total of 1200 Ross 308 one-day broilers were placed in the floor pens in a random block system with 6 treatments (2 x 3 factors design) and 4 replicates per treatment. The examined light intensity levels of 150 lx (high light intensity- HLI) and 20 lx (low light intensity-LLI) were applied to 3 stocking densities of broilers: 10, 13 and 15 birds/m². The duration of the photo period was 18 hours. The light source was incandescent bulbs of adequate intensity, according to the trial design. The control of the light intensity was performed at the level of broiler eyes in 3 positions, at right angle (*Lewis and Morris, 2006*). A four-phase program of nutrition on corn/soy based mixtures was applied. The bedding/litter was from chopped straw. Scoring foot pad (FP) and hock burns (HB) was performed at the end of the trial, on 42nd day, with a three-step scale (*Thomas et al., 2004*): no lesions (score 1), moderate lesions (score 2), severe lesions score 3). Investigation of the incidence and severity of the lesions was carried out on a sample of 240 broilers, i.e. 40 broilers per treatment with equal sex ratio in the sample. Tibia was measured on a sample of 72 broilers (12 broilers per treatment with equal sex ratio) after the examination of the slaughtering quality of the carcass (*Škrbić et al., 2018*) and the separation of other tissues from the bone. The bone weight and the length of the proximal to distal end of the bone were measured. Diaphysis cross-sectional area was calculated on the basis of measured anterior posterior and lateral medial diameter (*Vitorović et al., 1992*). Bone firmness was determined using a direct method of three-point-bending test, with a support width of 40 mm, using the IPNIS apparatus (*Mašić et al., 1985*). The specific breaking force was calculated as the ratio of the breaking force and the surface of the cross-section of the tibia diaphysis.

Data analysis was performed by the factorial ANOVA software package STATISTICA, version 8, StatSoft, Inc(www.statsoft.com). The mean values were tested with the Tukey test at the significance level $p < 0.05$.

Results and discussion

The morphometric parameters of the tibia of broilers grown in the conditions of 150 lx and 20 lx intensity of light in stocking densities of 10, 13 and 15 birds/m² are shown graphically (Figure 1).

The weight, length and surface area of the cross-section of the tibia diaphysis were not significantly affected by the factors examined. The measured weight of tibia of broilers grown in densities of 10 and 13 birds/m² was lower in HLI compared to LLI. On the contrary, broilers reared in a density of 15 birds/m² had slightly heavier tibia under conditions of higher intensity compared to lower

intensity of light. The length of the tibia was lower in all three stocking densities under conditions of high light intensity. It is noticeable that the difference between the average values of the length of the bone, as a light intensity effect, decreased with increasing stocking density. The effect of the high light intensity on the development of tibia, based on the calculated cross-section, is mostly expressed in conditions of stocking density of 15 birds/m².

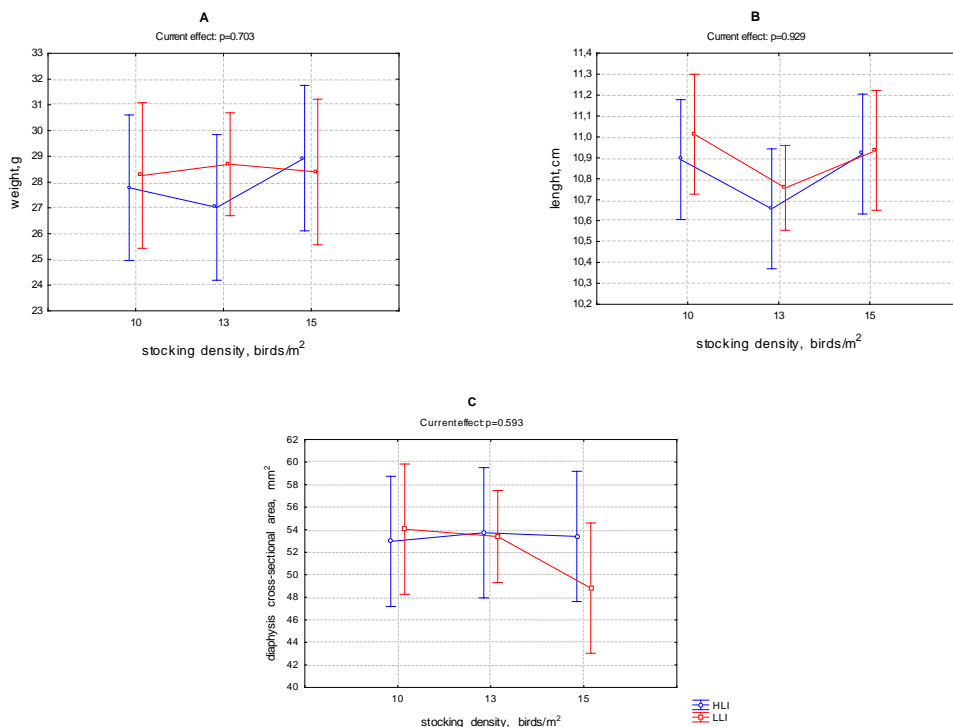


Figure 1. Morphometric parameters of tibiotarsus broilers in various conditions light intensity and stocking density; A-weight B-length C-diaphysis cross sectional area

Biomechanical parameters of the quality of tibiotarsus are shown in Figure 2.

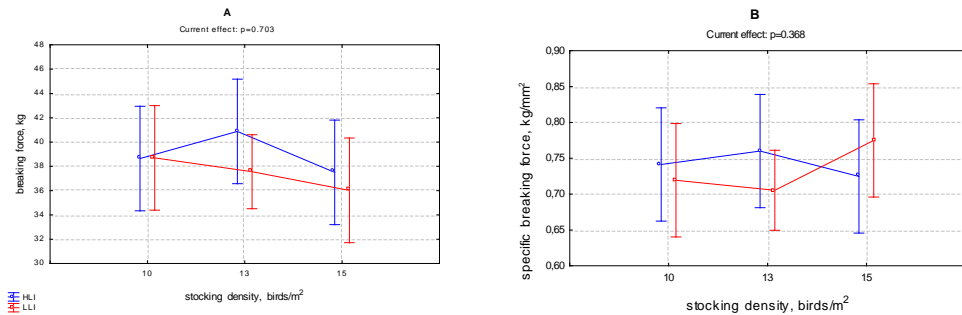


Figure 2. Biomechanical parameters of tibiotarsus broilers in various conditions light intensity and stocking density; A-breaking force B-specific breaking force

The firmness of the tibia expressed by breaking force was the highest in HLI treatment and the density of 13 birds/m². In conditions of lower intensity of light, the breaking force linearly decreased with increasing stocking density. The exposure of broilers to the light of increased intensity has led to a change in the indicated tendency, however without the statistical confirmation of the obtained results. As a result of lower breaking force and greater cross-sectional area of the tibia diaphysis in HLI treatment and 15 birds/m² density, a lower specific breaking force was calculated in relation to HLI treatments and a density of 10 and 13 birds/m².

Broilers of commercial genotypes show more locomotor problems and FP lesions compared to autochthonous breeds and therefore poorer foot health (*Alves et al., 2016*), which is associated with a rapid early increase and lower broiler activity (*Angel, 2007; Knowles et al., 2008*). The stocking density directly, through the available space for movement, affects the activity of broilers, and in this respect, the ability to move through the gait score (*Škrbić et al., 2009*). Results of *Škrbić et al. (2011)* point to the improvement of the measured absolute values of the quality parameters of tibia as a result of broiler activity in the lower stocking density. The high stocking density of broilers changes the intestinal absorption and the metabolism of calcium and phosphorus associated with the quality of tibia (*Sun et al., 2018*). The effect of the investigated light intensities in the stocking density of 10, 13 and 15 birds/m² on morphometric and biomechanical parameters of tibia was absent in this study. However, there are indications that in conditions of higher stocking density, in which there are poorer conditions for movement, high light intensity stimulates the activity of broilers and improves the individual properties of tibia compared to the same lighting conditions in lower stocking density. In our research, generally low stocking density did not cause great differences in the housing conditions and environment between treatments, which may be the reason for the insufficient differences between treatments. In support of this, *Sherlock et*

al. (2010) show that in the semi-intensive conditions of growing gradual changes in the intensity of light did not affect the activity of broilers.

The average scores and incidence of foot pad lesions estimated according to severity of damage in light intensity and stocking density are shown in Table 1.

Table 1. The effect of the light intensity in different broiler stocking densities on the foot pad score (frequency and average assessment)

Light intensity Stocking density	LLI			HLI			P		
	10	13	15	10	13	15	Light intensity	Stocking density	Interaction
Foot pad %									
1	41.67	25.00	0	75.00	58.33	0			
2	25.00	16.67	0	25.00	33.33	8.33			
3	33.33	58.33	100	0	8.34	91.67			
Average score	1.92 ^{ab} ± 0.90	2.33 ^{bc} ± 0.89	3.00 ^c ± 0.00	1.25 ^a ± 0.45	1.50 ^a ± 0.67	2.92 ^c ± 0.29	0.000	0.000	0.099

The average FP scores were significantly affected by the intensity of light and stocking density, while the interaction of these two factors was not statistically confirmed. A better average FP score was found in broilers housed in conditions of higher intensity of light, and this difference was statistically confirmed only in the density of 13 birds/m², where average scores of FP lesions were 2.33 in LLI treatment and 1.5 in HLI. The greater prevalence of FP dermatitis in treatment with 5 lx versus 20 lx, is stated by *Rault et al. (2017)* and explained by the lower activity of broilers and, consequently, by the greater susceptibility of broilers that are still to the incidence of contact dermatitis. However, in light intensity conditions 0.5; 5 and 10 lx, *Olanrewaju et al. (2015)* have not found differences in the incidence of FP dermatitis. By examining the predisposing factors for the development of FP lesions in broiler flocks in Denmark, *Kyvsgaard et al. (2013)* have found a smaller main effect of the stocking density on the development of FP lesions compared to interaction with the season. Since moisture levels are the primary cause of contact dermatitis (*de Jong et al., 2014*), in conditions of high humidity of litter, the effects of the examined treatment on FP dermatitis are lost (*Fidan et al., 2017*). In this way, the inconsistency of the results of the effects of certain factors on the occurrence of FP lesions can be explained.

The effect of HLI and LLI in the tested stocking densities of broilers on the average score and frequency of incidence of hock burns estimated according to the severity of the damage are shown in Table 2.

Table 2. The effect of the light intensity in different broiler stocking densities on the hock burn score (frequency and average assessment)

Light intensity Stocking density	LLI			HLI			P		
	10	13	15	10	13	15	Light intensity	Stocking density	Interaction
Hock burns %									
1	66.67	58.33	33.33	100	75.00	8.33			
2	25.00	8.33	33.33	0	8.33	41.67			
3	8.33	33.33	33.33	0	16.67	50.00			
Average score	1.42 ^{ab} ± 0.67	1.75 ^{abc} ± 0.97	2.00 ^{bc} ± 0.85	1.00 ^a ± 0.00	1.42 ^{ab} ± 0.79	2.42 ^c ± 0.67	0.520	0.000	0.100

There were no significant differences in the average HB score between the investigated levels of light intensity. The effect of the light of increased intensity did not lead to significant differences in the average HB scores observed by groups of broilers of the same stocking density. Starting from the absence of a significant effect of the intensity of light on the firmness of the broiler tibiotarsus, the incidence and severity of hock burns can be considered expected. In support of this, *Kristensen et al. (2006)* in which they confirm a positive correlation between the gait score, the indicators of the leg problems, and the hock burns. Because of the lameness of broilers, the incidence of hock burns is higher. The established differences in the HB score are the result of the effect of the stocking density. However, we must conclude that the difference between groups of different stocking density is determined only in conditions of increased light intensity.

Conclusion

The light of increased intensity showed no significant effect on the development and firmness of the tibia of broilers grown in the stocking densities of 10, 13 and 15 birds/m². There are indications that in the highest stocking density, the light of increased intensity can improve the individual properties of tibia.

The differences in the average scores for hock burns were determined as the result of the stocking density observed only in conditions of a high light intensity.

The average score for foot pad lesions was under significant main effect of both investigated factors. The differences in the average FP scores between the light intensity treatments was confirmed in the stocking density of 13 birds/m².

In general, the results of the study indicate that the high light intensity may influence increase in the tibia firmness and reduce the incidence of contact lesions,

or alleviate the adverse effect of stocking density on foot health, with the need for further research in conditions of higher stocking density.

Efekti intenziteta svetlosti u različitim gustinama naseljenosti na parametre tibije i pojavu lezija kod brojlera

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Rezime

Mogućnost upotrebe svetlosti jačeg intenziteta u menadžmentu brojlerske proizvodnje za poboljšanje zdravlja nogu brojlera, ispitana je u ogledu sa ciljem utvrđivanja efekta dva nivoa intenziteta svetlosti u različitim gustinama naseljenosti brojlerskih pilića. Istraživanje je sprovedeno na 1200 Ross 308 brojlera po slučajnom blok sistemu sa 6 tretmana (2 x 3 faktorijalni ogled) i 4 ponavljanja. Ispitivani nivoi intenziteta svetlosti 150 lx (HLI) i 20 lx (LLI) su primenjeni u 3 gustine naseljenosti brojlera: 10, 13 i 15 birds/m². Procena lezija na tabanskom jastučiću (FP) i skočnom zglobu (HB) je izvršena 42. dana, na uzorku od 240 brojlera. Osteometrijska i biomehanička merenja na tibiji su izvršena nakon klanja i obrade, na uzorku od 72 brojlera. Svetlost jačeg intenziteta nije usloвила signifikantan efekat na razvoj i čvrstoću tibije brojlera gajenih u gustinama naseljenosti 10, 13 i 15 grla/m². Postoje indicije da u najvećoj ispitivanoj gustini naseljenosti, svetlost jačeg intenziteta može poboljšati pojedine osobine tibije. Razlike u prosečnoj oceni HB utvrđene su kao rezultat efekta gustine naseljenosti koji je ispoljen samo u uslovima jačeg intenziteta svetlosti. Prosečna ocena FP lezija je bila pod značajnim glavnim (main) efektom oba ispitivana faktora. Razlike u prosečnoj oceni FP između tretmana intenziteta svetlosti potvrđene su u gustini naseljenosti 13 grla/m².

Ključne reči: brojler, intenzitet svetlosti, gustina naseljenosti, tibija, lezije

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MICROBIAL CONTAMINATION OF POULTRY LITTER DURING FATTENING PERIOD

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Original scientific paper

Abstract: The results of the research into the microbiological contamination of litter used by broiler chickens are presented. Litter samples were taken prior to the introduction of chicks (day 0) and in 7-day intervals until the end of the fattening period. The total numbers of aerobic mesophilic microorganisms, yeasts, moulds and *Clostridium perfringens* spores, and the presence of bacteria of the *Salmonella* genus were determined. The total microbial count in newly laid litter was 7 log₁₀ CFU/g, which increased to 9 log₁₀ CFU/g by the 4th week. However, at the end of the 5th week, it was at the same level as in newly laid litter. *C. perfringens* spores, presumably originating from chicks' faeces, were first detected on day 7. In the next 7 days their number increased, reaching 3-4 log₁₀ CFU/g, and remained at approximately same levels until the end of the research. The initial mould contamination was 5-6 log₁₀ CFU/g. However, from day 21 moulds were not isolated, but only yeasts of the *Saccharomyces* genus. It is supposed that these were deposited with chicks' faeces, due to their presence in complete broiler feed. No bacteria of the *Salmonella* genus were ever isolated from the litter. In conclusion, the total numbers of microorganisms in deep litter reach their peak in approximately a month, which is followed by their decrease. Deep litter is a favourable environment for probiotic yeast cultures. Added to feed intended for broilers, they can positively influence the microbial composition of litter, providing healthier environment to fattening broilers.

Key words: poultry litter, broiler fattening, total number of microorganisms, yeast, mould, *Clostridium perfringens*

Introduction

Bedding material for animal housing is of organic or inorganic origin, primarily made from various by-products of agriculture and wood industry: baled hay, chopped straw, peanut or rice hulls, wood shavings and chips, sawdust, shredded paper etc. (Torok *et al.*, 2009). When used in intensive broiler production it is expected to absorb moisture, dilute chicken manure and protect the birds from the thermal effects of the floor. Litter is defined as the combination of bedding material, wasted feed and water, manure, feathers, and other detritus from the chicken (Tabler *et al.*, 2009; Ritz *et al.*, 2005; Torok *et al.*, 2009). Owing to the close link between chicken welfare and bedding, it should be of adequate quality and chosen carefully. The birds are continuously in close contact with litter, which may heavily affect their health and influence production performance owing to ammonia, dust particles, high humidity, pH, temperature (Torok *et al.*, 2009) and, primarily, to microorganisms originating from the environment and the chicken's guts.

Microbial species which can be detected in bedding are hypothesized, rather than known. Certain data originate from research using standard culture methods, which enable the isolation and identification of cultivable, known microbial species, but not of some fastidious ones existing in the environment (Lu *et al.*, 2003). In spite of the logical expectation that enteric bacteria are prevalent in litter, enteric bacteria such as enterococci and coliforms account only for the minority of the total bacteria (0.1% and 0.11%, respectively), which suggests that they can barely survive in poultry litter (Lu *et al.*, 2003). Research on 340 bacterial clones originating from broiler litter revealed that the microbiota consisted predominantly of gram-positive bacteria (87%), members of *Lactobacillaceae*, *Bacillus*, *Staphylococcus*, *Clostridiaceae*, *Enterococcaceae*, *Corynebacteriaceae*, *Micrococcaceae*, *Microcinaceae* and *Proteobacteria* groups. Staphylococci made up for the majority of aerobic bacteria (Martin and McCann 1998; Lu *et al.*, 2003). Certain microbes in bedding material are not only potential causative agents of bird diseases, but are also human pathogens threatening people involved in poultry production. For example, carried by dust particles into the air fungal spores may reach concentrations 10^6 - 10^9 /m³ of air, which may lead to respiratory diseases in birds and farmers, such as allergic alveolitis or hypersensitivity pneumonitis (Kotimaa *et al.*, 1991). *C. perfringens* is the causative agent of necrotic enteritis and gangrenous dermatitis of broiler chickens (Lu *et al.*, 2003; Tan *et al.*, 2013).

The assessment of the total number of microorganisms in poultry litter revealed neither increase with time (resulting from increased faecal content), nor that it was directly proportional to the number of flocks successively grown in the building (Schefferle, 1965; Thaxton *et al.*, 2003): the average number of aerobics plunged after four flocks were kept on the bedding, after which it remained similar, whilst

anaerobic bacteria number was approximately the same until as many as 28 flocks were raised (Thaxton *et al.*, 2003). In some other research, the populations of bacteria and fungi reached maximum about a month after the bedding has been laid, after which they declined and remained similar until the end of the investigation (Martin and McCann, 1998; Terzich *et al.*, 2000).

Broiler litter may be utilized as a fertilizer (Terzich *et al.*, 2000), owing to potentially valuable nutrients, almost 30% of crude protein content and high levels of minerals (Lu *et al.*, 2003). Adequately used, chicken litter is a precious resource, which improves soil fertility, aeration and its water-holding capacity (Omeira *et al.*, 2006). However, when used as a fertilizer, it poses a serious risk of introducing pathogenic bacteria into soil (Trawinska *et al.*, 2016), including zoonotic agents. In addition, non-pathogenic, animal staphylococci and enterococci, part of microbiota, may contribute to the transmission of antibiotic resistance to human commensals (Lu *et al.*, 2003). Poultry litter can also be used as feed supplemented to cattle (Smith *et al.* 1974; Terzich *et al.*, 2000; Lanyasunya *et al.*, 2006; Ghaly and MacDonald 2012; Rankin, 2018), in spite of the risk of possibly present microorganisms such as *Campylobacter*, *Salmonella*, *Listeria monocytogenes* etc. (Lu *et al.* 2003). However, bacteria loads can significantly be reduced at high temperature (60°C), which is easily achievable by compressing and wrapping manure to prevent oxygen entry and preserve nitrogen content (Lanyasuny *et al.*, 2006). Knowing the microbial composition of animal waste, including poultry litter, restricts its impact on the environment and human and animal health, contributing to the management of animal diseases (Lu *et al.*, 2003).

This research was aimed at the assessment of microbial contamination levels, that is the total aerobic mesophilic bacteria, yeasts and moulds in bedding material and litter sampled from a broiler farm in Vojvodina. In addition, the numbers of *Clostridium perfringens* spores were determined, given that necrotic enteritis is one of the leading health problems on poultry farms in Serbia.

Materials and Methods

Bedding material subjected to this research was made of peat, cellulose pellet, wood chips and a pH stabiliser. It was laid in two broiler houses with 6,550 chicks in each. The specific weight of the fresh bedding was 320-340 kg/m³. The chicks were of *Gallus gallus domesticus* species, ROSS 308 hybrid. They were fed on standard complete feed for fattening chickens, in compliance with the technological normative for the hybrid.

For microbial examination the bedding material was sampled at the beginning of the experiment and litter in 7-day intervals during 5 weeks, in April and May 2018. Litter was sampled randomly, from under the nipple-drinkers and

along the length of the flock house, and pooled. Two pooled samples were taken from both houses at each timepoint.

For microbiological analysis, 20 g of each sample was put into a sterile 500-mL BagPage (Interscience, France) and 180 g of Buffered Peptone Water (BPW) was added. The suspension was homogenized in Stomacher BagMixer® 400 (Interscience, France) set to the maximum speed, after which a series of ten-fold dilutions were made by transferring 1 mL of suspension into 9 mL of BPW. The analyses were done in compliance with the procedures described in the following standards: Horizontal method for the enumeration of microorganisms, Part 1: Colony count at 30°C by the pour plate technique (*ISO 4833-1:2014*), Horizontal method for the enumeration of yeast and moulds (*ISO 21527-2:2011*) and Horizontal method for the detection, enumeration and serotyping of *Salmonella* (*ISO 6579:2017*). To eliminate the sulphite-reducing bacteria which are non-sporogenic, in order to more precisely determine the numbers of *C. perfringens* on sulphite cycloserine agar (Biokar Diagnostics, France), the ten-fold dilutions of samples were thermally treated at 80°C for 15 minutes. This is how the total number of *C. perfringens* spores were determined using Horizontal method for the enumeration of *Clostridium perfringens* - Colony-count technique (*ISO 7937:2010*).

Results and Discussion

The levels of microbial contamination of the bedding material in two broiler houses were determined at the beginning of the broilers' fattening period – prior the introduction of the flocks, and in seven-day intervals throughout the 35 days of fattening. The results referring to the total number of microorganisms, the numbers of *C. perfringens* spores, yeast and mould were expressed quantitatively, as log₁₀ colony-forming units per gram of material (CFU/g), and are shown in Tables 1 and 2.

Table 1. Average total aerobic microorganisms, *Clostridium perfringens*, yeast and mould counts in bedding material (day 0) and litter (day 7-35) in broiler house No.1

Day	Sample No.	C o u n t s (log ₁₀ CFU/g)			
		Total microorganisms	<i>Clostridium perfringens</i> spores	Yeasts	Moulds
0	1	7.48	<10	<100	5.40
	2	7.40	<10	<100	5.30
7	1	8.18	2.90	8.20	5.48
	2	8.20	2.78	8.43	6.51
14	1	8.65	4.30	8.18	<3
	2	8.70	4.18	8.43	<3
21	1	9.23	4.70	8.18	<3
	2	9.28	4	8.30	<3
28	1	9.30	4.30	7.40	<3
	2	9.48	3.70	7.30	<3
35	1	7.48	4.78	<4	<3
	2	7.30	5.48	4.90	<3

Table 2. Average total aerobic microorganisms, *Clostridium perfringens*, yeast and mould counts in bedding material (day 0) and litter (day 7-35) in broiler house No. 2

Day	Sample No.	C o u n t s (log ₁₀ CFU/g)			
		Total microorganisms	<i>Clostridium perfringens</i> spores	Yeast	Mould
0	1	7.397	<10	<100	6
	2	7.30	<10	<100	6.18
7	1	8.20	2.70	<100	7.18
	2	8.18	2.81	<100	7.38
14	1	8.60	3.30	7.15	6.53
	2	8.78	3.60	7.15	7.13
21	1	9.26	4.17	8.30	5.30
	2	9.18	4.30	8.48	6.70
28	1	9.48	4.30	8.18	<3
	2	9.48	3.78	8.30	<3
35	1	7.70	3.45	5	<3
	2	7.60	4.85	5.85	<3

The total number of microorganisms in bedding materials was 7 log₁₀ CFU/g. This included the total number of aerobic, mesophilic bacteria, yeast and moulds which form visible colonies at 30°C on Plate count agar (Biokar Diagnostics, France) during 72h of incubation in aerobic conditions. Given that a number of microbial species from the environment is non-cultivable in laboratory conditions and that strict anaerobes cannot be detected with this method, the real number of microorganisms was certainly higher. An additional hindrance for the precise determination of total microbial count were unsedimented particles of bedding

material which were transferred from the sample dilutions onto Petri plates, and may have been erroneously considered to be microbial colonies. In litter samples, from the end of the 1st to the end of the 4th week of research, the total number of microorganisms increased by 1-2 log₁₀ CFU/g. The values of total microbial counts in broiler litter detected in the current research (7-9 log₁₀ CFU/g) are in accordance with the results of investigations published previously. For example, by cultivation on brain heart infusion (BHI) agar *Lu et al. (2003)* detected 10⁹ aerobic bacteria per g of poultry litter, and *Tan et al. (2013)* approximately 5-7 log₁₀ CFU/g. *Martin and McCann (1998)* found total bacterial counts ranging between 1.2 x 10³ and 8.4 x 10⁷, and *Terzich et al. (2000)* from 1.72 x 10⁷ to 8.80 x 10¹¹ on Tryptic soy agar plates after 24 h incubation at 37°C.

From litter samples no bacteria of the *Salmonella* genus were isolated. *Salmonella* spp. counts are usually very low due to competition with other bacteria (*Omeira et al., 2006*), and since they are not part of the intestinal microbiome, they are highly unlikely to be found in litter (*Martin and McCann., 1998; Lu et al., 2003; Omeira et al., 2006; Wang et al., 2016*).

Clostridium perfringens was not detected in any of the 4 bedding material samples taken at the beginning of the research. *C. perfringens* spores were detected on day 7 (concentration 2 log₁₀ CFU/g) in litter samples from both houses, which suggests that they are deposited to litter from the broilers' faeces. Litter may be an environment in which *C. perfringens* grows on condition anaerobic conditions are achieved, that is, if its porosity declines, which happens when the moistness and the compactness of the litter increase. The number of *C. perfringens* spores increased on day 14 to reach 3-4 log₁₀ CFU/g and remained similar until the end of the experiment. *Tan et al. (2013)* detected similar numbers of *C. perfringens* in poultry litter in both gangrenous dermatitis (GD)-positive and GD-negative farms (from 1 to 3 log₁₀ CFU/mL and 2 to 4 log₁₀ CFU/g, respectively), but witnessed its tendency to decrease in the period from the 3rd to the 7th week. Decreasing numbers of *C. perfringens* has been explained by the its lower eliminated with chicken faeces (*Craven et al., 2001*). In addition, the enumeration pattern of *C. perfringens* in bedding does not always follow its occurrence pattern in faecal samples originated from chicks which are grown on the deep litter.

In the current research, the contamination level of the bedding material with moulds was 5-6 log₁₀ CFU/g (Figure 1).

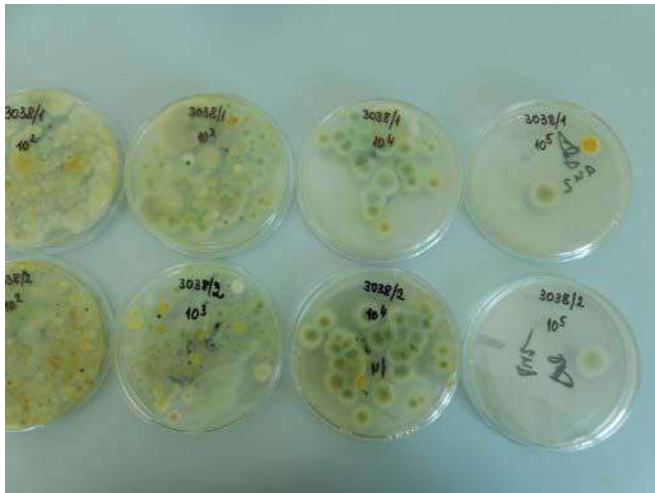


Figure 1. Mould colonies on DG18 agar isolated from ten-fold dilutions of bedding material (at the beginning of the experiment).

On day 14 from litter samples taken from house No. 2 moulds were isolated (Figure 2, plates in the top row), but from litter samples originating from house No. 1. on DG18 agar only yeasts were isolated (Figure 2, plates in the bottom row). Mould colonies were not noticeable, which is why their number was expressed as fewer than $3 \log_{10}$ CFU/g (less than 1,000/g), because 100 μ L of the first dilution ($1:10^{-2}$) was streaked on DG18 agar.

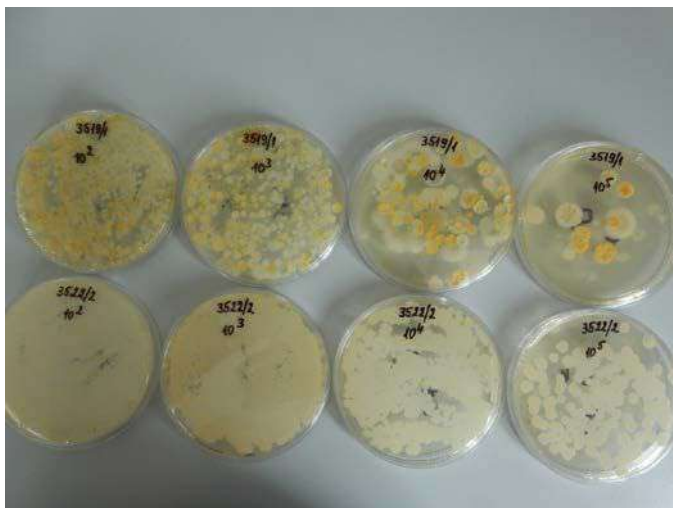


Figure 2. Mould colonies on DG18 agar isolated on day 14 from litter samples taken from house No. 2 (plates in the top row) and yeast colonies isolated from litter samples taken from house No. 1 (plates in the bottom row).

In litter samples taken from house No. 1 until the end of the experiment only yeast grow was detected, but not mould. With litter samples taken from house No. 2, an identical phenomenon occurred on days 28 and 35 (Figure 3). Only few investigations were conducted to detect the fungal contamination of poultry litter. The most frequent genus found was *Penicillium*, followed by *Alternaria*, *Cladosporium* and *Aspergillus* (Viegas et al., 2012). Fungal contamination of poultry litter (CFU/g) is in direct correlation with fungal contamination of the air (CFU/m³), which poses health risks to exposed workers and animals. If inhaled, dust particles containing fungal spores may cause irritation, or even allergic and/or toxic respiratory diseases (Viegas et al., 2012). Moreover, spreading of poultry litter as fertiliser on agricultural land is a potential public health concern due to the possible dissemination of keratinophilic (*Scopulariopsis* and *Fusarium* genus) and toxigenic fungi (*Aspergillus*, *Fusarium* and *Penicillium* genus).

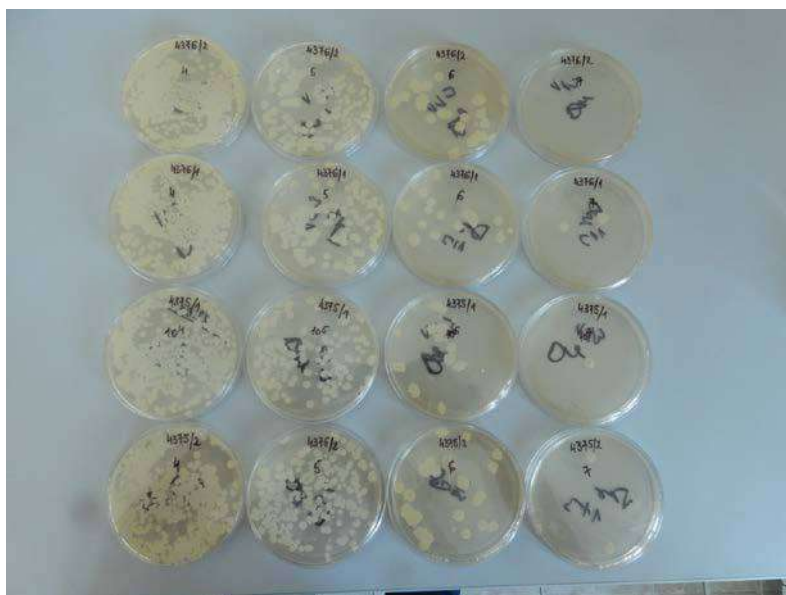


Figure 3. Mould colonies on DG18 agar isolated on day 28 from litter samples taken from both broiler houses

Based on cultural and microscopic characteristics (Figures 4A and 4B), the yeast isolate was identified as a species of the *Saccharomyces* genus. It was supposed to have ended up in the litter from the chicks' digestive tract, owing to the fact that it has been used as a probiotic culture in complete diet intended for fattening chickens. It is obvious that in litter the conditions are favourable for the survival of these yeasts, which account for the considerable part of total microbial

counts. These results point to the influence of probiotic cultures in complete broiler diets on the microbial composition in litter, which provides beneficial environment to chicken health.



Figure 4. A. Yeast colonies on DG-18 agar; B. Microscopic view of yeast in a Gram-stain preparation (100x oil immersion objective)

Microbes normally present in the chicks' gastrointestinal tract will end up in litter. It is expected that the increase in the quantity of fresh faecal material (as it is being deposited) leads to an increase in the total number of microbes in litter. However, in the last taken samples (day 35), the total number of microorganisms was similar to that detected in bedding material before it was inhabited by the chicks. This result is in line with some others reported previously which claimed that the bacteria numbers in litter after a while remain similar, not depending on the number of flocks that have been grown on it (*Thaxton et al., 2003*). It was recognised earlier that the numbers of bacteria and fungi reach a peak in a months' time, when they decrease and remain roughly unchanged (*Schefferle, 1965*), which was confirmed in the current research. Microbial species vary in conditions they need for growth regarding nutrition and atmosphere conditions. The number of enteric bacteria decreases with time because litter is not an appropriate environment for the survival of coliform bacteria (*Thaxton et al., 2003; Omeira et al., 2006*). In litter some species involved in composting organic material were identified, which also explains the absence of species pathogenic to people and animals (*Lu et al., 2003*). Thus, it is not obligatory to lay new bedding material, but the same may be reused, lacing fresh litter shavings on top of the old litter. Repeated use of the same deep litter even several times does not pose particular

risk to the chicks' health, but influences the composition of their gastrointestinal microbiome (Wang *et al.*, 2016). The digestive tract harbours a complex microbiota which is of immense importance to the functions of the immune system, defence against enteric pathogens and food digestion.

Conclusion

The total number of microorganisms in poultry litter reaches a peak in about a month from the beginning of the fattening period, which is followed by its decrease and maintenance at the levels established. Litter is not a favourable environment for coliform bacteria and moulds, and if sufficiently loose, does not enable the multiplication of *Clostridium perfringens*. Yeasts of the *Saccharomyces* genus survive successfully in litter, which is why their addition in the form of probiotic cultures to complete feed for fattening chicks influences the composition of the litter microbial composition, and thus indirectly provides more favourable environment conditions for chicks' health in a deep litter rearing system.

Acknowledgment

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Mikrobiološka kontaminacija prostirke tokom tova brojlera

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Rezime

U radu su prikazani rezultati ispitivanja mikrobiološke kontaminacije prostirke korišćene u tovu pilića. Prostirka je uzorkovana pre naseljavanja objekata i u nedeljnim intervalima tokom 35 dana tova pilića. Uzorci su ispitani na ukupan broj aerobnih mezofilnih mikroorganizama, kvasaca, plesni i spora *Clostridium perfringens*, kao i prisustvo bakterija roda *Salmonella*. Ukupan broj mikroorganizama u svežoj prostirci iznosio je $7 \log_{10}$ CFU/g, a do 4 nedelje tova pilića povećao se do $9 \log_{10}$ CFU/g. Međutim, na kraju pete nedelje tova, ukupan

broj mikroorganizama u prostirci bio je na nivou vrednosti ustanovljenih u svežoj prostirci. Spore *C. perfringens* su ustanovljene tek sedmog dana od naseljavanja objekta, što ukazuje da u prostirku dospevaju fecesom pilića. Za 14 dana broj spora *C. perfringens* se povećao do 3-4 log₁₀ CFU/g i na približno istim vrednostima zadržao do kraja ispitivanja. Sveža prostirka bila je kontaminirana plesnima u nivou od 5-6 log₁₀ CFU/g, ali od 21. dana iz uzoraka prostirke nisu izolovane plesni, već samo kvasci roda *Saccharomyces*. Pretpostavka je da su kvasci u prostirku dospeli fecesom pilića, jer se koriste kao probiotske kulture u smešama za njihov tov. Bakterije roda *Salmonella* nisu izolovane iz prostirke. Rezultati ispitivanja pokazuju da ukupan broj mikroorganizama u prostirci dostiže svoj pik za oko mesec dana, nakon čega se smanjuje. Prostirka je pogodna sredina za život probiotskih kultura kvasaca i njihovo dodavanje u smeše za tov brojlera može imati povoljan uticaj na sastav mikroorganizama u prostirci, a time i obezbeđenje zdravije životne sredine u podnom sistemu uzgoja brojlera.

Ključne reči: prostirka za piliće, tovní pilići, ukupan broj mikroorganizama, kvasci, plesni, *Clostridium perfringens*

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BIODIVERSITY OF TICKS OF SHEEP AND GOATS IN SEMI-INTENSIVE FARMING SYSTEM IN VOJVODINA

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Original scientific paper

Abstract: The present study was conducted in 90 sheep and goats flocks from the territory of Vojvodina in the two years period of March 2016 to October 2017, during the grazing season. Tick infestation was detected in 53.14% of examined animals. The most dominant was *Ixodes ricinus* (43.91%), followed by *Dermacentor marginatus* (31.91%), *Rhipicephalus bursa* (15.22%), *R. sanguineus* (8.72%), *Hyalomma savignyi* (3.72%), *Haemaphysalis punctata* (3.21%) and *D. pictus* (2.72%). The sex ratio of detected tick species showed a higher number of females in four species (*I. ricinus*, *H. punctata*, *R. sanguineus* and *D. marginatus*), while higher number of males were detected in two species (*R. bursa* and *Hy. savignyi*), and an equal number of ticks of the *D. pictus*. The population dynamics of recorded tick species showed two annual maxima, in spring (April-May) and in autumn (September-October). The considerable interchange between spring and autumn tick populations can be attributed mainly to environmental conditions

Key words: semiintensive sheep production, ticks biodiversity, seasonal distribution

Introduction

Semi-intensive sheep and goats production is a tradition in the northern region of Serbia (Vojvodina). Some parts of Vojvodina are abundant in grasslands, especially in the Banat region, where animals grazing is mainly semi-intensive. The rational use of pastures in the period of April-October makes the sheep and goats production sustainable and low input in this period of the year. The specific climate and the unique habitat includes high biodiversity of flora and fauna of the grasslands (Janković *et al.*, 1984).

Ticks represents one of the indispensable elements of that specific biotope. A diverse tick fauna present in this region mainly influences the health status of grazing ruminants. Ticks are known as vectors for a number of diseases (Papadopoulos *et al.*, 1996; Jongejan and Uilenberg, 2004; Sevinc *et al.*, 2013). However, in the semi-intensive breeding system, which is the most often practice in this region, is very difficult to avoid infections with different types of ticks that are contaminants of the grasslands and pastures (Pavlović *et al.* 2018; 2019). Tick infestations are common, especially during late spring and autumn months of the year (Harlan and Foster, 1990; Fourie and Horak, 1991; Milutinović, 1992)

Our research was carried out in those parts of Serbia which have never been examined for their tick fauna, but which border directly on areas known as enzootic foci of domestic ruminant piroplasmosis, tailreosis and other tick borne diseases (Dimitrić, 1999; Nieder *et al.*, 2013; Pavlović *et al.*, 2002; 2012; 2016b). These research covered the effects of climatic factors such as temperature, relative humidity and precipitation which usually vary throughout the year (Milutinović *et al.*, 1996a; 1996). Thus, environmental conditions change seasonally and are characterised by differences in temperature or precipitation, and may have an influence on the distribution of ticks to appear periodically (Daniel, 1978; Fourie *et al.*, 1988; L'Hostis *et al.*, 1995; Dautel *et al.*, 2008; Hornok, 2009).

The aim of the study was to considerate the following parameters: biodiversity, relative abundance, sex ratio, and the effects of environmental factors (temperature, relative air humidity and precipitation) on tick populations. Data on the monthly average temperature, relative air humidity and precipitation for the examined period were obtained from the Weather Bureau of the Republic of Serbia.

Materials and Methods

The present study was conducted in 90 sheep and goats flocks from the territory of Vojvodina in the two years period of March 2016 to October 2017, during the grazing season. A systematic investigation of the ticks was carried out at 12 places (in Srem, Banat and Bačka) where was the largest number of flocks. Usually, sheep and goats are keep together at same pastures and had a same tick fauna.

Ticks were collected by removing them from sheep and goats with tweezers and were placed in vials with 70% ethanol. The tick species and sex/gender were identified by morphometric characteristics. The main attribute of identification of tick family is a plain dorsal sclerotised scutum or shield, which is often ornate with patterns in white or gold against a brown or grey background and which distinguishes these ticks from other families. This sclerotised plate covers the entire dorsal surface of the male, but only one third of the female's dorsal

surface. Second one was the capitulum of hard ticks which just as the mouthparts and is visible from a dorsal view. The peritreme or groove is big and clearly visible around the stigmal plate. Grooves are deep, linear depressions in the body cuticle, usually on the ventral surface. Hard ticks can be easily differentiated by the shape of the basis capitulum and by the form of anal grooves (Pomerancev, 1950; Kolonin, 2009).

Results And Discusion

Tick infestation was detected in 53.14% of examined animals. The most abundant species was *Ixodes ricinus* (43.91%), followed by *Dermacentor marginatus* (31.91%), *Rhipicephalus bursa* (15.22%), *R.sanguineus* (8.72%), *Hyalomma savignyi* (*Hy.marginatum*) (3.72%), *Haemaphysalis punctata* (3.21%) and *D.pictus* (2.72%).

Out of the total number of ticks collected, 52.35% were females and 47.65% were males. The sex ratio of detected tick species showed a higher number of females in four species (*Ixodes ricinus*, *Haemaphysalis punctata*, *Rhipicephalus sanguineus* and *Dermacentor marginatus*), while higher number of males were detected in two species (*Rhipicephalus bursa* and *Hyalomma savignyi*), and an equal number of ticks of the *D.pictus*. This is in agreement with the research of the tick sex ratio that have been made around the world (Černý *et al.*, 1974; Milutinović, 1992; Milutinović *et al.*, 1997a; Anderson and Magnarelli, 2008; Kolonin, 2009).

Comparison of the obtained results with findings in other regions of Serbia indicated that there is a great similarity in the established tick species. Examination performed in small ruminants in eastern, northeastern, and southern parts of Serbia and the Prizren district (Kosovo) (Milutinović *et al.*, 1987; 1990; 1997b; 1998; Mišćević *et al.*, 1990; Pavlović *et al.*, 1995; 1999; Petrović *et al.*, 1996; Becskei *et al.*, 2015) pointed to the presence of the same tick species, including *Hy. savignyi*, *Ha. inermis*, *Boophilus calcaratus* and *Ornythonisus lachorenis*. Similary results established in the Belgrade area and northwest (Mačva district) where the *I. ricinus*, *R. sanguineus*, *D. pictus* and *D. marginatus* as the most abundant species at small ruminants (Milutinović *et al.*, 1992; 1996 a, b; 1997b; 1998; Dimitrić, 1999; Pavlović *et al.*, 1999; 2013; 2016a). The found species of ticks are most common in sheep and goats in the regions of the Western Balkans including Macedonia, Mediterranean and Central Europe (Omeragić, 2011; Dumitrache *et al.*, 2012; Mihalca *et al.*, 2012; Pavlović *et al.*, 1995; 2014; 2016a, c).

Climate conditions have a great influence on the population dynamics of ticks. Population dynamics of ticks is related to the impact of climate factors like air temperature, relative humidity and rainfall (Milutinović, 1992; Belozerow, 1982; Clark, 1995; Carrol and Kramer, 2003; Anderson and Magnarelli, 2008). The climate in the Vojvodina is moderate continental. The average annual

temperature is 10.9⁰C. The average winter temperature is -1⁰C and in July is 21.6⁰C. Annual rainfall is 686 mm, with 122 rainy days. The lowest point of the Danube is 70.83 m and the highest is 79.70 m above sea level. The highest recorded water level is +778 cm and the lowest is -134 cm.

The influence at climate condition to population dynamics of ticks was monitored from March to October. They showed two annual maxima, in spring (April-May) and in autumn (September-October) (Milutinović, 1992; Anderson and Magnarelli, 2008). The March was a period when the grazing season started and the first occurrence of *Ixodes ricinus*, *Ha. punctata* and *Dermacentor marginatus* (Clark, 1995; Carol and Kramer, 2003) was recorded. During April the following tick species were found: *I.ricinus*, *R.sanguineus*, *D.marginatus* and *Ha. punctata* (Černý et al., 1974;1982; Pavlović et al., 2019). Climate condition during April (average 10.02 °C temperature, 72% relative humidity, i.e. in early spring at the beginning of the rainy season (monthly mean precipitation: 35.21 mm) reached maximum numbers of *D.marginatus* and *Ha.punctata*. In May, we observed the occurrence of the following species: *D.pictus*, *R.bursa*, *Hyalomma savignyi* and *Ha.inermis* (Milutinović et al., 1996b; Dimitrić, 1999; Hornok, 2009). Climate conditions in May, temperature of 15.55 °C, relative humidity of 71.5%, and 71.19 mm precipitation - induced population peak for a species which requires higher relative humidity - *I.ricinus*, *Hy.savignyi*, *R.bursa* and *D.pictus* (Dyk and Boučková, 1968; Tovornik, 1976; Zahler and Gothe,1995; Estrada-Peña et al., 2011).

In June, the population peak is observed for the *R.sanguineus*, which are the most common types both in July and August. The autumn population peak in September (with temperature of 15.95°C, 73.5% relative humidity and 51 mm precipitation) we saw an increase in the population of two species of ticks: *I.ricinus* and *D.marginatus*, while in October (17.9°C temperature, 73% relative humidity and 32.42 mm precipitation) we observed the emergence of the following species: *Ha.punctata*, *Ha. inermis*, *R.sanguineus* and *R. bursa* while the two species of the genus *Dermacentor* - *D. marginatus* and *D. pictus* were rarely encountered (Černý et al.,1982). The influence of climate condition and some part of it (temperature, humidity and precipitation) to population dynamics of ticks we presented at figure 1, figure 2, figure 3 and figure 5.

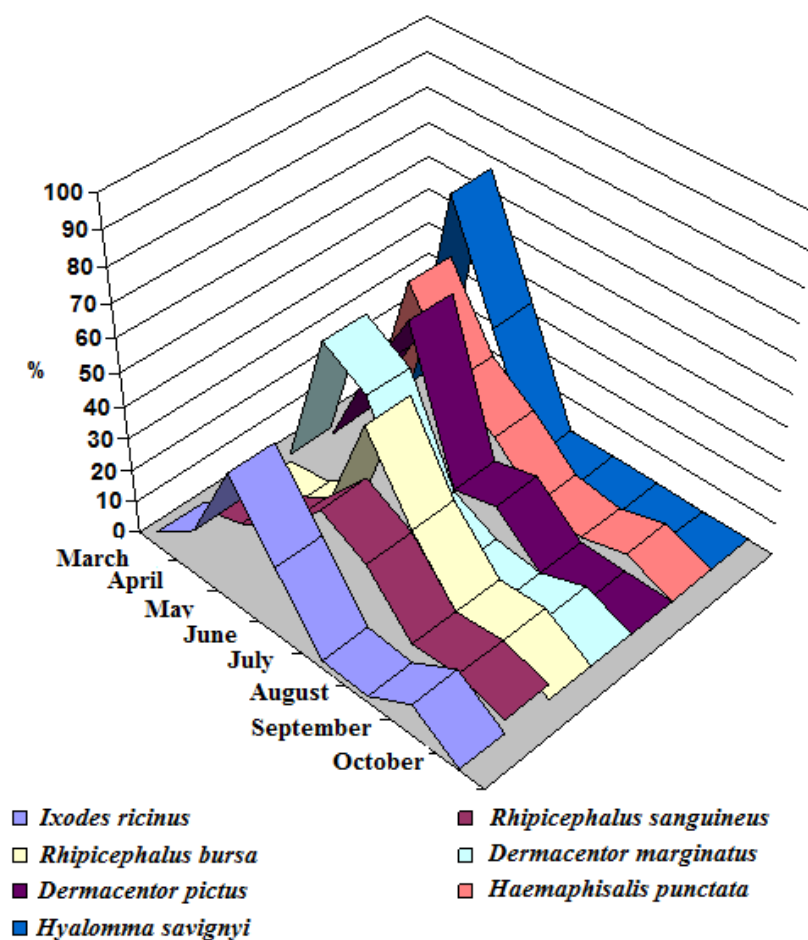


Figure 1. The influence at climate condition to population dynamics of ticks

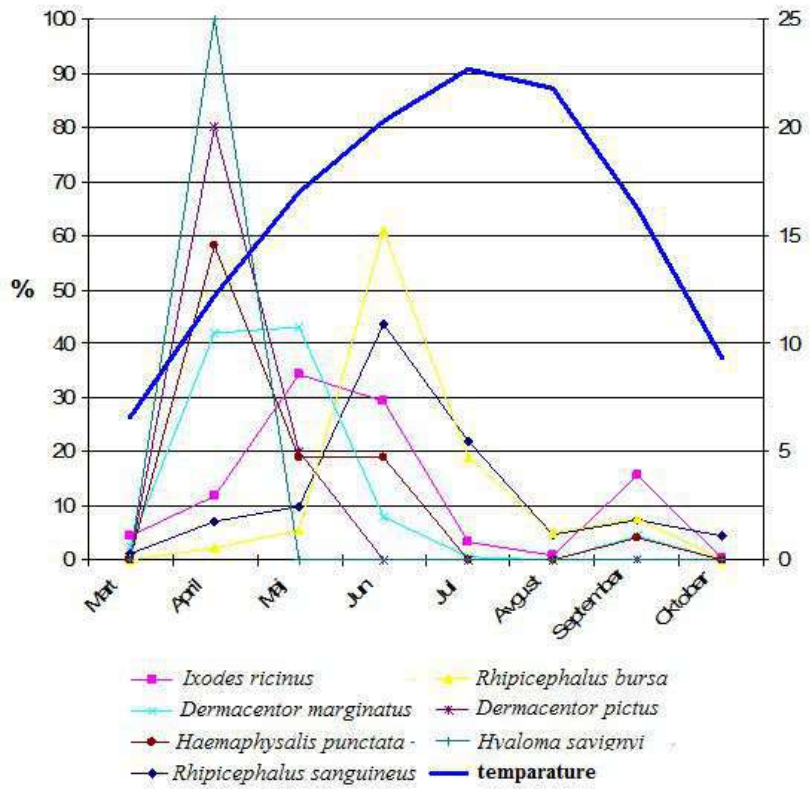


Figure 2. The influence of temperature to population dynamics of ticks

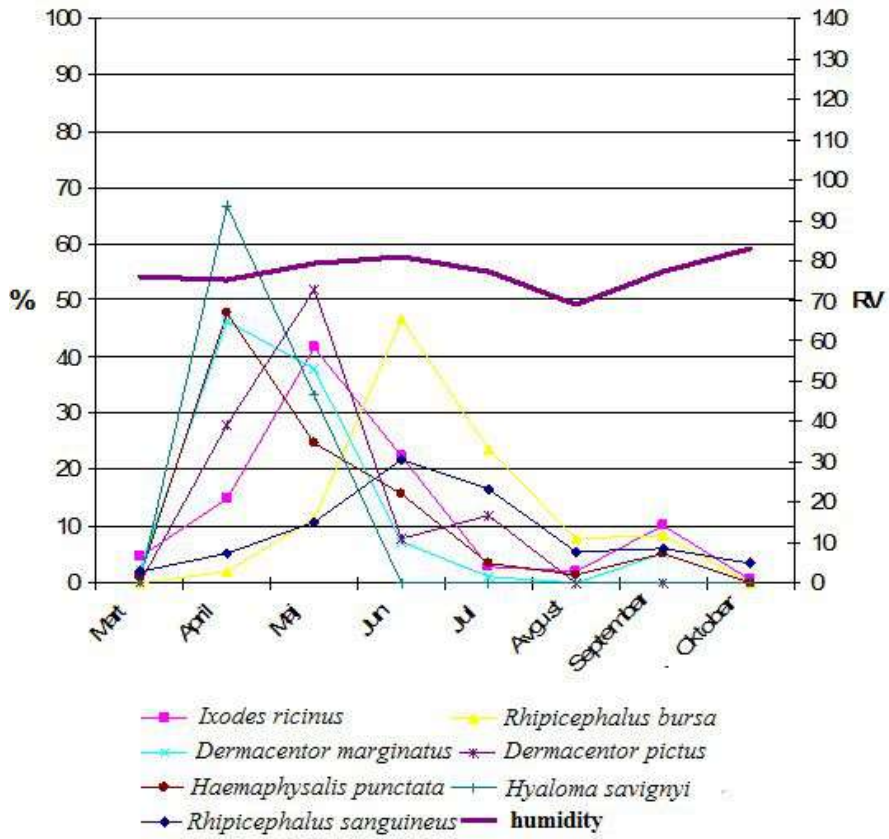


Figure 3. The influence of humidity to population dynamics of ticks

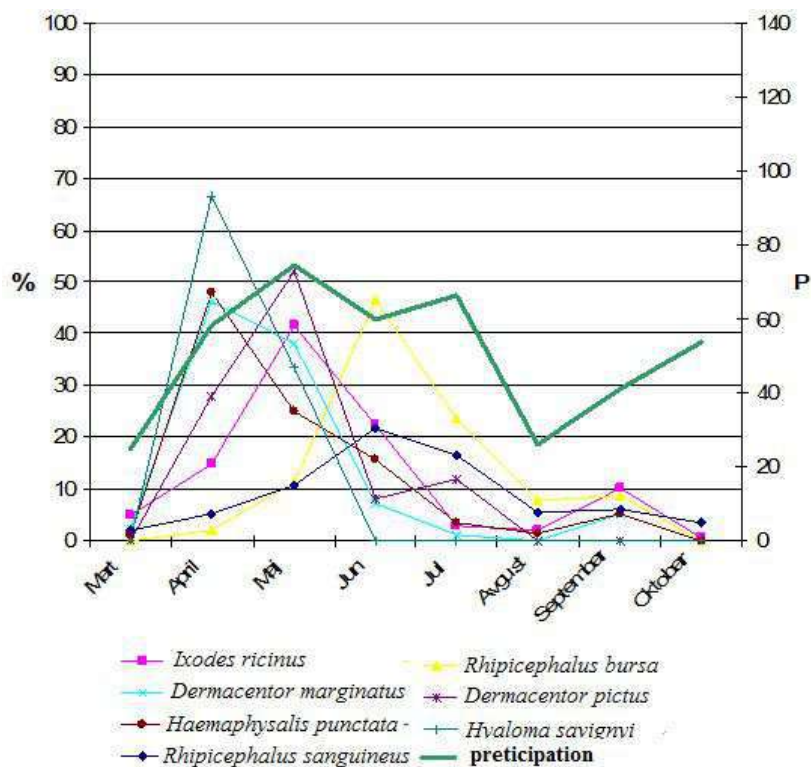


Figure 4. The influence of precipitation to population dynamics of ticks

Our results confirmed the results of the similarly studies carried out in northeast, eastern and south-eastern part Serbia (Milutinović et al., 1987; 1996; 1998; Mišević et al., 1990). This data is in correlation with the results of other examinations of the seasonal dynamics of ticks in Europe (Belozerow, 1982; Černý et al., 1974; Daniel, 1978; L'Hostis et al., 1995; Maroli et al., 1996; Papazahariadou et al., 2003; Estrada-Peña et al., 2011). This agrees with the data published about ticks in various part of Russia, Central Europe and the West Balkans (Belozerow, 1982; Dautel et al., 2008; Kolonin, 2009; Omeragić, 2011; Sevinc et al., 2013; Pavlović et al., 2014; 2016c). The authors point out that the low temperature, high humidity and rainfall significantly affect the life cycle of ticks, particularly the *I. ricinus* species.

Conclusion

Based on the obtained results, it can be seen that ticks represent a significant problem of small ruminant production in Vojvodina. The method of

holding allows permanent infections with these parasites and the climatic conditions favor their development and maintenance on grazing surfaces. Climate conditions have a great influence on the population dynamics of ticks which had two peaks - at late Spring and early Autumn. Fauna, seasonal dynamics and sex ratio of ticks found do not differ much from the results obtained in other regions of Serbia. Likewise, the presence of ticks points to the ever-present possibility of infections of the often zoonotic character that the ticks carry.

Biodiverzitet krpelja ovaca i koza držanih u poluintenzivnom sistemu odgoja u Vojvodini

Ivan Pavlović, Snežana Ivanović, Milan P. Petrović, Violeta Caro-Petrović, Zsolt Becskei, Mila Savić, Ferenc Csordás

Rezime

Studija je rađena tokom dvogodišnjeg perioda od marta 1996. do oktobra 2017. godine, tokom pašne sezone, na 90 stada ovaca i koza u Vojvodini. Infestacija krpeljima je ustanovljena kod 53,14% životinja. Dominantna vrsta krpelja je bila *Ixodes ricinus* (43,91%), slede *Dermacentor marginatus* (31,91%), *Rhipicephalus bursa* (15,22%), *R. sanguineus* (8,72%), *Hyalomma savignyi* (3,72%), *Haemaphysalis punctata* (3,21%) i *D. pictus* (2,72%). Odnos polova je bio u korist ženki kod četiri vrste (*I. ricinus*, *H. punctata*, *R. sanguineus* i *D. marginatus*), dok su mužjaci bili brojniji kod dve vrste (*R. bursa* i *Hy. savignyi*). Jednak broj mužjaka i ženki je ustanovljen kod samo jedne vrste krpelja - *D. reticulatus*. Populaciona dinamika je pokazala da nađene vrste krpelja imaju dva sezonska maksimuma u proleće (april-maj) i u jesen (septembar-oktobar). Brojnost populacije tokom ovih sezonskih maksimuma je u direktnoj korelaciji sa životnim uslovima (temperatura, vlaga).

Ključne reči: poluintenzivno ovčarstvo, biodiverzitet krpelja, sezonska distribucija

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GARLIC AS ALTERNATIVE FOR ANTIBIOTICS IN DIET FOR GROWING PIGS

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Original scientific paper

Abstract: The effects of the use of antibiotics and garlic powder in the nutrition of weaning piglets were compared. The trial included 120 weaned piglets of the same genotype (Large White) distributed in three feeding treatments. In the first study period (day 27-56), a feed mixture with 20% protein was used, while in the second period (day 57-84), a mixture with 18% protein. The control group was fed with mixtures containing antibiotics in the amount of 0.2%, while the two experimental groups were fed with mixtures containing fermented garlic powder (FGP) in the concentration of 0.05% and 0.2%. The obtained results showed that the use of FGP, instead of antibiotics, resulted in better gain in second trial group, and also better feed conversion ratio, in both trial groups by 3.84% and 6.93% respectively, during the entire research period.

Key words: piglets, growth promoters, nutrition, production results

Introduction

Garlic (*Allium sativum*) is widely used as culinary or medicinal supplement for the prevention and treatment of various heart and metabolic diseases (Konjufca et al., 1997; Amagase et al., 2001). Garlic contains a sulfur volatile active component that has antibacterial, anti-inflammatory and antioxidant biological properties (Wilson and Demming-Adams, 2007), it has been examined as a potential alternative to antibiotics in pig production. The alternative growth promoters including herbs, prebiotics and/or eubiotics deserve special attention (Grela et al., 2011). Garlic inclusion levels widely varied in those studies, being added at levels as low as 0.1% to as high as 4% to piglet diets. Cullen et al. (2005) have suggested that a better feed conversion could be observed when the garlic was supplemented at levels of 1 or 10 g/kg to the pigs. Various experiments also reported that garlic bulb, paste, oil, powder, husk and leaves could positively

influence the animal production performance (*Birrenkott et al., 2000; Chowdhury et al., 2002*).

Those functions are mainly attributed to the bioactive components of garlic, including sulphur-containing compounds such as alliin, diallylsulphides and allicin (*Amagase et al., 2001*). *Tatara et al. (2005)* have reported that process of ageing could convert the odorous, harsh and irritating compounds in garlic into stable and safe sulphur compounds such as S-allyl cysteine and S-allyl mercaptocysteine. Some authors (*Kakimoto et al., 2000*) also suggested that fermented garlic powder had an antioxidative activity several times greater than that of raw garlic as well as antidiabetes activity, anticancer activity, immunity enhancing activity and cholesterol-reducing activity. Some previous studies have suggested that the supplementation of fermented garlic powder could be beneficial the swine (*Yan et al., 2010; Yan et al. 2011; Wang et al., 2011*). Therefore, the objective of this study was to evaluate the effects of fermented garlic powder on growth performance in growing pigs.

Material and methods

The trial included 120 pigs of the same genotype (Large White) distributed in three feeding treatments (Table 1). Immediately after the piglets were weaned, groups of 10 piglets were formed on the basis of uniform initial weight, taking into account that in each group the sex ratio is the same. There were 4 repetitions per treatment. All piglets were placed in solid wall boxes, with lattice floor each containing 10 feeding places. Average initial weight of piglets was from 7.56 to 8.73 kg. All piglets came from 12 different mothers, and same father. In the initial period of the experiment, animals were fed during 39 feeding days, with a starter mixture containing 20% of the crude protein, and in the final period of the experiment, which lasted 17 feeding days, the meals were formulated to contain 18% of the crude protein.

The first group of piglets, control, was fed with mixtures based on the use of antibiotic Neodox in concentration of 0.2%, and the other two group of piglets with mixtures where instead of antibiotics, fermented garlic powder (FGP) was included in a concentration of 0.05% and 0.2 % of the diet (Table 1). Food and water were *ad libitum*.

Table 1 . Composition of diets for weaned piglets in the trial

	Starter, Day 27-56			Grower, Day 57-84		
Group	C (control)	T ₁ (trial)	T ₂ (trial)	C (control)	T ₁ (trial)	T ₂ (trial)
Ingredients, g/kg						
Maize	528.00	529.50	528.00	-	-	-
Amiloprotex*	-	-	-	638.00	639.50	638.00
Barley	100.00	100.00	100.00	100.00	100.00	100.00
Triticale	-	-	-	50.00	50.00	50.00
Milk replacer	20.00	20.00	20.00	-	-	-
Ekofish meal	40.00	40.00	40.00	30.00	30.00	30.00
Soybean meal	200.00	200.00	200.00	70.00	70.00	70.00
Oil	10.00	10.00	10.00	10.00	10.00	10.00
Mineral-vitamin premix 1**	100.00	100.00	100.00	-	-	-
Mineral-vitamin premix 2**	-	-	-	100.00	100.00	100.00
Antibiotic	2.00	-	-	2.00	-	-
FGP	-	0.50	2.00	-	0.50	2.00
Calculated nutrient composition, g/kg of feed						
Crude protein	207.40	207.40	207.40	180.50	180.50	180.50
Lysine	13.10	13.10	13.10	11.10	11.10	11.10
Methionine	5.20	5.20	5.20	4.00	4.00	4.00
Cysteine	3.30	3.30	3.30	3.00	3.00	3.00
Threonine	8.50	8.50	8.50	7.00	7.00	7.00
Tryptophan	2.20	2.20	2.20	1.90	1.90	1.90
Crude fibre	36.70	36.70	36.70	54.80	54.80	54.80
Crude fat	44.30	44.30	44.30	70.80	70.80	70.80
Calcium	12.00	12.00	12.00	9.82	9.82	9.82
Phosphorus	7.26	7.26	7.26	6.55	6.55	6.55
DE content, MJ/kg	14.05	14.05	14.05	13.62	13.62	13.62

*Amiloprotex is mixture of maize and full fat soybean, in ratio 70:30, heat treated prior to mixing into diet

**The commercial premixes (10% premix for piglets) without any antibiotics or phytoadditives included

During the research period, the following production indicators were monitored: body weight, average daily gain, average daily food consumption and food conversion, by trial periods. The data obtained were processed using the software package "STATISTICA" (Stat Soft Inc, 2012). ANOVA was used while

the Tukey test served to determine the statistical significance of the differences between individual means values.

Results and discussion

Production performances were shown in Table 2. During the starter and grower period, it was found that there were no significant differences in average daily gain or feed conversion. Major statistical significance was noted in feed intake, it was much lower in trial compared to control (C) group. The introduction of garlic powder instead of antibiotics in the mixture has led to increasement of gain in second trial group (T₂). Second group had an increase of 5.15 g/d compared to the C group and 11.01 g/d compared to T₁. Experimental groups had lower feed intake per feeding day, by 5.94% and 4.39%. The use of FGP in the mixture caused the feed conversion to improve by 3.84% and 6.93% compared to diet based on antibiotics.

Table 2. Production performance

	Treatments			SEM	p
	C	T ₁ 0.05%	T ₂ 0.2%		
Starter period (27-66d)					
FI, g/d	518.07 ^a	486.24 ^b	486.26 ^b	0.042	p<0.05
ADG, g/d	234.42	230.77	241.92	0.037	0.820
FCR, g/g	2.21	2.12	2.01	0.181	0.134
Grower period (66-83d)					
FI, g/d	992.95 ^a	943.59 ^b	951.79 ^b	0.086	p<0.05
ADG, g/d	470.59	460.29	471.18	0.076	0.921
FCR, g/g	2.11	2.05	2.02	0.194	0.173
Whole period (27-83d)					
FI, g/d	662.23 ^a	625.08 ^b	627.58 ^b	0.040	p<0.05
ADG, g/d	306.10	300.04	311.15	0.009	0.746
FCR, g/g	2.16	2.08	2.02	0.092	0.051
Mortality, %	0.50	0.25	-	0.019	0.364

SEM, Standard error of the means; FI, feed intake; ADG, average daily gain; FCR, feed conversion rate; ^{a, b, c} In a row, the least squares means with a different superscript differ significantly (p<0.05)

Some previous study also showed that garlic supplementation can improve intestinal health and nutrient digestibility owing to its antimicrobial effect (*Chen et al. 2008; Ao et al. 2010; Grela and Klebaniuk, 2007*). However, some studies have asserted that garlic supplementation has no detectable effect on the growth performance of pigs (*Freitas et al. 2001; Bampidis et al. 2005; Chen et al. 2008*). This inconsistency may be attributable to the different garlic levels and animals

used in each study. Moreover, it has been previously demonstrated that the fermentation step could provide several advantages, such as improved flavor and enrichment with desirable metabolites generated by microorganisms (Buckenhiuskes *et al.* 1990). Kakimoto *et al.* (2000) also reported that FGP has an anti-oxidative activity several times more potent than that of intact garlic, as well as higher anti-diabetes activity, liver protective activity, anti-cancer activity, immunity enhancing activity, and cholesterol-reducing activity.

Conclusion

The overall results obtained showed that fermented garlic powder can be used as a substitute for antibiotics in diets for growing pigs. Some further research, on a larger number of animals, could be done in order to conclude does FGP implementation only significantly benefits conversion or it can influence some other production parameters. Also economic cost could be implemented into calculation for further research.

Beli luk kao alternativa za antibiotike u ishrani prasadi u odgoju

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Rezime

Ispitivani su uticaji korišćenja antibiotika i fermentisanog belog luka u ishrani prasadi u odgoju. Ogled je sproveden na 120 prasadi, genotipa Veliki jorkšir, podeljenih u dve grupe tokom celog perioda istraživanja. U prvom periodu istraživanja korišćena je smeša hraniva sa 20% proteina, dok je u drugom korišćena smeša sa 18% proteina. Prva kontrolna grupa je hranjena smešama sa antibiotikom u količini od 0,2%, dok su ogledne grupe hranjena smešama sa belim lukom u koncentraciji od 0,05% i 0.2%. Dobijeni rezultati su pokazali da korišćenjem belog luka, umesto antibiotika, dolazi do poboljšanja prirasta u drugoj oglednoj grupi, kao i konverzije hrane u obe ogledne grupa za 3.84%;6.93% u toku celog perioda istraživanja.

Ključne reči: prasad, promoteri rasta, ishrana, proizvodni rezultati

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CHARACTERIZATION OF THE GENETIC STRUCTURE OF THE BROWN TROUT (*SALMO TRUTTA*) FROM “BRADULJICA” FISH FARM, SERBIA

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Abstract: Due to the ecological concerns and preservation of genetic resources, the characterization of genetic structure of the brown trout (*Salmo trutta*) was carried out on the "Braduljica" fish farm. DNA was isolated from fin clips of 10 individuals, and after that molecular PCR-RFLP methods were used for distinguishing between Atlantic and Danubian lineages based on control region of the mitochondrial DNA (CR mtDNA) and lactate dehydrogenase gene of the nuclear DNA (LDH nDNA). Based on phenotypic characteristics it was estimated that out of 10 individuals included in this study five belonged to the allochthonous Atlantic lineage and remaining five belonged to the native Danubian lineage of brown trout. However, results of molecular analyses showed a high percentage of allochthonous genes among the individuals, which confirms the hybridization between these two lineages. Also, the results showed that the selection based on the phenotype is not adequate. In order to continue with proper broodstock management, it is necessary to eliminate allochthonous individuals of the Atlantic lineage from the broodstock.

Key words: *Salmo trutta*, CR mtDNA, LDH nDNA, PCR, RFLP, selection.

Introduction

Brown trout (*Salmo trutta*) is one of the most important and widely distributed freshwater fish, which inhabits the waters of Eurasia and North Africa as an autochthonous salmonid species (Behnke, 1986; Elliott, 1994). It inhabits cold waters, usually the upper river sections, although it can also be found in plain rivers and lakes with clean and cold water.

In the last decades, based on genetic analyses, it has been concluded that the trout of a specific geographical area show significant similarities at the genetic level. The presence of five main phylogenetic groups i.e. lineages of the brown trout were determined: the Danubian (Da), the Atlantic (At), the Adriatic (Ad), the Mediterranean (Me) and the Marmoratus (Ma). Statement made by *Bernatchez et al. (1992)* is still relevant: “for the purpose of clarification of the phylogeographical structure of the brown trout populations, the results have not been completed, as some parts of the areal have not been sufficiently examined”. One of such insufficiently explored territories is the Balkan Peninsula, which, in addition to the Apennine and the Pyrenees, was of great importance as a refugium during the Ice Ages in Pleistocene (*Hewitt, 1996; Hewitt, 1999*). Ichthyofauna of the Balkan Peninsula is very complex, because each river basin, lake or mountain stream has its own distinctive character (*Banarescu, 2004*). In the area of the Balkan Peninsula, perhaps the largest phenotypic diversity in the brown trout population is present (*Kottelat, 1997*).

Genetic research of the brown trout shows the presence of three phylogeographical lineages on the territory of Serbia: the Danubian (Da), the Adriatic (Ad) and the Atlantic (At) (*Marić et al., 2006*). Haplotypes of Da lineage are autochthonous for the locations of the Black Sea basin, while haplotypes of Ad lineage are autochthonous for the locations of the Adriatic and Aegean basins. However, haplotypes of At lineage are of allochthonous origin. It is assumed that the At lineage was introduced into waters in Serbia through an anthropogenic factor (*Marić et al., 2006*). According to research by *Bernatchez et al. (1992)* and *Weiss et al. (2001)* the Atlantic lineage is autochthonous only in the upper parts of the Danube River basin.

In Serbia, out of the total area under fish farms (13500-14000 ha), only 0.1% is under the trout fish farms, and the rest is used for common carp (*Cyprinus carpio*) fish farms. Trout fish farms are located south of the Sava and the Danube rivers in the mountainous regions (*Marković et al., 2009*).

Brown trout is farmed in a small percentage for consumption, and more for the fish stocking of open waters. Some of the problems in open water aquaculture are: inadequate introduction of fish into waters (mainly using fish from other basins and allochthonous species), illegal fishing/poaching, destruction of habitats and absence of regular monitoring of fish resources. These processes lead to the extinction of local populations, hybridization and loss of genetic variability within populations (*Ryman et al., 1995; Laikre and Ryman, 1996*).

It is well established that hereditary basis and proper selection are among the most important factors for successful agricultural production. The selection is mainly conducted on the basis of phenotypes and production data for the given animal, but in some animal species, this is not possible. In the case of aquaculture, specifically in breeding of brown trout, the individuals of different lineages are very similar in the phenotypic view, and the hybridization between lineages can

occur. In theory, the brown trout of certain lineage has a characteristic phenotype, however this is not always the rule, so the colour and shape of the body can also depend on the ecological characteristics of aquatic environment in which fish live. In such cases, for the proper selection, a more precise method is needed, i.e. it is necessary to determine genetic structure, because only in this way the phylogenetic lineages can be precisely distinguished.

The aim of this paper is to use such a method for the characterization of the genetic structure of brown trout originating from the "Braduljica" fish farm in the vicinity of Ivanjica, using PCR (Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism) methods. In the fish farm, breeding of brown trout has been routinely performed in the last 30 years, exclusively by phenotypic selection, without precise data on the phylogenetic origin of the parent material.

The initial hypothesis tested in this study is whether the phenotype is sufficient and reliable indicator of the origin of brown trout.

Materials and Methods

DNA sampling and isolation

Sampling of fish was done at the trout fish farm "Braduljica", which is located on the river Braduljica in the vicinity of Ivanjica. River Braduljica is the left tributary of the River Studenica, Ibar River basin. Annually, around 200,000 brown trouts are grown/spawned in this fish farm, and subsequently introduced into rivers Moravica, Studenica and the surrounding rivers. There is a mixture of different genotypes of brown trout on the farm because part of the fish are of allochthonous origin (At lineage), and part of the autochthonous (fish collected from nearby streams). Based on the phenotype characteristics, five individuals were considered to be originating from the At lineage (samples marked A1 to A5) and five samples originating from the nearby river and considered to be originating from the Da lineage (samples marked from P1 to P5). All sampled brown trout individuals were three years old and were used as a broodstock. Before sampling, anesthetization of fish with several drops of clove oil was carried out, which was added to a vessel with 10 liters of water. Then, the small piece of the anal fin was cut and placed in 96% ethanol, which is the standard method of tissue sampling for the isolation of the fish DNA, without sacrificing the animal. Sampling was carried out in July 2015, and laboratory processing of DNA samples was done in October of the same year.

DNA isolation was performed using the specialized kit Zymo Research Genomic DNA™ -Tissue MiniPrep (Irwin, USA). Following the DNA extraction,

DNA concentration checks of samples were performed using the nanodrop apparatus Implen P300 (Munich, Germany).

Polymerase Chain Reaction (PCR)

Polymerase chain reaction began with the preparation of PCR Mix, from the Taq PCR Kit produced by Kapa Biosystems (Wilmington, USA) whose ingredients are: 171,5 µl MilliQ H₂O, 25 µl buffer A, 12,5 µl MgCl₂, 10 µl dNTP, 10 µl of primer F solution, 10 µl of primer R solution, 1.5 µl Taq polymerase. This amount was enough for 10 analysis. Once the mix was made, the ingredients were evenly mixed on the vortex. Subsequently, 24 µl of PCR mix was added to 1 µl of the eluted DNA sample to a separate volume vessel (200 µl). The amplification of the desired fragments was performed in Eppendorf Mastercycler Nexus GSX1 (Hamburg, Germany) according to the protocol by *Marić et al.*, (2010).

Control region of mitochondrial DNA: Step 1: 94°C - 3 min; Step 2: 94°C - 45 sec; Step 3: 54°C - 45 sec; Step 4: 72°C - 1 min 20 sec; Step 5: 72°C - 10 min; Step 6: 10°C - ∞; Steps 2-4 are repeated 32 times.

Lactate dehydrogenase of nuclear DNA gene: Step 1: 94°C - 3 min; Step 2: 94°C - 45 sec; Step 3: 62°C - 45 sec; Step 4: 72°C - 1 min; Step 5: 72°C - 10 min; Step 6: 10°C - ∞; Steps 2-4 are repeated 32 times.

The oligonucleotide sequences used in the amplification of CR mtDNA and LDH nDNA gene are listed in Table 1.

Table 1. Sequences of primers used in PCR method

Primer	Sequence (5'-3')
Ldhxon3F	GGCAGCCTCTTCCTCAAAACGCCCAA
Ldhxon4R	CAACCTGCTCTCTCCCTCCTGCTGACGAA
28RIBa	CACCCTTAACTCCCAAAGCTAAG
cytR	GTGTTATGCTTTAGTTAAGC

The first two oligonucleotide sequences were designed for partial amplification of the nuclear LDH (LDH-C1) gene in the study of *McMeel et al.* (2001), while the other two were used for the amplification of CR mtDNA (*Snoj et al.*, 2000; *Bernatchez and Danzmann* 1993).

Restriction Fragment Length Polymorphism (RFLP)

For the RFLP method, two endonucleases were used:

1. For amplified CR mtDNA, *SatI* (Thermo Fisher Scientific) was used, which specifically cuts the following nucleotide sequence:

```

5'  G C ↓ N  G C  3'
3'  C G  N ↑ C  G  5'

```

2. For cutting the LDH gene, *BseLI* (Thermo Fisher Scientific) was used, which specifically cuts the following nucleotide sequence:

```

5'  C C N N N N N ↓ N N G G  3'
3'  G G N N ↑ N N N N N C C  5'

```

Both endonucleases were used according to the manufacturer's specification, according to the following protocols:

1. Endonuclease *SatI*: PCR product CR - 10 µL, MilliQ water - 18 µL, 10X G buffer - 2 µL, *SatI* - 2 µL. This mixture was gently stirred (without the use of a vortex) and incubated for 3 hours at 37°C.

2. Endonuclease *BseLI*: PCR product LDH - 10 µL, MilliQ water - 18 µL, 10X Tango buffer - 2 µL, *BseLI* - 2 µL. This mixture was gently stirred (without the use of a vortex) and incubated for 3 hours at 55°C.

RFLP fragments were separated by electrophoresis on agarose gel. A reference marker of 3 kbp was used to determine the length of the RFLP fragments. The voltage on the electrophoresis device was constantly at 120 V. The power supply used was the Serva BluePower 500 and the BlueMarine 200 tub (Heidelberg, Germany). RFLP fragments stained with ethidium bromide were visualized using ultraviolet light on the Vilber Lourmat EBox VX5 (Marin la Vale, France).

Results and Discussion

The *SatI* restriction enzyme cut the amplified CR mtDNA of the At lineage at a specific site C₄₃₄, while the control region of the Da lineage remained uncut. The mtDNA control region of 1088 bp length of At lineage was cut into two fragments of length 654 and 434 bp. The *BseLI* restriction enzyme cut an amplified nDNA fragment i.e. LDH gene at the position G₃₅₃ on allele LDH-C1*90, specific for the At lineage. After cutting, the LDH gene of the At lineage was divided into two fragments of 353 and 75bp lengths. Allele LDH-C1*100 of Da lineage remains uncut. The analysis of CR mtDNA and LDH nDNA gene showed that both brown trout lineages were present, as well as their hybrids (Figures 1a, 1b, 2a, and 2b).

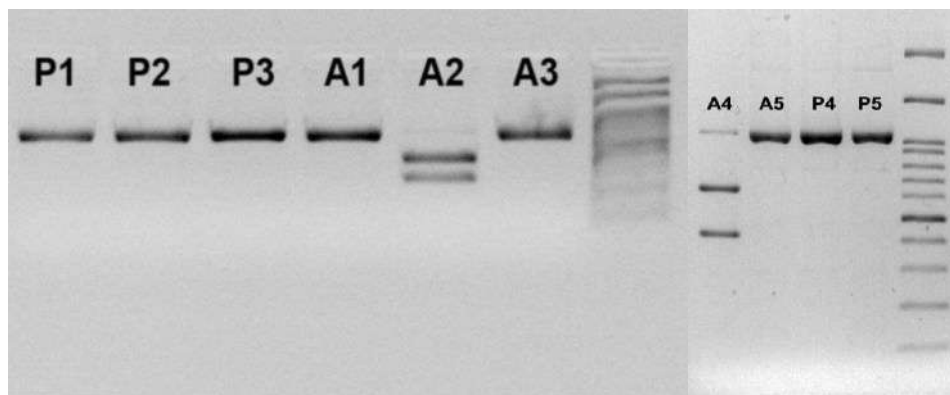


Figure 1. 1a (left) and 1b (right): Restriction of mtDNA control region with *Sat I* enzyme.

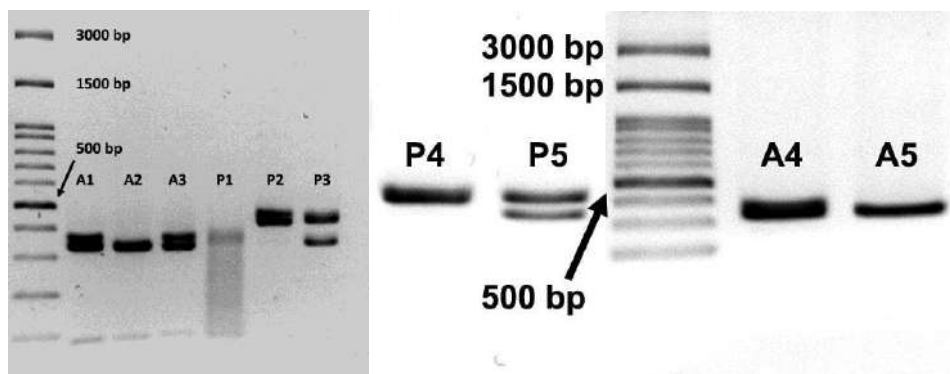


Figure 2. 2a (left) and 2b (right): Restriction of nDNA partial LDH gene with *BseII* enzyme

The analysis of CR mtDNA showed that only two individuals belonged to the At lineage, both A2 and A4 (Figures 1a and 1b). Since mtDNA is inherited exclusively through the maternal lineage, nuclear LDH gene inherited biparentally was used to obtain more precise results. An analysis of the LDH gene revealed that eight individuals had an allochthonous LDH-C1*90 allele characteristic for the At lineage (A1, A2, A3, A4, A5, P1, P3 and P5) (genotypes LDH-C1*90/90 and LDH-C1*90/100) (Figures 2a and 2b). Six individuals were homozygous for LDH-C1*90 allele, while two individuals (P3 and P5) were heterozygous (LDH-C1*90/100). Four individuals (P2, P3, P4 and P5) had the autochthonous LDH-C1*100/100 allele characteristic for the Da lineage with only two individuals homozygous for LDH-C1*100/100 allele (P2 and P4) (Table 2).

Table 2. Frequency of alleles and genotypes of 10 brown trout specimens originating from fish farm "Braduljica"

Genetic markers	Number of specimens	Frequency
At mtDNA	2/10	20%
Da mtDNA	8/10	80%
LDH-C1*90	16/20	80%
LDH-C1*100	4/20	20%
Genotypes		
LDH-C1*90/90	6/10	60%
LDH-C1*90/100	2/10	20%
LDH-C1*100/100	2/10	20%

By crossing the At and Da lineages, the F₂ generation can have mtDNA of one lineage and nuclear alleles of the other lineage. The degree of hybridization between the At and the Da lineages can be more accurately determined using multiple genetic markers, but even with the use of mtDNA and a single nuclear marker, it can be concluded that the degree of hybridization is very high in the fish farm "Bladuljica", with only two individuals (P2 and P4) suitable for the broodstock. If these results are compared with the study conducted in the fish farm "Bled" in Slovenia, it is noticeable that the structure is somewhat different, since the LDH-C1*90/90 genotype is predominant in the "Braduljica" fish farm, with a frequency of 60%, while in the "Bled" fish farm, the dominant genotype is hybrid LDH-C1*90/100 (55%) (Marić *et al.*, 2010). Similarly, by comparing mtDNA, the Da lineage (80%) is dominant in the fish farm "Braduljica" while in the fish farm "Bled" the percentage of individuals belonging to the autochthonous Da lineage is 57.5% (Marić *et al.*, 2010). The result obtained from the "Bled" fish farm can be considered more reliable than ones from this study, because the study was done on a larger sample (40 fish, 20 males and 20 females). Fish stocking of natural aquatic ecosystems is one of the basic conservation activities, which helps in restoration of water ecosystems, however, it must be taken into account which broodstock material will be used. In order for the fish farm "Braduljica" to continue with adequate selection and broodstocking, it is necessary to eliminate individuals of the At lineage from the broodstock.

The hybridization with Atlantic brown trout in the Danube basin was also indicated according to the research by Kohout *et al.* (2013) in the eastern Balkans.

The PCR-RFLP method is often used to determine the population structure of different fish species in freshwater and marine ecosystems around the globe. A study very similar to this one is the discovery of genetic differences between Atlantic and Pacific herring at several locations in the Atlantic and Pacific oceans (Norwegian Sea, Iceland, Barents Sea and near Vancouver; Shaw *et al.*, 1999). The PCR-RFLP method was also used for the identification of hybrid species (Hashimoto *et al.*, 2010). For example, in South America, two species *Leporinus macrocephalus* and *Leporinus elongatus* can give hybrid offsprings. In the study by

Hashimoto et al. (2010), partial gene sequences of the mitochondrial 16S subunit of ribosomal RNA and for nuclear α -tropomyosin were used. After RFLP analysis of the endonuclease *NsiI*, the 300 bp fragment remains uncut in the *L. elongatus* species, whereas mentioned endonuclease cuts the amplified fragment into two smaller fragments of approximately 190 and 110 bp, in the species *L. macrocephalus* (Hashimoto et al., 2010). Hybrid, after restriction, has all three fragments, which confirms that it has inherited one allele from *L. elongatus*, which is not cut by restriction endonuclease and one allele from *L. macrocephalus*, which is cut into two fragments. It is interesting that after the restriction of the mitochondrial gene, *L. elongatus* may be distinguished from the species *L. macrocephalus*, but not the specimen created by crossing the hybrid crossbreeding and *L. macrocephalus*, because endonuclease cuts both types so that two fragments of the same length appear. In fish farms in Denmark, the PCR-RFLP method has been used for the genotyping of different brown trout lineages (Hansen et al., 1997). Namely, samples were taken from 11 fish farms in which the individuals had already gone through the selection process because there was a suspicion that fish from fish farms that inadvertently escape or are deliberately used to introduce them into river waterways can affect (and disrupt) the genetic structure of wild populations, so that samples from fish farms were compared with wild populations from eight large Danish rivers. The study used 11 different endonucleases that cut mtDNA into two segments, and it was concluded that in all 11 fish farms the populations had suffered a significant loss of genetic variability, compared to wild populations.

The informativeness of the PCR-RFLP method is evident also from the study of Wolf et al. (2000) in which 23 fish species were detected.

Conclusion

The initial hypothesis, that the phenotypic selection was reliable and that of the 10 sampled brown trout, five belonged to the Atlantic (At) and five to the Danubian (Da) lineages was rejected. The hypothesis was based on the differences in the phenotypic appearance between these two lineages. Based on genetic analysis, it was found that eight individuals contained allochthonous genes related to the Atlantic lineage. Although it is a small sample, it can be said that the hybridization of these two lineages is very high, which is not adequate and acceptable, because in this fish farm brown trout is bred for the purpose of conservation of genetic resources as well as to introduce fish stock in the surrounding rivers. In this way, the allochthonous genes are spread and the genetic structure of the native Danubian lineage is disturbed.

Eventhough we can distinguish to which lineage one individual/specimen belongs based on phenotype, from the given research it is obvious that it is not

always accurate. In order to improve fisheries and aquaculture in our country, it is necessary to continue research in other fish farms as well as to increase the size of the sample and the number of markers analyzed in order to obtain higher precision of the results. With mtDNA, the use of large number of microsatellite nuclear loci would be very useful because they are very precise markers for determining the genetic structure of the population.

Presently, genetic analysis is increasingly available and can be used in other livestock production. These methods are extremely useful in conserving animal genetic resources and applying adequate selection methods. They could also be used in the detection of the individuals used for breeding/reproduction that carries lethal and semi-lethal genes, which would reduce the losses and increase the economic benefits.

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Određivanje genetičke strukture potočne pastrmke (*Salmo trutta*) iz ribnjaka „Braduljica“, Srbija

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Rezime

U cilju zaštite životne sredine i genetičkih resursa potočne pastrmke (*Salmo trutta*) u radu je ispitivana genetička struktura ove vrste na ribnjaku „Braduljica“ u blizini Ivanjice. DNK je izolovana iz odsečaka peraja 10 jedinki, a nakon toga molekularne PCR-RFLP metode su korišćene za razlikovanje između atlantske i dunavske linije na bazi kontrolnog regiona mitohondrijalne DNK (KR mtDNK) i jedarnog gena za laktat dehidrogenazu (LDH nDNK). Na osnovu fenotipskih karakteristika procenjeno je da je pet jedinki pripadalo alohtonoj atlantskoj liniji, a preostalih pet autohtonoj dunavskoj liniji potočne pastrmke. Međutim, rezultati molekularnih analiza pokazali su prisustvo visokog procenta alohtonih gena u analiziranom uzorku, što potvrđuje hibridizaciju između ove dve linije. Takođe, rezultati ukazuju na to da selekcija na osnovu fenotipa nije adekvatna. Kako bi ribnjak nastavio da se bavi gajenjem potočne pastrmke za potrebe poribljavanja okolnih reka, neophodno je da se eliminišu jedinke atlantske linije iz matičnog fonda.

Ključne reči: *Salmo trutta*, KR mtDNK, LDH nDNK, PCR, RFLP, selekcija

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GROWTH PERFORMANCE AND MORPHOLOGY OF IN 28-84 DAY-OLD VIETNAMESE LOCAL NOI CHICKEN

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Abstract: Noi chicken is one of the most popular native breeds in the South of Vietnam because their good meat quality and disease resistance. Therefore, although price of their products (eggs and meat) is always high, they are always preferred. However, there have not been many studies on the morphology of this breed yet. In the current study, a total of 355 Noi chickens (164 males and 191 females) at 28 days old were selected and randomly allotted to individual cages until they reached the market weight at 84 days old. During the experiment, Noi broilers were fed *ad libitum* a diet with 17% crude protein and 3.000 kcal/kg ME produced by GreenFeed Vietnam Joint Stock Company. Average daily gain, feed conversion ratio and some morphological dimensions were recorded at two different stages of age (28-56 and 56-84 days old). Results were demonstrated that (i) significant differences in feed intake ($P=0.006$), body weight ($P=0.019$), beak length ($P=0.014$), thigh length ($P=0.004$), shank length ($P=0.003$), breast diameter ($P=0.000$), and thigh diameter ($P=0.001$) were found between males and females as well as between different stages; and (ii) body weight and some dimensions will increase rapidly at the first stage. An understanding on these economic traits helps researchers and producers have a more detailed glance on developing of each part of Noi chicken at different stages of age which they are the basic scientific foundation for further studies on this breed.

Key words: Noi broilers, economic traits, measurements

Introduction

Chicken industry in Vietnam has been developing rapidly and strongly in recent years. Many poultry companies have exported commercial chicken meat and eggs to China, Cambodia, Japan market, etc. However, similar to other countries owing native breeds, Vietnamese customers tend to choose the products of these breeds because of their specific quality (taste, flavor, toughness, stiffness, sweetness, etc.).

Noi is known as one of the native chicken breeds who are easy to raise in different conditions of environment, micro-climate, nutrition, and nursing from rural to urban areas for improving farmers' income and meals quality as well as sometimes for fighting games. Noi chickens have strong tough muscles, good natural disease resistance and produce delicious meat. Thus, this breed is chosen as a potential genetic for the sustainable poultry production system in Vietnam. In earlier studies, (i) some performance characteristics (feather color, shank color, eyes color, beak color, comb color) and estimation of allele frequencies of 2 microsatellite markers in chromosome 7 (Linh, 2013), (ii) genetic association of some candidate genes with eggs performance (Vu and Ngu, 2016), (iii) effects of garlic on growth performance (Thuong, 2014), (iv) influence of different ME and CP levels on growth and laying rate (Quyen, 2008) in Noi chicken were investigated. Recently, Khoa et al. (2019) reported some quantitative genetic traits including body weight, average daily gain, feed intake, feed conversion ratio, and some dimensions in 0-28 day-old Noi chicken. Nowadays, with the increasing demand of domestic customers, Noi chicken breed needs to be studied in many ways/methods to produce high quality products. It is also the reason why this study was subsequently conducted and focused on some indicators of growth performance, feed conversion ratio and morphology, as a basic foundation for further studies on this breed.

Materials and methods

This study was conducted at the Can Tho Center for Breeds of Seed, Livestock and Fish, where it is cooperating with Can Tho University in many scientific research activities, located at O Mon district, Can Tho city, Vietnam.

A total of 355 Noi individuals (164 males and 191 females) at 28 days old were selected from a resource population of 1.200 birds investigated in previous studies (Vu and Ngu, 2016; Khoa et al., 2019). All they were kept in private cage with dimensions of 47cm length, 40cm front height, 38cm back height and 40cm

width. Each cage had a separate feeder and drinker. Cages were put inside of the opened housing system.

Table 1. Nutrient component of the experimental feed

Items	31-84 days old (GF1324)*
Crude protein (min), %	17
Humidity (max), %	14
Crude fiber (max), %	5
Methionine and cysteine (min), %	0.7
Metabolism energy (min), kcal/kg	3.000
Calcium, %	0.8-1.2
Phosphorus, %	0.6-1.0
Total lysine (min), %	1

*Main ingredients: soybean cake, fish meal, rice bran, broken rice, corn, wheat bran, cassava, amino acids, vitamins and minerals (Source: GreenFeed Vietnam Joint Stock Company)

Whole flocks were fed *ad libitum* a diet containing 17% crude protein and 3.000 kcal ME (Table 1). During the experiment, multivitamins and beta-glucan were supplied some days at time points of stress (suddenly high temperature, vaccinating, catching, etc.).

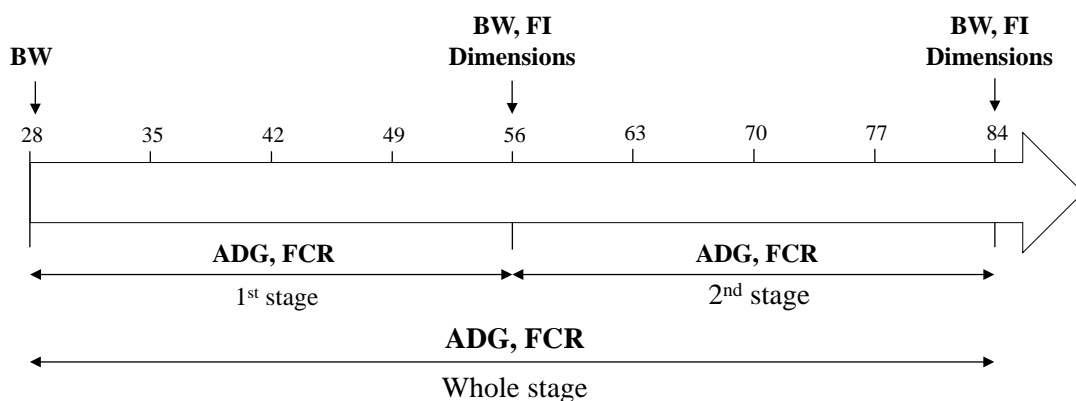


Figure 1. Scheme for sampling

At the end of each month, body weight (BW, g/head), average daily gain (ADG, g/head/day), feed intake (FI, g/head/day), feed conversion ratio (FCR) as well as some parameters such as beak length (BL, mm), skull length (SL, mm), skull width (SW, mm), neck length (NL, mm), back length (BaL, mm), wings length (WL, mm), thigh length (TL, mm), shank length (ShL, mm), keel length (KL, mm), breast diameter (BD, mm), and thigh diameter (TD, mm) (FAO, 2012)

were recorded and calculated. Increasing percentage (IP) of observed traits was calculated as below:

$$\text{IP (\%)} = \frac{\text{Measurement at the end of the month} - \text{Measurement at the beginning of the month}}{\text{Measurement at the beginning of the month}} \times 100$$

Collected data were statistically analyzed by using R program (ver. 3.4.2). General Linear Model was applied to analyze effects of gender or old stage on observed traits.

Results and discussion

Average daily gain, feed intake and feed conversion ratio

The obtained results revealed that (i) there was high significant difference in ADG, FI and FCR between males and females at each various stage of age ($P=0.000$) (Table 2). It is easy to recognize that BW of males was always higher than that of females. This was probably due to much more feed consumption of males. However, males showed lower FCR than females. This was normal biological development in poultry production industry in which males were used economically to produce meat; (ii) significant difference between two stages was found for FI ($P=0.006$) (Table 2). Feed consumption gradually increased in aging of broilers.

Table 2. Effects of aging and sex on ADG, FI, and FCR in Noi broilers

Traits	28-56 days old			28-84 days old			56-84 days old			P
	Trống	Mái	P	Trống	Mái	P	Trống	Mái	P	
ADG	21.19±3.22	17.32±3.30	0.000	22.28±3.13	17.88±3.70	0.000	23.38±4.37	18.44±5.09	0.000	0.195
FI	60.68 ^b ±8.68	51.15 ^a ±8.32	0.000	70.48 ^d ±8.91	58.22 ^b ±10.65	0.000	80.27 ^e ±13.11	65.30 ^c ±15.98	0.000	0.006
FCR	2.88±0.23	2.98±0.25	0.000	3.17±0.18	3.28±0.21	0.000	3.46±0.25	3.58±0.29	0.000	0.827

Under intensive management condition in Northwest Ethiopia, ADG, FI and FCR of the native chicken breeds such as Tilili, Gellilia, Debre-Ellias, Mello-Hamusit, Gassay, Guangua, and Mecha from 35-56 days old were 8.8-11.5, 36.4-47.3, and 3.3-4.9, respectively. At 154 days old, they weighed 1,038-1,257 kg/head of BW although during the experiment they were offered a standard starter ration for a period of 56 days and then, a commercial grower ration for an additional period of 84 days *ad libitum* (Hassen et al., 2006). In another early study, at the stages of 0-

42 days old and 49-84 days old, Thai native chickens were respectively fed a diet with 15% and 19% of crude protein as well as one ME level of 2,900 kcal/kg for both stages. As a result, (i) at the stage of 28-56 days old, ADG (15.46-15.87 vs 17.32-21.19) and FI (33.00-49.76 vs 51.15-60.68) in the Thai showed lower than in the Noi, while FCR was similar between two breeds (2.09-3.24 vs 2.88-2.98); and (ii) at the stage of 56-84 days old, FCR (3.89-4.66 vs 3.46-3.58) of the Thai was higher than the Noi (*Jaturasitha et al., 2002*). It was clear that Noi chicken breed had advantages in terms of ADG and FCR compared with some native ones in the world but strategies for developing this breed has not been noticed and focused in national poultry breeding programs yet.

Body weight and morphology

Table 3. Length of measured traits at different time points of age

Traits	28 days old				56 days old				84 days old			
	Mean (n= 355)	Male (n= 164)	Female (n=191)	P	Mean (n= 355)	Male (n= 164)	Female (n= 191)	P	Mean (n= 325)	Male (n= 149)	Female (n= 176)	P
BW	253.79±53.25	265.68±54.70	243.59±49.89	0.000	787.94±137.21	857.32±119.21	728.38±123.15	0.000	1300.95±247.20	1424.09±232.88	1196.71±208.41	0.000
CV%	0.21	0.21	0.20		0.17	0.14	0.17		0.19	0.16	0.17	
BL	21.44±1.11	21.57±1.10	21.32±1.10	0.036	29.40±1.70	29.84±1.62	29.02±1.67	0.000	34.19±1.81	35.00±1.71	33.51±1.61	0.000
CV%	0.05	0.05	0.05		0.06	0.05	0.06		0.05	0.05	0.05	
SL	23.24±1.37	23.41±1.47	23.09±1.26	0.030	28.94±1.47	29.38±1.47	28.55±1.37	0.000	32.03±1.62	32.47±1.59	31.67±1.55	0.000
CV%	0.06	0.06	0.05		0.05	0.05	0.05		0.05	0.05	0.05	
SW	22.06±1.08	22.27±1.17	21.87±0.95	0.000	28.72±1.56	29.46±1.37	28.08±1.44	0.000	32.74±1.86	33.43±1.72	32.16±1.77	0.000
CV%	0.05	0.05	0.04		0.05	0.05	0.05		0.06	0.05	0.06	
NL	81.44±7.93	82.41±8.59	80.60±7.24	0.032	132.99±9.97	136.25±11.32	130.19±7.63	0.000	158.55±14.26	162.19±14.28	155.47±13.53	0.000
CV%	0.10	0.10	0.09		0.07	0.08	0.06		0.09	0.09	0.09	
BaL	115.99±7.32	117.18±7.20	114.97±7.30	0.004	168.76±11.31	171.96±10.66	166.02±11.15	0.000	205.39±16.79	209.84±15.89	201.63±16.65	0.000
CV%	0.06	0.06	0.06		0.07	0.06	0.07		0.08	0.08	0.08	
WL	273.66±20.48	278.32±19.35	269.66±20.62	0.000	413.75±26.31	422.46±26.44	406.27±23.84	0.000	490.72±35.12	497.23±37.27	485.21±32.27	0.002
CV%	0.07	0.07	0.08		0.06	0.06	0.06		0.07	0.07	0.07	
TL	110.29±8.95	111.33±7.94	109.40±9.66	0.042	168.97±11.51	173.66±10.14	164.95±11.11	0.000	199.53±13.63	201.50±13.85	197.86±13.25	0.016
CV%	0.08	0.07	0.09		0.07	0.06	0.07		0.07	0.07	0.07	
ShL	26.13±1.74	26.46±1.61	25.85±1.80	0.001	41.71±3.64	43.46±3.21	40.20±3.30	0.000	52.28±4.99	52.89±5.02	51.77±4.92	0.044
CV%	0.07	0.06	0.07		0.09	0.07	0.08		0.10	0.09	0.09	
KL	71.58±6.52	72.55±6.90	70.75±6.08	0.010	115.27±8.10	118.35±7.69	112.63±7.50	0.000	146.25±11.11	148.21±11.09	144.59±10.89	0.003
CV%	0.09	0.10	0.09		0.07	0.07	0.07		0.08	0.07	0.08	
BD	159.47±10.06	161.32±9.72	157.88±10.11	0.001	241.15±15.52	247.95±13.66	235.32±14.66	0.000	291.63±19.36	295.10±19.27	288.69±19.00	0.003
CV%	0.06	0.06	0.06		0.06	0.06	0.06		0.07	0.07	0.07	
TD	52.48±6.20	53.40±6.07	51.69±6.23	0.009	83.35±6.35	86.14±5.58	80.96±6.00	0.000	101.87±8.69	103.53±8.84	100.47±8.33	0.001
CV%	0.12	0.11	0.12		0.08	0.06	0.07		0.09	0.09	0.08	

BW: body weight (g/head); BL: beak length (mm); SL: skull length (mm); SW: skull width (mm); NL: neck length (mm); BaL: back length (mm); WL: wings length (mm); TL: thigh length (mm); ShL: shank length (mm); KL: keel length (mm); BD: breast diameter (mm); TD: thigh diameter (mm).

Table 4. Percent increase of measured traits

Traits	28-56 days old			56-84 days old			28-84 days old			P ^a sex	P ^a period
	Mean (n=355)	Male (n=164)	Female (n=191)	Mean (n=325)	Male (n=149)	Female (n=176)	Mean (n=325)	Male (n=149)	Female (n=176)		
BW	534.15±107.34	577.57±99.94	496.87±99.37	511.39±152.93	560.54±157.17	469.54±136.28	1045.95±217.87	1153.04±203.72	955.28±186.43	0.008	0.019
%	217.52±55.61	226.62±56.75	209.71±53.53	64.83±17.13	64.95±16.24	64.73±17.91	420.68±96.98	436.20±94.95	407.54±96.99		
BL	7.96±1.62	8.23±1.70	7.73±1.51	4.89±1.51	5.12±1.52	4.70±1.48	12.82±1.84	13.50±1.71	12.26±1.75	0.000	0.014
%	37.33±8.21	38.35±8.57	36.45±7.79	16.77±6.39	17.24±6.18	16.39±6.55	60.11±9.88	62.63±9.35	58.03±9.84		
SL	5.71±1.48	5.83±1.54	5.60±1.43	3.25±1.59	3.14±1.72	3.34±1.48	8.72±1.84	8.97±1.89	8.53±1.78	0.330	0.238
%	24.78±7.26	25.17±7.71	24.45±6.85	11.37±5.94	10.83±6.25	11.81±5.66	37.87±9.20	38.47±9.54	37.40±8.91		
SW	6.66±1.48	6.90±1.54	6.46±1.40	4.11±1.71	4.19±1.75	4.04±1.68	10.68±1.92	11.22±1.99	10.25±1.74	0.005	0.056
%	30.34±7.25	31.14±7.60	29.65±6.88	14.41±6.51	14.41±6.67	14.41±6.40	48.64±9.65	50.39±10.23	47.22±8.94		
NL	51.55±11.88	52.27±10.75	50.93±12.77	27.24±14.81	29.33±15.27	25.45±14.21	76.84±15.64	79.84±16.23	74.33±14.72	0.191	0.614
%	64.67±18.83	65.08±18.49	64.33±19.17	20.86±11.91	22.12±12.18	19.78±11.61	95.76±24.09	97.78±25.42	94.06±22.86		
BaL	52.77±11.02	54.10±10.97	51.64±10.97	36.56±17.36	37.55±16.29	35.74±18.21	89.34±18.30	93.33±17.07	86.04±18.68	0.064	0.203
%	45.86±10.86	46.60±10.64	45.23±11.03	21.93±11.12	21.93±10.00	21.93±12.01	77.77±18.21	79.96±16.69	75.96±19.24		
WL	140.08±23.81	143.40±23.56	137.24±23.71	78.41±38.62	72.65±41.99	83.35±34.87	216.54±40.34	217.52±41.45	215.73±39.52	0.115	0.058
%	51.70±11.06	52.21±10.41	51.27±11.59	19.22±10.09	17.35±10.52	20.82±9.45	79.90±18.92	77.95±17.28	81.51±20.07		
TIL	58.80±10.50	60.91±10.44	56.99±10.23	30.96±15.30	27.93±15.58	33.46±14.64	88.89±16.04	89.77±15.21	88.18±16.70	0.467	0.004
%	53.79±11.54	55.34±11.22	52.46±11.68	18.65±10.07	16.28±9.37	20.60±10.23	81.42±18.26	80.54±16.37	82.14±19.68		
SHL	15.57±3.50	16.34±3.69	14.92±3.19	10.89±5.38	9.92±5.60	11.68±5.08	26.28±5.39	26.74±5.42	25.90±5.35	0.878	0.003
%	60.00±14.55	62.15±15.06	58.15±13.87	26.71±14.45	23.43±13.85	29.36±14.42	101.88±23.90	102.12±22.98	101.68±24.68		
KL	43.69±8.40	44.74±8.19	42.79±8.49	31.23±12.77	30.82±13.23	31.58±12.40	74.62±12.76	75.53±13.45	73.89±12.16	0.437	0.369
%	62.00±15.24	62.75±15.33	61.37±15.17	27.48±12.19	26.53±12.06	28.25±12.29	105.38±23.52	104.17±24.52	106.37±22.71		
BD	81.93±12.66	85.70±12.00	78.67±12.34	51.94±23.99	46.94±21.01	56.08±25.41	131.89±20.95	133.35±20.11	130.71±21.61	0.487	0.000
%	51.70±9.72	53.67±10.14	50.00±9.03	21.57±9.88	19.07±9.20	23.64±10.12	83.06±16.02	82.32±14.45	83.65±17.22		
TD	30.88±7.42	32.74±7.40	29.28±7.07	19.93±10.52	17.67±10.90	21.78±9.84	49.35±11.61	48.55±12.29	50.04±10.99	0.047	0.001
%	60.65±19.31	63.20±19.61	58.45±18.82	24.67±13.96	21.12±13.82	27.57±13.43	96.82±30.61	93.62±31.19	99.58±29.91		

Significant difference for BW and all dimensions were found between two sexes at various time points of age, in which male was always higher than the female for these observed traits ($P < 0.05$) (Table 3). At 84 days old, BW of Noi broiler (female 1,196.71 and male 1,424.09) was higher than the Thai native one (1,156.05) (*Jaturasitha et al., 2002*), Chum Long Dau broiler (female 1,095.79 and male 1,298.50) (*Thanh, 2012*) and Ho chicken breed (female 1,124.51 and male 1,297.21) (*Doan and Luu, 2006*), but it was similar to Ninh Hoa Ri breed (female 1,195.65 and male 1,571.79) (*Thinh et al., 2017*) and Long Cam one (female 1,069.41 and male 1,440.34) (*Mui et al., 2012*). These evidence indirectly point to the advantages of carcass traits of Noi chicken and their potential for domestic poultry meat production industry in the future.

Data in Table 4 indicated that (i) significant difference between the males and females in the ratio of increasing dimensions such as BW ($P=0.004$), BL ($P=0.030$), TL ($P=0.019$), ShL ($P=0.010$), BD ($P=0.000$), and TD ($P=0.021$) at the first stage (28-56 days old), WL ($P=0.004$), TL ($P=0.000$), ShL ($P=0.001$), BD ($P=0.000$), and TD ($P=0.000$) at the second stage (56-84 days old) as well as BW ($P=0.019$), BL ($P=0.014$), TL ($P=0.004$), ShL ($P=0.003$), BD ($P=0.000$) and TD ($P=0.001$) throughout the whole growing stage (28-84 days old) were found; (ii) growth and development of Noi chickens were noticed at the first stage in which dimensions showed double compared with the second stage; moreover (iii) a total of 8/12 dimensions (BW, NL, WL, TL, ShL, KL, BD and TD) has an percent increase over 50% (51.70-217.52%) at the first stage.

The measurement of weight and some dimensions on body helps us predict the development of muscle and skeleton, which they may be important data for setting up healthy diets and appropriate nursing modes to maximize genetic potential at different growing stages of Noi chickens.

Conclusion

It can included that growth rate and feed conversion ratio in Noi chicken were better than some other native chicken breeds in Vietnam and other countries. Furthermore, reasonable high nutritional diets as well as most appropriate nursing should be noted during the stage of 28-56 days old to maximize genetic potential for meat and skeleton traits in Noi chicken in the future.

Porast i morfologija pilića vijetnamske lokalne noi rase starosti od 28-84 dana

Do Vo Anh Khoa, Nguyen Thi Hong Tuoi, Nguyen Thi Dieu Thuy, Shin Okamoto, Kataro Kawabe, Nguyen Thi Kim Khang, Nguyen Tuyet Giang, Takeshi Shimogigri

Rezime

Noi rasa živine je jedna od najpopularnijih autohtonih rasa na jugu Vijetnama, zbog njihovog dobrog kvaliteta mesa i otpornosti na bolesti. Stoga, iako je cena njihovih proizvoda (jaja i mesa) uvek visoka, ovi proizvodi se preferiraju od strane krajnjih potrošača. U ovom istraživanju, ukupno 355 noi pilića (164 muških i 191 ženski) u starosti 28 dana su odabrani i nasumično smešteni u pojedinačnim kavezima dok nisu dostigli tržišnu težinu u uzrastu od 84 dana. U toku eksperimenta, noi brojleri su hranjeni ad libitum obrokom sa 17% sirovih proteina i 3.000 kcal/kg ME proizvođača GreenFeed Vietnam a.d. Prosečni dnevni prirast, konverzija hrane i neke morfološke dimenzije evidentirani su u dva različita uzrasta (28-56 i 56-84 dana starosti). Rezultati su pokazali (i) značajne razlike u unosu hrane ($P = 0,006$), telesnoj težini ($P = 0,019$), dužini kljuna ($P = 0,014$), dužini bataka ($P = 0,004$), dužini tarzusa ($P = 0,003$), obimu grudi ($P = 0,000$), i obimu bataka ($P = 0,001$) između muških i ženskih pilića, kao i između različitih uzrasta; i (ii) telesna težina i neke dimenzije se ubrzano povećavaju u prvoj fazi. Razumevanje ovih ekonomskih osobina pomaže istraživačima i proizvođačima da detaljnije sagledaju razvoj svakog dela noi pilića u različitim fazama starosti koje su osnovna naučna osnova za daljnje studije o ovoj rasi.

Ključne reči: Noi brojleri, ekonomske osobine, merenja

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Milan M. Petrović¹, Stevica Aleksić¹, Milan P. Petrović¹, Milica Petrović², Vlada Pantelić¹, Željko Novaković¹, Dragana Ružić-Muslić¹

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Review paper

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

Zdenka Škrbić, Zlatica Pavlovski, Miloš Lukić, Veselin Petričević

Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia

Corresponding author: Zdenka Škrbić, e-mail address

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