UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

CONTENTS

Original scientific paper

VOL 32, 3
Founder and publisher
INSTITUTE FOR ANIMAL HUSBANDRY
11080 Belgrade-Zemun
Belgrade 2016

Journal for the Improvement of Animal Husbandry

UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

Belgrade - Zemun 2016

Biotechnology in Animal Husbandry 32 (3), p 239-320, 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636

Address of the Editor's office
Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080 Belgrade-Zemun, Republic of Serbia Tel. 381 11 2691 611, 2670 121; Fax 381 11 2670 164;

e-mail: biotechnology.izs@gmail.com; www.istocar.bg.ac.rs

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According to CEON bibliometrical analysis citation in SCI index 212, in ISI 9, impact factor (2 and 5) of journal in 2012: 0,667 and 0,467, - M51 category

Annual subscription: for individuals -500 RSD, for organizations 1200 RSD, -foreign subscriptions 20 EUR. Bank account Institut za stočarstvo, Beograd-Zemun 105-1073-11 Aik banka Niš Filijala Beograd.

Journal is published in four issues annually, circulation 100 copies.

The publication of this journal is sponsored by the Ministry of Education and Science of the Republic of Serbia.
Printed: "Mladost birošped", Novi Beograd, St. Bulevar AVNOJ-a 12, tel. 381 11 2601-506

Biotechnology in Animal Husbandry 32 (3), p 239-249 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.2'636.083.1
DOI: 10.2298/BAH1603239O

THE STATE OF WELFARE ON SERBIAN DAIRY FARMS

Dušica Ostojić Andrić[1], Slavča Hristov[2], Milan M. Petrović[1], Vlada Pantelić[1], Dragan Nikšić[1], Violeta Caro Petrović[1], Branislav Stanković[2]

[1]Institute for Animal Husbandry, Auto put 16, 11080 Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade-Zemun, Republic of Serbia
Corresponding author: Dušica Ostojić Andrić, andricdusica.iah@gmail.com
Original scientific paper

Abstract: The aim of this study was to analyze the overall welfare state on Serbian dairy farms, as well to suggest measures for its improvement. The assessment was done according to Welfare Quality® Assessment Protocol for Dairy Cows on 16 selected commercial farms in which the cows of Simmental and Holstein-Friesian breeds were reared (N=4833). Welfare state on each farm was evaluated by relevant measures that indicated insurance of appropriate feeding, housing, health and behavior as basic principles of welfare. Overall score (0-100 points) enabled finally categorization of farms into one of four welfare category (not classified, acceptable, enhanced and excellent).Based on results, half of the farms were assigned to acceptable, and other half to enhanced welfare category. Housing conditions on the majority of farms (63%) were assessed as unacceptable (≤20 points) due to poor hygiene and discomfort. Cows were kept tied continuously on more than one third of farms which together with lack of pasture (17 days/year on average) restricting their comfort and freedom of movement. This may be also linked to low scored behavioral insurance (32points), especially inability to express its natural forms (6.7 points).Health condition was estimated as acceptable, but endangered welfare by high incidence of laminitis (38%), distocya (4.2%) and mortality (6.7%). Commonly performed dehorning procedure (79%) without anesthetic/analgesic application caused pain and stress in affected animals. Overall assessment score (2.5/5) showed the need for improvement in all areas of dairy cows' welfare, especially in terms of their housing and management.

Keywords: welfare assessment, feeding, housing, health, behavior

Introduction

Broom (1986) describes welfare as a state of well-being of the animals, which is created as a response to its attempts to cope with the impacts of the

environment. It means to establish control over the mental and physical stability. Since the response to a particular challenge of the environment can be one or more of combat strategies (behavioral, physiological, immunological, etc.), there is a wide range of indicators of animal welfare that can be used to assess and determine the level of its quality. Analysis of the state of welfare is the first step in defining strategies for improvement, which is of great significance considering that the concern about the welfare of farm animals is not only to the benefit of animals, but also people with concurrent positive effects on environmental protection (*Gregory 1993; Scanga et al., 1998; Cook, 2004; Hill et al., 2007; Lindenlauf et al., 2010*).

At the present time, taking into account the gravity and exposure to impacts that threaten the animal welfare, as well as the number of farmed animals, the issue of welfare of dairy cows is second to the welfare of broiler chickens in Europe *(EFSA, 2009*). Defining, implementation, analysis of the relevance and development of standards for the protection of animal welfare on cattle farms have become a very important topic in the late 20th and early 21st century. Protecting the welfare of dairy cows is a complex issue, which involves a range of different aspects and requires urgent action in changing the genetic selection and system of management. The most interested parties are consumers of animal products and agricultural producers, but also all those who are directly or indirectly involved in the production of food. In addition, the protection of the welfare of dairy cows is associated with environmental issues, sustainable development, and a whole range of medical, hygienic, economic and social problems of a society.

In Serbia, in 2009, the Animal Welfare Law was passed and related regulations for the protection of animal welfare on farms, during transport and during their stay at the slaughterhouse, however, the technical and scientific analysis of the application of these regulations are not yet completed. Previous studies in the field of welfare of cattle in our country are mostly fragmented and analyze certain aspects of the quality of animal welfare (*Hristov et al., 2006, 2008, 2011; Ostojić Andrić et al., 2011, 2012, 2015, 2016)*. Bearing in mind that, in our country, the interest in the welfare of farm animals is growing, not only among consumers of animal products, but also the producers, these investigations are becoming more necessary.

The aim of this study was to analyze the factors of importance for ensuring the animal welfare, to show the average condition/status of welfare on dairy farms in Serbia, as well as to indicate the key risks to the welfare and propose measures for its improvement.

Materials and Methods

The study was conducted on a total of 16 farms with different housing (tied, free) and different capacity (small, medium, large) in which the cattle of

Simmental and Holstein - Friesian breeds were reared. Minimum number of cows in the sample was 30 and the average per farm was 64 animals in two repetitions - during winter and summer season. The welfare assessment was done by the *Welfare Quality® Assessment Protocol for Dairy Cows (2009)* that is specifically designed to assess relevant indicators of welfare from the viewpoint of the animals themselves. The protocol includes 29 indicators used to determine the 12 criteria: the absence of long-term hunger and thirst, comfort, thermal comfort, freedom of movement, lack of injuries and illness, absence of pain due to management procedures, expressing social and other behaviours, good human - animal relations and a positive emotional state. By aggregation of these criteria the values of 4 basic principles of welfare are determined: good nutrition, good housing, good health and appropriate behavior.Welfare state was (partialy and overall) determined by classifying each criteria and principle into one of four categories of welfare quality according to score (0-100 points) and given descriptive rating scale (1-4): 1-unacceptable (<20 points), 2-acceptable (20-55 points), 3-enhanced (55-80 points) and 4-excellent (>80 points).

Data processing and categorization of welfare quality of the investigated dairy farms was conducted using software specially developed under the Protocol, and the respective statistical parameters were analyzed with the program StatSoft. Inc. (2004), Statistica for Windows version 7.

Results and discussion

Overall welfare assessment

The overall assessment of the welfare quality of the dairy farms in Serbia was conducted by collecting data relating to the principles of good nutrition, good housing, good health and appropriate behaviour. Software analysis showed the overall state of welfare, i.e. the categorization of farms as unacceptable (score 1), acceptable (score 2), enhanced (score 3) and the welfare of excellent quality (score 4).

Table 1. Categorization of Serbian dairy farms (N=16) according to overall welfare assessment

Farms (1 - 16)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Overall welfare assessment (descriptive rating scale from 1 to 4)	2	2	2	3	2	3	3	3	3	3	2	2	3	2	3	2

Research results (Table 1) show that one-half of the surveyed farms is classified as acceptable (score 2) and the other half is classified into the category of welfare of enhanced quality (score 3). None of the studied farms are classified into categories of unacceptable (score 1) or excellent (score 4) quality on the basis of which it can be argued that on the observed farms the conditions are provided that meet more than the basic needs of animals in terms of nutrition, health, comfort in housing and expression of behavior of cows. By comparison, a survey conducted by the same methodology on farms in the EU (*Welfare Quality Network, 2012*) showed a great similarity with the results presented in this research. In 2012, the share of farms with an acceptable quality of welfare in the EU was 47%, with acceptable quality 51%, and with unacceptable quality of welfare 2%, while in our country none of the evaluated farms are classified in the latter category.

Good feeding

According to the results presented in Table 2, nutrition of dairy cows, at least when it comes to the lack of long-term starvation and thirst, is not a problem on our farms as opposed to Europe, whose score on average is lower. Most of the observed farms (44%) showed the value of this principle in the range from 90 - 100 points and only 6.25% showed the value of less than 20 points. The average value of the principle of good nutrition on farms in the EU stood at 52.3, and in Serbia 76 points with similar variation - S (28.5 vs. 25.31, respectively). Greater deviations from optimal are present in tied system compared to the free range system, especially when it comes to the share of cows of fattened condition (4.27%). It is known that, same as malnutrition, enhanced fattening of cows can also lead to problems in breeding, especially in terms of reduced reproductive capacity, difficulties in calving and fatty degeneration of the liver (*Reid et al., 1986*). Therefore, it is obvious that in our conditions, greater attention should be paid to proper balanced diet and its qualitative rather than quantitative aspects.

Table 2. Animal welfare status of Serbian dairy farms (Total score and categorization of welfare principles/criteria)

Welfare principles/criteria	$\bar{x}$	*SD*	S^2	*Min*	*Max*	*Welfare Categories*
I Good feeding	75.97	25.31	640.39	12.20	100.00	Enhanced
1. Absence of prolonged hunger	78.84	19.51	380.77	40.30	100.00	Enhanced
2. Absence of prolonged thirst	88.41	29.58	875.02	3.00	100.00	Excellent
II Good housing	36.59	19.37	375.18	7.30	65.40	Acceptable
1. Comfort around resting	25.77	12.50	156.32	2.70	45.10	Acceptable
2. Freedom of movement	56.94	39.25	1540.58	15.00	100.00	Enhanced
III Good health	41.17	8.11	65.78	23.90	56.60	Acceptable
1. Absence of injuries	51.57	14.85	220.40	21.00	81.10	Acceptable
2. Absence of diseases	59.53	21.67	469.70	30.20	100.00	Enhanced
3. Absence of pain induced by management procedures	41.00	28.86	833.03	20.00	100.00	Acceptable
IV Appropriate behavior	31.93	13.77	189.50	15.40	81.10	Acceptable
1. Expression of social behaviour	98.68	1.42	2.01	95.00	100.00	Excellent
2. Expression of other behaviours	6.73	20.23	409.38	0.00	79.10	Unacceptable
3. Good human-animal relationship	64.10	20.25	410.20	24.40	93.90	Enhanced
4. Positive emotional state	50.74	20.90	436.80	10.20	92.70	Acceptable
Overall welfare assessment, average value (1-4)	2.47	0.51	0.26	2.00	3.00	Acceptable

Good housing

Poor housing conditions are certainly one of the most significant welfare problems in our country. This is at the same time field of welfare in which, according to the results of the research, there are largest deviations in relation to the situation in EU countries. The housing conditions on the largest number of dairy farms (31.25%) were evaluated as unacceptable while in the EU the largest number of farms (50%) rated acceptable to the enhanced quality score (*Welfare Quality Network, 2012*). This observation is further confirmed by the fact that in our study none of the farms is rated excellent category while in the EU 2% of farms are classified within this category.

Analysis of indicators of housing conditions suggests that the main reason for this condition is primarily poor cow comfort. The comfort conditions are

estimated as poor on the basis of high share of cows that lie outside the bed (36.54%), which may be the result of inadequate or insufficiently sized beds. In addition, the farms surveyed showed a very poor state of hygiene of dairy cows with a high percentage of cows with contaminated parts of the lower leg (84.64%), rump (71.34%) and udder (60.07%). This indicates inadequate hygiene of cow beds and facilities, insufficient amount of bedding, but it can also be an indicator of the disorder in rumen digestion (*Huxley and Whay, 2006*). Extended time of cow's lying down of 6.25 seconds was also one of the indicators of cows' discomfort and in this case constituted a high incidence of laminitis (37.45%).

Although the freedom of movement of dairy cows on nearly half of the observed farms was scored as excellent, one third of farms showed unacceptable scores in evaluation of this criterion. The main reason for the limited freedom of movement is tied system of housing applied on six of the sixteen examined farms and especially rare use of grazing which was practiced only on two farms. By comparison, in the EU, in recent years the freedom of movement of dairy cows has significantly improved resulting in a maximum score for this welfare criterion in 2012 (*Welfare Quality Network, 2012*). The importance of ensuring freedom of movement is reflected in its positive impact on the comfort and health of dairy cows as well as to the expression of normal behavior patterns.

Good health

The health status of dairy cows on 81.25% of farms was scored as acceptable and on 18.75% as enhanced. The above-mentioned results are very encouraging when compared with estimates of this principle in EU countries (*Welfare Quality Network, 2012*). In fact, despite of poorer housing conditions established on farms in our country, the average score is similar to the health status assessed on farms in the EU where the housing conditions are significantly more acceptable in terms of welfare. A possible reason for this phenomenon is the increased average milk yield of cows on European farms and greater exposure to selective pressures. However, it was found that certain diseases and disorders in the examined farms in Serbia represent a risk to the welfare of farmed animals. Such is the case with the incidence of dystocia and laminitis (4.18% and 37.45%), which combined with a high mortality rate (6.70%) represent serious welfare problems.

In Serbia, one of the major welfare problems certainly is dehorning of calves, done without the use of analgesics and anesthetics, which leads to activation of the chain reaction of pain - stress - distress and endangering physical condition and behavior of animals (*Anderson and Muir, 2005*). Given the objective of dehorning, animals grown in free systems are more exposed to this danger. In most European countries the use of anesthetics and analgesics, to a lesser extent, is applied as standard procedure and is expected to soon become part of the protocol in Serbia.

Appropriate behavior

The values of this principle criteria (Table 2) indicate that opportunities for securing appropriate behavior on farms in Serbia, on average, are lower than the same in the EU. The greatest number of the examined farms (87.5%) is estimated in the range from 21 to 50 points, which corresponds to acceptable score. The average value of this principle was about 32 points while the farms in the EU (*Welfare Quality Network, 2012*) show slightly higher value (43 points) with a similar variability - S (13 vs. 15 points, respectively). Similar to the results of the assessment presented here, also in the EU the largest number of farms (59%) are scored as acceptable in regard to this principle, but compared to farms in Serbia, where only 6.25% of farms showed enhanced score for this criterion, 35% of farms in the EU are scored as enhanced.

The highest deviation in the negative sense, was identified in the expression of behavior characteristic for grazing on pastures, which the majority of examined farms (87.5%) did not practice. This can be considered a high risk to the welfare, given the importance and positive impact of grazing in terms of providing good health and productivity (*Krohn, 1994; White et al., 2001*).

The interaction between animals and people, i.e. their experience of people and mutual interactions have a major impact on health, productivity and welfare of farm animals, which is why they are considered as a significant indicator in the assessment of their welfare (*Hemsworth and Coleman, 2011; Waiblinger et al., 2003*). The average value of the criterion the good man-animal relationship was 64.1 points, with 43.75% of the farms scored as appropriate and as many as 25% of farms as excellent in regard to this criterion. According to the results of the *Welfare Quality Network (2012)*, average rating of this criterion on farms in the EU is 51.5 points, with only 8% of the farms scoring excellent and 50% of the farms scoring acceptable. This suggests that the relationship between breeders and cows in Serbia is satisfactory and on average even better than on the farms in the EU.

Conclusion

The results of the research of welfare on dairy farms in Serbia indicate that the quality of welfare in general is satisfactory, and that the dairy cows on average have slightly higher than minimum of their needs satisfied in terms of nutrition, housing conditions, securing appropriate health status and behavior. However, there is considerable room for improvement of the current situation, particularly with regard to the identified welfare risks. As the most significant welfare problems on dairy farms in Serbia the following can be emphasized: inadequate

housing conditions, health disorders, dehorning, problems of inadequate nutrition and expression of normal behaviour.

In regard to the identified major risk factors and current trends in the dairy cattle as recommendations for improvement of welfare, the following measures are proposed:

- Ensuring the appropriate space and comfort for keeping dairy cows;
- Ensuring the adequate size, quality and hygiene of the cow beds;
- Ensuring the greater freedom of movement of cows using the free housing system and grazing
- Optimal balancing of diet and continuous monitoring of physical fitness as an important indicator of many factors of welfare risk;
- Application of anesthetic and analgesic medications when dehorning as a pain management procedure;
- Prevention and control of diseases of the locomotor system, in the first place laminitis;
- Prevention and control of mastitis, metabolic and reproductive disorders;
- Improvement of breeding-selection program by defining the optimum balance between production and non-production traits;
- Education of farmers about the importance of ensuring the welfare of dairy cows and farm animals in general;
- Compliance with legal regulations and the constitution of national institutions for monitoring and controlling the quality of the welfare of farm animals;
- Consumer information and development of *animal - friendly* market as a direct support system in which production is carried out with the concern for animal welfare.

Acknowledgment

Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 31053.

Stanje dobrobiti na mlečnim farmama u Srbiji

Dušica Ostojić Andrić, Slavča Hristov, Milan M. Petrović, Vlada Pantelić, Dragan Nikšić, Violeta Caro Petrović, Branislav Stanković

Rezime

Cilj ovog istraživanja bio je da se analizira stanje dobrobiti na mlečnim farmama u Srbiji, kao i da se predlože mere za njegovo unapređenje. Ocena stanja dobrobiti obavljena je putem Protokola za ocenu kvaliteta dobrobiti mlečnih krava na 16 odabranih komercijalnih farmi na kojima su gajene krave simentalske i holštajn-frizijske rase (N=4833). Stanje dobrobiti na svakoj od farmi procenjivano je na osnovu relavantnih pokazatelja koji ukazuju na stepen obezbeđenja odgovarajuće ishrane, uslova držanja, zdravlja i ponašanja kao osnovnih principa dobrobiti. Ukupan skor (0-100 poena) omogućio je konačnu kategorizaciju farmi u jednu od četiri kategorije stanja kvaliteta dobrobiti (nezadovoljavajuću, prihvatljivu, odgovarajuću i odličnu). Prema rezultatima istraživanja, jedna polovina farmi svrstana je u kategoriju prihvatljivog, a druga polovina farmi u kategoriju odgovarajućeg kvaliteta dobrobiti. Uslovi držanja su na većini farmi (63%) ocenjeni nezadovaljavajuće (≤20 poena) zbog loše higijene i diskomfora. Na više od trećine farmi krave su držane vezano tokom cele godine, što udruženo sa slabom primenom ispaše (17 dana/godini prosečno) značajno ograničava njihov komfor i slobodu kretanja. Ovo je svakako u vezi i sa niskom ocenom obezbeđenja odgovarajućeg ponašanja (32 poena), posebno kada je u pitanju mogućnost ispoljavanja njegovih prirodnih oblika (6.7 poena). Zdravstveno stanje u proseku je ocenjeno kao prihvatljivo, ali su visoka incidenca laminitisa (38%), otežanih telenja (4.2%) i mortalitet (6.7%) prepoznati kao glavni činioci rizika po dobrobit. Uobičajena praksa izvođenja obezrožavanja (79%) bez primene anestetika/analgetika na farmama u Srbiji uzrok je bola i stresa kod životinja. Prosečan skor celokupno ocenjene dobrobiti (2.5/5) ukazuje na potrebu za unapređenjem u svim segmentima obezbeđenja dobrobiti mlečnih krava, posebno menadžmenta i uslova držanja.

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Received 19 August 2016; accepted for publication 21 September 2016

Biotechnology in Animal Husbandry 32 (3), p 251-260 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.2'575
DOI: 10.2298/BAH1603251B

CHARACTERISATION OF EXON 9 OF SOLUTE CARRIER FAMILY 11 MEMBER A1 GENE IN VECHUR CATTLE

Anu Bosewell[1], Naicy Thomas[2], Thazhathu Veettil Aravindakshan[1]

[1]School of Applied Animal Production and Biotechnology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur 680651, India
[2]Department of Animal Breeding, Genetics and Biostatistics, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur 680651, India
Corresponding Author: anubosewell@gmail.com
Original scientific paper

Abstract: The solute carrier family 11 member A1 (*SLC11A1*) gene has been associated with natural resistance to intracellular pathogens such as Brucella, Salmonella, Leishmania and Mycobacterium in several species including bovine and plays a critical role in elimination of pathogens by generating hydroxyl free radicals. The objective of the present study was to investigate the polymorphism in exon 9 of *SLC11A1* gene in Vechur cattle, one of the dwarf cattle of India which is known for its disease resistance. A 198 bp fragment containing exon 9 of the gene was amplified by polymerase chain reaction (PCR). The amplicons upon single strand conformation polymorphism (SSCP) analysis revealed two different banding patterns. A novel non synonymous SNP (g.46C>T) with predominance of CC genotype was also detected in Vechur cattle. These results suggest that there exists a considerable genetic variation at *SLC11A1* locus and further association studies may help in development of a PCR based genotyping assay to select cattle with better immunity to intracellular pathogens.

Key words: *SLC11A1*, Vechur, Disease resistance, Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP)

Introduction

Dairy industry is affected by various infectious diseases such as Brucellosis, Paratuberculosis, Salmonellosis caused by various intracellular pathogens. These infections has a significant economic impact and causes substantial financial losses every year. Due to a more efficient host immunological defence, native breeds are found to be resistant against various pathogens. These resistance may be associated with one or more host genes. Thus, it is increasingly

important to study those natural resistance against disease in different bovine breeds as it contributes to the control and eradication of diseases through genetic selection.

One of the candidate genes studied in livestock is *SLC11A1* gene. *SLC11A1* gene formerly known as Natural Resistance Associated Macrophage Protein 1 *(NRAMP1)* gene encodes a protein with 12 transmembrane domains which is involved in the transport of divalent cation such as Fe^{2+}, Mn^{2+}, Zn^{2+}, Mg^{2+} and Co^{2+} ions *(Vidal et al., 1996; Goswami et al., 2001)*. The *SLC11A1* gene delivers increased concentration of iron from cytosol into the phagolysosome which catalyses Fenton and Haber-Weiss reaction generating oxygen intermediates favouring bacterial killing *(Goswami et al., 2001)*. *SLC11A1* has many pleiotropic effects on macrophage function that includes enhanced tumor necrosis factor-α, interleukin- 1β, inducible nitric oxide synthase and MHC class II expression which are important in resistance to intra cellular pathogens such as *Mycobacterium tuberculosis (Awomoyi, 2007).*

Various studies suggested variations in both coding and noncoding sequences of *SLC11A1* gene in many species. Mutations in the coding regions result in alteration of SLC11A1 function leading to variation in disease resistance by increasing or decreasing the rate of transcription. The polymorphisms in the promoter could lead to altered expression of the gene *(Nicholas, 2012).* A mutation at position 169 of the protein (glycine to aspartic acid) had resulted in loss of function of SLC11A1 in mice *(Vidal et al., 1993)*. Microsatellite polymorphisms detected in the 3'UTR of the *SLC11A1* gene in ruminants were found to be associated with resistance to *Brucella abortus, Mycobacterium bovis and Mycobacterium avium subsp. paratuberculosis (Barthel et al., 2001; Borriello et al., 2006; Capparelli et al., 2007a; Kadarmideen et al., 2011; Korou et al., 2010; Martinez et al., 2008, 2010; Pinedo et al., 2009; Reddacliff et al., 2005; Taka et al., 2013; Taka et al., 2015).*

According to the National Bureau of Animal Genetic Resources (NBAGR), out of 39 recognised cattle breeds in India, Vechur cattle is the only recognised cattle breed from Kerala. The Vechur Cattle was named after the village Vechur in Kottayam district of the state of Kerala in India, where it was supposed to have evolved. The extremely small size, low feed requirements and high disease resistance make these cattle reliable. Vechur cows were observed to have low incidence of diseases and are less prone to mastitis, parasites, or foot and mouth disease *(Iype and Venkatachalapathy, 2001).*

Since Vechur cattle, the indigenous cattle of Kerala, are well known for their disease resistance, the objective of the present study was to characterize the exon 9 of *SLC11A1* gene in Vechur cattle.

Materials and Methods

Sample collection and isolation of genomic DNA

Blood samples were collected from the animals maintained at the Vechur Cattle Conservation Centre, Mannuthy. Five millilitres each of blood samples were collected in vaccutainers coated with EDTA from the jugular vein of 60 Vechur adult cattle. DNA was isolated by the standard phenol choloform extraction method *(Sambrook and Russell, 2001)* with minor modifications.

PCR amplification of exon 9 of SLC11A1 gene

A pair of primers were (EX9F 5'TCCTTTTACCTTCGTAGTCTCG 3' and EX9R 5' GAATATATGGGGTGTGCCTCA 3') were designed using the bioinformatics tool Primer 3 *(Untergasser et al., 2012)* based on the reference sequence (GenBank Acc. No. DQ493966.1). A region corresponding to 198 bp of exon 9 of *SLC11A1* gene was amplified by PCR. The PCR reaction was carried out in 25 µl mixture containing 1.5 mM of $MgCl_2$, 0.5 U of Taq DNA Polymerase, 200 µM of dNTP, 10 pM of each forward and reverse primer and 50 ng of genomic DNA as template. The thermal cycling profile for the reaction includes initial denaturation for 3 min at 95ºC, followed by 35 cycles of denaturation at 95ºC for 30 sec, annealing at 55 ºC for 30 sec, extension at 72ºC for 30 sec and final extension at 72ºC for 5min. The amplified products were then resolved in 2% agarose gel electrophoresis and visualised in UV transilluminator (Bio Rad, USA) after staining with ethidium bromide.

Single strand conformation polymorphism analysis

SSCP was conducted by mixing 10 µl of PCR product with 15 µl of denaturing buffer (9.5 ml formamide, 0.4 ml of 0.5M EDTA, 2.5 mg xylene-cyanole and 2.5 mg bromophenol blue). The mixture was then incubated at 95ºC for 10 min and immediately chilled on ice. Denatured PCR products were separated by 12% polyacrylamide gel electrophoresis (acrylamide:bisacrylamide = 29:1). The gel was run at 130 voltage for 17 hrs time at 4 ºC, in a vertical electrophoresis apparatus (Hoefer, USA). The SSCP patterns were visualised using silver nitrate staining, photographed and analysed.

Nucleotide sequencing of SSCP alleles

Representative PCR products showing different banding patterns in SSCP were sequenced using respective forward and reverse primer to detect variations in nucleotides. Sequencing was performed by automated sequencer using Sanger's dideoxy chain termination method at Sci Genom Labs Pvt. Ltd., Cochin, India, and aligned with other sequences in GenBank employing BLASTn. For comparative analysis, the *SLC11A1* gene reference sequences from 8 mammalian species were retrieved from the GenBank database. Multiple Sequence Alignment was performed using the bioinformatics tool Clustal Omega. The *SLC11A1* gene sequences of different species were analysed using the 'MegAlign' tool of

Lasergene Software (DNASTAR, Madison, WI, USA) to generate phylogenetic tree.

Statistical analysis

The genotypes were identified by observing the SSCP patterns of each sample in the gels which was further confirmed by sequencing. The genotype frequency and allele frequency were calculated using the formulae:

$$\text{Genotype frequency} = \frac{\text{No of individuals with 'particular genotype'}}{\text{Total no of individuals in the population}}$$

$$\text{Allele frequency} = \frac{\text{No of copies of a given allele}}{\text{Sum of counts of all alleles in the population}}$$

Results and Discussion

Single strand conformation polymorphism analysis

In order to ascertain the polymorphism in 198 bp fragment (comprising of partial intronic region (8 and 9) and complete exon 9), the PCR product was subjected to SSCP-PAGE. Silver staining revealed two unique SSCP banding patterns. Pattern 1 consisted of two bands and pattern 2 consisted of three bands which were identified as CC and CT respectively (Figure1a). Sequencing results confirmed the presence of a novel SNP with C to T transition in the exon 9 of *SLC11A1* gene (Figure1b). In *SLC11A1* gene, there are reports of coding region mutations in various exons. *Bagheri et al. (2015)* reported a novel mutation in exon 11 of the *SLC11A1* gene of Holstein dairy cattle and this SNP had a significant effect on the occurrence of clinical mastitis. SNP in exon 4 and intron 4 were reported by *Cheng et al. (2015),* suggested that exon 4 polymorphism was corresponding to a non synonymous mutation (alanine to threonine), which was associated with tuberculosis in Holstein cattle. *Korou et al. (2010)* identified two polymorphic regions in 3`UTR of the *SLC11A1* gene and their significant association with the detection of Mycobacterium avium subsp. paratuberculosis (MAP) antibody by ELISA in goats.

a) b)

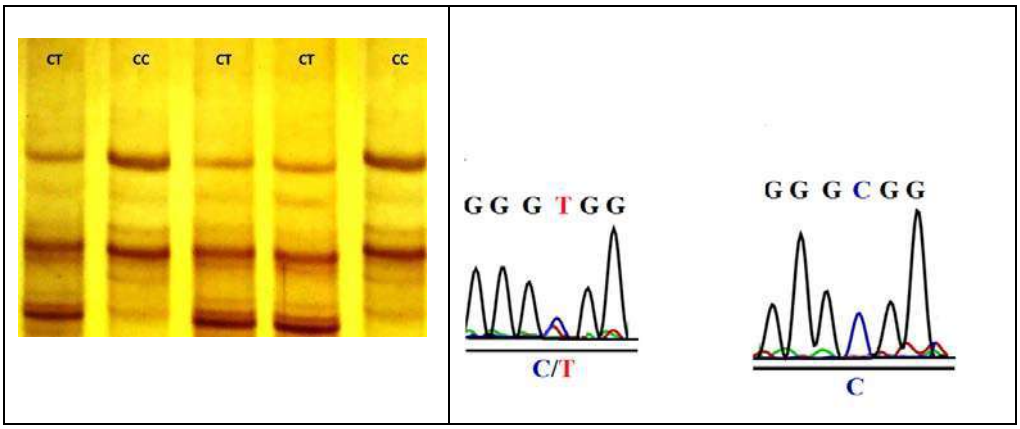

Figure 1. PCR-SSCP analysis of exon 9 of *SLC11A1* gene. (a) SSCP banding pattern of 198 fragment of exon 9 of *SLC11A1* gene (b) Sequence maps showing SNP g.46C>T

Using the Expasy Translate tool the amino acid sequences (partial protein sequence corresponding to the exon 9) were predicted, for each genotype. The predicted sequence suggested that the novel SNP, g.46C>T causes a non synonymous mutation, with substitution of alanine (GCG) by Valine (GTG).

The frequency of CC genotype was found to be 0.6 and CT was 0.4 in the population studied. The allele frequencies of C and T allele were found to be 0.8 and 0.2 respectively. From these values we can infer that the CC genotype showed high frequency and the C allele was found to be predominant.

Nucleotide sequence analysis

Clustal Omega analysis of the variant pattern of SSCP showed an SNP at 888th position compared to the reference sequence (GenBank Acc. No. NM_174652.2). The sequences were subjected to identity/divergence analysis using MegAlign tool. The 198 bp sequences obtained from C and T alleles of Vechur cattle were compared with the sequences of other mammalian species available in the NCBI database (Figure 2). The designated T allele showed 100 percent identity with the sequence of other cattle whereas 99.4 percent similarity with designated C allele (Figure 3). Most distant species identified was Sus scrofa (94.3%).

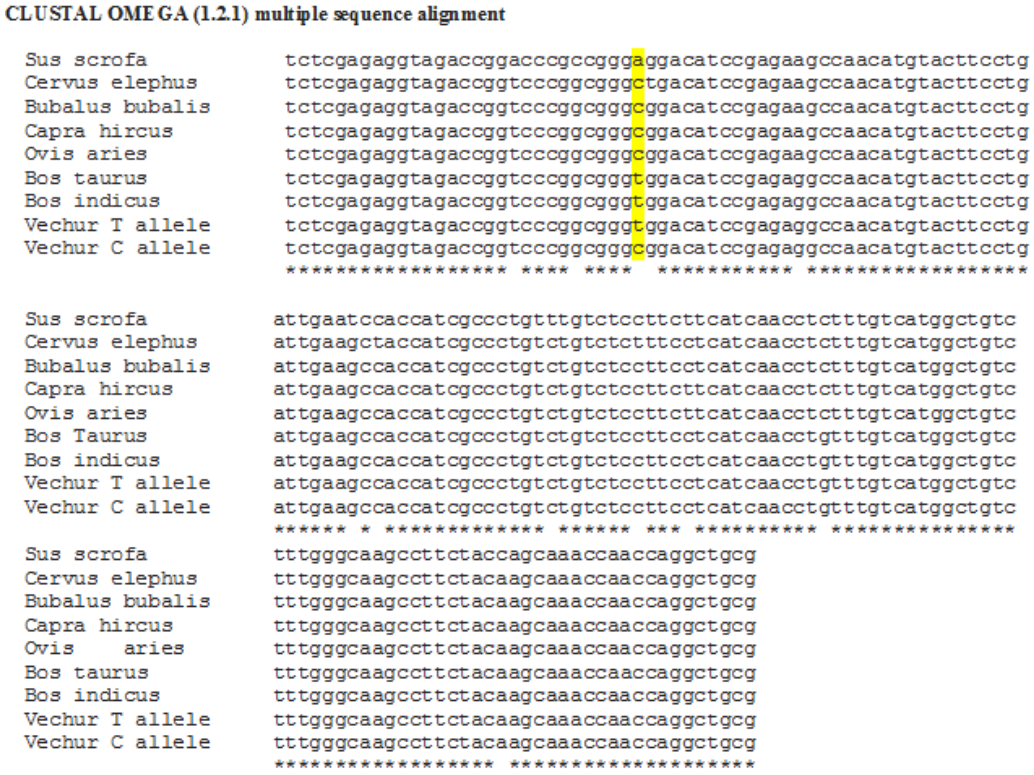

Figure 2. Alignment of nucleotide sequence of exon 9 of *SLC11A1* gene of *Bos tuarus, Bos indicus, Capra hircus, Ovis aries, Cervus elephus, Sus scrofa* and *Bubalus bubalis* using Clustal Omega

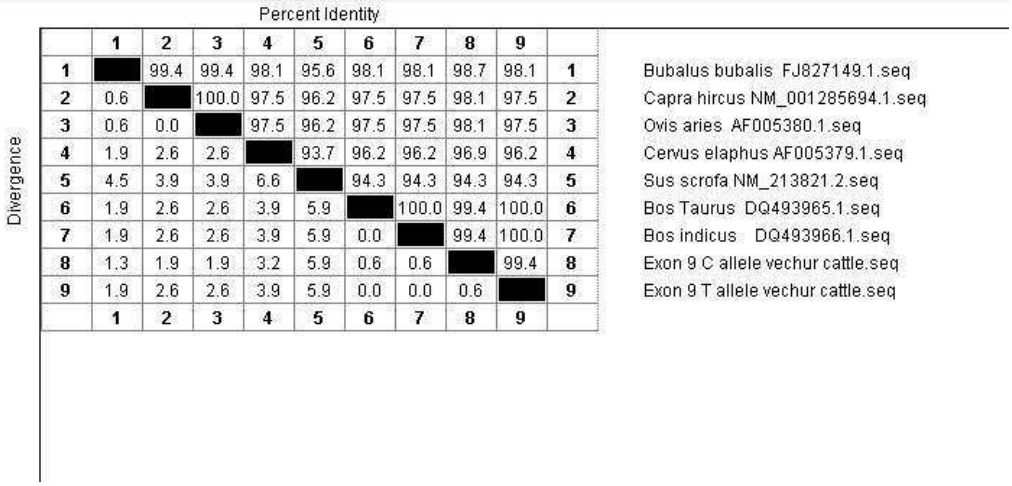

Figure 3. SLC11A1 nucleotide percent identity and divergence in eight species

Phylogenetic analysis

The phylogenetic tree from 198 bp fragment revealed close evolutionary relationship between designated C and T alleles and other cattle as they formed a common cluster (Figure 4). This was expected as they had a high percent similarity in their sequences. The phylogenetic tree showed two major branches at the primary node for pig and ruminants. Three unique branches were formed for cattle, buffalo and goat.

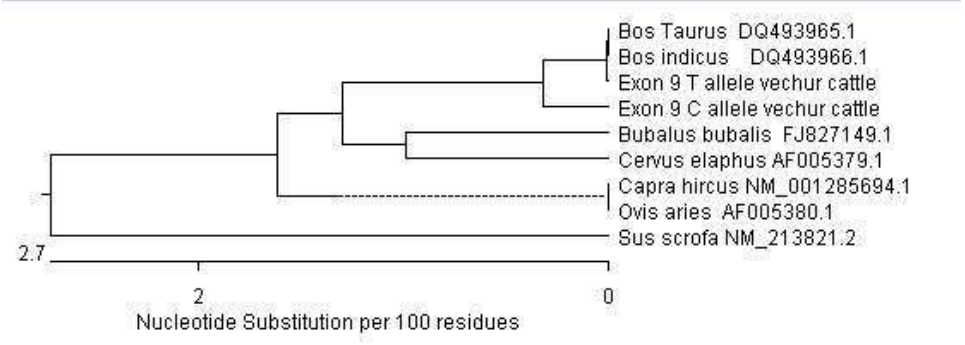

Figure 4. Phylogenetic tree on the basis of nucleotide sequences of complete exon-9 of *SLC11A1* gene of Vechur cattle and other mammalian species

Conclusion

The *SLC11A1* gene plays an important role in the innate immunity. The *SLC11A1* gene functions as metal ion transporter (divalent metals such as Fe^{2+}, Mn^{2+}, Zn^{2+}) involved in host defence against infections by acting as important cofactors for the production of toxic hydroxyl radicals. In the present study we identified a novel non synonymous single nucleotide variation in exon 9 of *SLC11A1* gene in Vechur cattle. We think that from this point the research on this field should be directed toward the identification of polymorphism in the coding and in the promoter regions of *SLC11A1* gene to further associate the eventual genotypes with phenotypes traits (resistant/susceptibility). The detected SNP can be used for further association analysis with disease incidence/resistance caused by intracellular pathogens in other cattle breeds.

Acknowledgement

We are thankful to the Kerala Veterinary and Animal Sciences University for providing the facilities for the conduct of research.

Karakterizacija eksona 9 membranskog transportnog proteina - SLC11A1 goveda vehur rase

Anu Bosewell, Naicy Thomas, Thazhathu Veettil Aravindakshan

Rezime

A1 (SLC11A1) - membransko transportni A1 (*SLC11A1*) gen se povezuje sa prirodnom otpornošću na intracelularne patogene kao što su Brucella, Salmonella, Leishmania i Micobacterium u nekoliko vrsta uključujući goveda i igra kritičnu ulogu u eliminisanju patogena stvaranjem slobodnih radikala hidroksila. Cilj ovog istraživanja je bio da se ispita polimorfizam u eksonu 9 SLC11A1 gena u vehur goveda, vrsta patuljaste stoke u Indiji, poznata po otpornosti na bolesti. A 198 bp fragment, koji sadrži ekson 9 gena je pojačan korišćenjem polimeraze lančane reakcije (PCR). Analiza usaglašenost polimorfizam (SSCP) otkrila je dva različita trakasta obrasca. Novi, nesinonimi SNP (g.46C>T) sa dominantnim CC genotipom je takođe detektovan u goveda rase vehurke. Ovi rezultati pokazuju da postoji značajna genetska varijacija na SLC11A1 lokusi i dalja istraživanja ovih veza mogu da pomognu u razvoju testova genotipizacije zasnovanih na PCR metodama sa ciljem selekcije stoke sa boljim imunitetom na intracelularne patogene.

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Received 7 June 2016; accepted for publication 27 July 2016

Biotechnology in Animal Husbandry 32 (3), p 261-269 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.43'636.08
DOI: 10.2298/BAH1603261L

ESTIMATION OF GENETIC PARAMETERS AND BREEDING VALUES FOR LITTER SIZE IN THE FIRST THREE PARITY OF LANDRACE SOWS

Dragomir Lukač[1], Vitomir Vidović[1], Teodora Vasiljević[2], Oliver Stanković[3]

[1]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21 000 Novi Sad, Serbia
[2]Patent co. doo, Vlade Ćetkovića 1a, 24211 Mišićevo, Serbia
[3]Patent IEC, Moše Pijade bb, 25220 Crvenka, Serbia
Corresponding author: Dragomir Lukač, dragomir.lukac@stocarstvo.edu.rs
Original scientific paper

Abstract: The aim of this study was to estimate the genetic parameters and breeding values for reproduction traits of Landrace sows in the first three parities by Animal model. Records of 2238 first parity; 2125 second parity and 1872 third parity Landrace sows farrowing between 2007 and 2012 were included in the analysis. The traits included in the analyses were total pigs born (TB), number of pigs born alive (NBA), number of pigs weaned (NW) and litter weaning weight (LW) in the parities. The genetic parameters were estimated using a multivariate analyses Animal model using REML procedure. Estimates of heritability for TB were 0.03, 0.05 and 0.18, for NBA were 0.04, 0.02 and 0.17, for NW were 0.08, 0.08 and 0.01 and for LW were 0.09, 0.11 and 0.03 for parities 1 to 3. Genetic and phenotypic variance were increased from the first to the third parity. Between the majorities of studied reproductive traits were the recorded positive genetic and phenotypic correlations, except between LW and other analyzed properties where they recorded a high correlation negative in third parity. Means of estimated breeding values of reproductive traits from first parity to third parity was indecreased.

Key words: genetic parameters, breeding values, liter size, parity

Introduction

From the viewpoint of profit to producers, improvement of productive traits in pigs is a very important issue. Sow productivity is recognized as a key factor affecting the efficiency and economic viability of the pig industry and is a leading concern of commercial producers and breeders (*Kim, 2001*). In current pig breeding programs, great emphasis is placed on improving reproduction traits in

dam lines (*Hanenberg et al., 2001*). The breeding goal is generally to increase the number of piglets weaned per sow per year. The number of piglets born or born alive per litter is still the only reproduction trait used in most breeding programmes (*Rydhmer, 2000*).

Because of the negative genetic correlations between many production and reproduction traits, the improvement of productive traits ignoring reproductive traits causes poor genetic progress populations. To wit, knowledge of genetic parameters for reproductive traits is essential to estimate accurate breeding values by accounting for all correlations available in a multivariate BLUP analysis. Estimates of genetic parameters for sow productivity traits are generally of low heritability (*Popovac et al.*, 2012; *Dube et al., 2012*; *Radojković et al*., 2012; *Roehe et al., 2009*). Low estimates of heritability for these traits do not discourage genetic selection in the populations studied. The heritability estimates give an indication of the rate of genetic progress that can be achieved when genetic selection is applied (*Dube et al., 2012*).

Estimates of genetic parameters can be biased by involuntary and directional selection from parity to parity. In order to account for this possible bias, reproductive traits of the sow recorded in parities one to three were treated as separate traits. The objective of this study was to estimate the genetic parameters and breeding values for Landrace sow reproduction traits in the first three parities by Animal model. Such results are prerequisite parameters in the breeding value estimation.

Material and Methods

Reproductive data were obtained for the first three parities of purebred Landrace sows breed. Litter size records proceeded from 6235 litters born (2238 first parity; 2125 second parity and 1872 third parity) from 2238 sows and from 94 sire landrace breed (3882 liters) and 73 sires Yorkshire breed (2353 liters), farrowed between January 2007 and December 2012 were used. Only sows with complete litter records were included. The traits included in the analyses were total pigs born (TB), number of pigs born alive (NBA), number of pigs weaned (NW), and litter weaning weight (LW) in the first, second and third parity.

Statistical analyses were conducted with statistical software program Statistica 12 for Windows, significance of fixed effects, included in the model were tested using PROC GLM procedures. Farrowing season was defined as a four month period: I season (November, December, January, February); II season (March, April, September, October); III season (May, June, July, August), and was fitted for all traits. Lactation length was grouped into five intervals: till 20 days; from 30 days; from 21 to 29 days – three intervals, each three days long.

The model for estimate genetics parameters included year-season, sire breed and lactation length as fixed effects, and additive genetics effects of the animal as random effect:

$$Y_{ijklm} = \mu + A_i + YS_j + SB_k + LG_l + e_{ijklm}$$

where Y_{ijklm} - represents the values of reproductive traits; μ - average mean; A_i = animal; YS_j = year-season; SB_k = sire breed; LG_l - lactation length; e_{ijklm} - random error

Genetic parameters of reproduction traits were estimated using the restricted maximum likelihood (REML) method based on an animal model using the WOMBAT software (*Meyer, 2007*) with multivariate analyses. The model can be represented in matrix terms by

$$y = Xb + Za + e$$

where ***y*** = vector of observations; X = incidence matrix of fixed effects; b = vector of fixed effects; Z = incidence matrix of random effects; a= vector of random effects; e = vector of residuals.

Results and Discussion

The mean, standard deviation and coefficient of variation for each trait in the first three parity are summarized in Table 1. Means and standard deviations of TB, NBA, NW and LW increased from first parity to third parity, that is, from 9.47 ± 2.64 to 11.12 ± 2.78, from 8.89 ± 2.58 to 10.55 ± 2.70, from 8.32 ± 1.30 to 10.49 ± 3.43, and from 56.54 ± 12.71 to 69.45 ± 24.67, respectively. Coefficient of variation for TB and NBA from first parity to third parity decreased from 27.83 to 24.99 and from 29.00 to 25.62, while the for NW and LT increased from 15.62 to 32.71 and from 22.48 to 35.52. This indicates that reproductive performance of sows for TB and NBA in the population was improved after the first parity, and that variation of individuals was decreased, thereby the variation for NW and LT increased. In research *Ziedina et al. (2011)*, the average number of NBA in the first parity was 9.1 and in the second 10.3 piglets per litter, thereby, 92% of the sow's had 6 - 19 piglets and only 8% up to five piglets per litter. The average number of NW in the first parity was 9.1 and in the second 10.00 piglets per litter, and LW 21-day is indecreased from 57.1 to 64.5 kg. In research *Oh et al. (2006)*, number of TB, NBA, NW and LW piglets has increased from first parity to later parities, from 11.22 to 12.50, from 10.55 to 11.65, from 9.08 to 9.35, and from 57.73 to 63.95, respectively.

Table 1. Summary statistics for reproductive traits by first, second and third parity in a Landrace sows

Parity	Traits	Mean	SD	CV	Min	Max
1	TB	9.47	2.64	27.83	3	18
	NBA	8.89	2.58	29.00	3	16
	NW	8.32	1.30	15.62	2	16
	LW	56.54	12.71	22.48	10	126
2	TB	10.57	2.87	27.14	3	23
	NBA	10.23	2.70	26.44	3	18
	NW	10.08	3.07	30.47	2	24
	LW	68.51	22.35	32.63	12	175
3	TB	11.12	2.78	24.99	3	21
	NBA	10.55	2.70	25.62	3	18
	NW	10.49	3.43	32.71	2	24
	LW	69.45	24.67	35.52	13	201

TB - total piglets born, NBA - number of piglets born alive; NBW - number of piglets weaned; LW - litter weaning weight

Genetics and phenotypic variances and erro for the first, second and the third parity are summarised in Table 2. Genetics variances for TB, TB, NBA, NW and LW in the first parity were 0.24, 0.25, 0.13 and 13.02, and have increased to the third parity to 1.28, 1.20, 0.98 and 19.08, respectivel, which is in direct relation with the increase in the standard deviation of the traits. In research *Paura et al. (2014)*, the genetics variance for NBA was 0.77 and 0.84 and for LW was 16.98 and 14.18 in first parity and later parities Landrace sows. Phenotypic variances for TB, TB, NBA, NW and LW in the first parity were 6.37, 6.20, 1.67 and 135.78, and have increased to the third parity to 6.94, 6.94, 5.57 and 491.12.

Table 2. Genetic and phenotypic variances for reproductive traits on first, second and third parity in a Landrace sows

Parity	Traits	Genetic variance	Phenotypic variance
1	TB	0.24 (0.18)	6.37 (0.24)
	NBA	0.25 (0.04)	6.20 (0.23)
	NW	0.13 (0.06)	1.67 (0.64)
	LW	13.02 (5.38)	135.78 (5.24)
2	TB	0.36 (0.28)	7.14 (0.31)
	NBA	0.18 (0.24)	6.87 (0.29)
	NW	0.52 (0.28)	6.14 (0.27)
	LW	62.16 (26.29)	524.35 (23.40)
3	TB	1.28 (0.50)	6.94 (0.37)
	NBA	1.20 (0.48)	6.94 (0.37)
	NW	0.98 (0.26)	5.57 (0.28)
	LW	19.08 (25.68)	491.12 (25.61)

TB - total piglets born, NBA - number of piglets born alive; NBW - number of piglets weaned; LW - litter weaning weight

Heritability, genotypic and phenotypic correlations and are phenotypic variances summarized in Table 3. The heritabilities for TB were 0.03, 0.05 and 0.18, for NBA were 0.04, 0.02 and 0.17, for NW were 0.08, 0.08 and 0.01 and for LW were 0.09, 0.11 and 0.03 for parities 1 to 3. To wit, for TB and NBA there was an increase of heritability with increasing parity, while the NW and LW heritability decreased, which is consistent with results *Hanenberg et al. (2001)*. In research *Radojković et al. (2011)*, introduction into the analysis of the results pertaining to the second, third and subsequent parities lead mainly to detection of lower values of heritability coefficients for TB, NBA and NBW. Heritability estimates of TB, NBA, NW and LW in this study were slightly lower than those previously reported *Oh et al. (2006)* (0.27 for TB, 0.25 for NBA, 0.16 for NW and 0.20 for LW in first parity and 0.15, 0.15, 0.08, 0.11, respectively in later parities). In research *Ziedina et al. (2011)*, the heritability estimates in the first parity were 0.07, 0.16 and 0.36 and in second parity were 0.07, 0.39 and 0.17 for NBA, NW, LW-21 days, respectively. *Paura et al. (2014)* obtained the heritability for the NBA 0.05 and 0.10 and for LW 0.23 and 0.20 in first parity and later parities Landrace sows. In research *Hamann et al. (2004)* heritabilities for NBA in first and later parity in Landrace sow were 0.15 and 0.11, respectively. In study *Chen et al. (2003)* low heritability estimates of 0.08 to 0.10 for NBA, 0.07 to 0.09 for LW, and 0.02 to 0.06 for NW were reported. *Hermesch et al. (2000)* are reported heritability estimates for NBA of 0.08, 0.09, and 0.08 in the first, second and third parity.

Between the majority of studied reproductive traits were the recorded positive genetic and phenotypic correlations, except between LW and other analyzed properties, where they the recorded a high correlation negative. Obtained genetic correlations were higher than phenotypic correlations in all parities. Genetic correlations between TB and NBA were 0.94, 0.99 and 0.98, between TB and NW were 0.75, 0.64 and 0.82, between TB and LW were 0.10, -0.28 and -0.83, between NBA and NW were 0.69, 0.61 and 0.77, between NBA and LW 0.00, -0.04 and -0,92 and between NW and LW were 0.09, 0.16 and -0.84 for parities from 1 to 3. Phenotypic correlations between TB and NBA were 0.91, 0.94 and 0.93, between TB and NW were 0.63, 0.58 and 0.78, between TB and LW were 0.02, -0.18 and -0.60, between NBA and NW were 0.57, 0.60 and 0.66, between NBA and LW 0.02, -0.01 and -0,84 and between NW and LW were 0.72, 0.10 and -0.80 for parities from 1 to 3.

In research *Oh et al. (2006)*, genetic correlations between sow reproductive traits in the first parity were estimated as 0.95, 0.78 and 0.62 between TB and NBA, NW and LW, 0.86 and 0.74 between NBA, NW and LW, and 0.85 between NW and LW. In the results of the same authors, phenotypic correlations was 0.86, 0.48 and 0.38 between TB and NBA, NW and LW, 0.59 and 0.51 between NBA, NW and LW, and 0.80 between NW and LW. In research *Radojković et al. (2005)*, genetic correlations between sow reproductive traits were in range from low

(0.230) to complete (1.197). *Hermesch et al. (2000)* reported estimates of genetic correlations between NBA and LW for the first, second and third parities were -0.14, -0.15, and -0.75, respectively. Genetic correlations between NBA, LW and NW have been reported *Chen et al. (2003)* that ranged from 0.10 to 0.15 between NBA and LW, 0.07 to 0.20 between NBA and NW, and 0.65 to 0.75 between NW and LW.

Table 3. Heritability (diagonal), genetic (below diagonal) and phenotypic correlations (above diagonal) for reproductive traits on first, second and third parity in a Landrace sows

Parity	Traits	TB	NBA	NW	LW
1	TB	**0.03 (0.01)**	0.91 (0.04)	0.63 (0.02)	0.02 (0.01)
	NBA	0.94 (0.06)	**0.04 (0.01)**	0.57 (0.02)	0.02 (0.01)
	NW	0.75 (0.04)	0.69 (0.03)	**0.08 (0.03)**	0.72 (0.01)
	LW	0.10 (0.03)	0.00 (0.03)	0.09 (0.03)	**0.09 (0.01)**
2	TB	**0.05 (0.02)**	0.94 (0.00)	0.58 (0.03)	-0.18 (0.03)
	NBA	0.99 (0.02)	**0.02 (0.01)**	0.60 (0.03)	0.01 (0.03)
	NW	0.64 (0.04)	0.61 (0.01)	**0.08 (0.02)**	0.10 (0.00)
	LW	-0.28 (0.01)	-0.04 (0.03)	0.16 (0.09)	**0.11 (0.04)**
3	TB	**0.18 (0.06)**	0.93 (0.00)	0.78 (0.03)	-0.60 (0.03)
	NBA	0.98 (0.01)	**0.17 (0.06)**	0.66 (0.03)	-0.84 (0.03)
	NW	0.82 (0.07)	0.77 (0.02)	**0.01 (0.02)**	-0.80 (0.01)
	LW	-0.83 (0.08)	-0.92 (0.06)	-0.84 (0.01)	**0.03 (0.01)**

TB - total piglets born, NBA - number of piglets born alive; NBW - number of piglets weaned; LW - litter weaning weight

Statistics of breeding values for each reproductive trait are presented in Table 4. Means of estimated breeding values of reproductive traits from first parity to third parity was indecreased. The breeding values for TB were -0.043, -0.029 and 0.011, for NBA were -0.044, -0.030 and 0.012, for NW were -0.046, -0.002 and 0.023 and for LW were -0.126, 0.206 and 0.404 for parities 1 to 3. Also, standard deviations of breeding values in later parities were larger than in the first parity.

Table 4. Statistics for breeding value estimates of reproductive traits on first, second and third parity in a Landrace sows

	First parity				Second parity				Third parity			
	TB	NBA	NW	LW	TB	NBA	NW	LW	TB	NBA	NW	LW
Mean	-0.043	-0.044	-0.046	-0.126	-0.029	-0.030	-0.002	0.206	0.011	0.012	0.023	0.404
SD	0.428	0.445	0.436	1.864	0.412	0.419	0.435	3.981	0.659	0.647	0.478	2.482
Max	0.997	0.995	0.999	6.476	0.996	0.988	1.164	16.123	2.177	1.961	0.992	7.200
Upper 1%	0.975	0.976	0.989	4.807	0.966	0.961	1.012	13.113	1.682	1.611	0.973	6.444
Upper 5%	0.874	0.882	0.920	3.845	0.826	0.848	0.878	9.984	1.292	1.276	0.901	5.245
Upper 10%	0.777	0.795	0.830	3.282	0.697	0.724	0.779	8.321	1.124	1.113	0.817	4.506
Upper 25%	0.576	0.606	0.587	2.343	0.487	0.418	0.561	5.495	0.840	0.825	0.591	3.224
Median	-0.126	-0.119	-0.126	-0.297	-0.120	-0.112	-0.111	-0.192	0.115	0.109	0.112	0.151
Lower 25%	-0.505	-0.527	-0.510	-2.414	-0.539	-0.561	-0.534	-4.328	-0.830	-0.813	-0.644	-3.073
Lower 10%	-0.741	-0.750	-0.749	-3.169	-0.755	-0.807	-0.732	-5.980	-1.098	-1.098	-0.865	-4.235
Lower 5%	-0.864	-0.865	-0.866	-3.625	-0.876	-0.897	-0.834	-7.164	-1.259	-1.261	-0.933	-4.956
Lower 1%	-0.977	-0.975	-0.973	-4.303	-0.957	-0.979	-0.957	-9.594	-1.602	-1.589	-0.987	-6.074
Min	-0.996	-0.998	-0.998	-4.877	-0.993	-0.996	-0.999	-11.418	-1.739	-1.779	-0.998	-6.736

Conclusions

Sow reproductive traits had the low heritability in the first three parity (0.03, 0.05 and 0.18 for TB, 0.04, 0.02 and 0.17 for NBA, 0.08 and 0.01 for NW and 0.09, 0.11 and 0.03 for LW) and response to selection may therefore be slow. Since sows reproductive traits are lowly heritable, genetic selection may not always yield substantial additive gains. However, because of their economic importance an attempt should always be made to keep these traits at their optimum levels.

Acknowledgments

Research was financially supported by the Ministry of Science and Technological Development, Republic of Serbia, with in the project TR 31032.

Ocena genetskih parametara i oplemenjivačke vrednosti veličine legla u prva tri pariteta landras krmača

Dragomir Lukač, Vitomir Vidović, Teodora Vasiljević, Oliver Stanković

Rezime

Cilj ovog istraživanja je bio da se ocene genetski parametari i oplemenjivačka vrednost reproduktivnih osobina Landras krmača u prva tri pariteta

Animal modelom. U radu je analizirano 2238 prvih pariteta, 2125 drugih pariteta i 1872 trećih pariteta landras krmača oprasenih između 2007. i 2012. godine. Analizirane osobine su bile ukupan broj rođene prasadi (TB), broj živorođene (NBA) i zalučene (NW) prasadi i masa legla na zalučenju (LW). Genetski parametri su ocenjeni multivarijantnim Animal modelom upotrebom REML procedure. Ocenjene heritabilnosti za TB su bile 0,03, 0,05 i 0,18, za NBA 0,04, 0,02 i 0,17, za NW 0,08, 0,08 i 0,01 i za LW 0,09, 0,11 i 0,03 od 1 do 3 pariteta. Genetske varijanse za TB, NBA, NW i LW u prvom paritetu su bile od 0,24, 0,25, 0,13 i 13,02 i povećavale su se do trećeg pariteta do 1,28, 1,20, 0,98 i 19,08. Između većine ispitivanih reproduktivnih osobina su zabeležene pozitivne genetske i fenotipske korelacije, osim između LW i drugih osobina, gde su zabeležene negativno jake korelacije u trećem paritetu. Procenjene oplemenjivačke vrednosti reproduktivnih osobina su se povećavale od prvog do trećeg pariteta.

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Received 20 April 2016; accepted for publication 7 June 2016

Biotechnology in Animal Husbandry 32 (3), p 271-284 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.593
DOI: 10.2298/BAH1603271O

BIOCHEMICAL CHARACTERIZATION OF THE NIGERIAN INDIGENOUS GUINEA FOWL (Numida meleagris)

Abel Olusegun Oguntunji[1]*, Kolawole Luke Ayorinde[2], Taiwo Olayemi Aremu[1]

[1]Department of Animal Science and Fisheries Management,
Bowen University, P.M.B. 284, Iwo, Osun State, Nigeria.
[2]Department of Animal Production,
University of Ilorin, Ilorin, Kwara State, Nigeria.
*Corresponding author: abelmendel@yahoo.co.in
Original Scientific Paper

Abstract: Blood protein polymorphism has been extensively used for characterization and estimation of genetic diversity in farm animals. A study on biochemical characterization and estimation of genetic diversity of Nigerian indigenous guinea fowls *(Numida meleagris)* was conducted using four blood proteins {Haemoglobin (Hb), Carbonic anhydrase (CA), Transferrin (Tf) and Albumin (Alb)}. Cellulose acetate electrophoresis indicated that all the protein markers were polymorphic; expressing two co-dominant genes and two genotypes at their respective locus. Heterozygouse genotypes were prevalent at Hb, Tf and Alb loci while homozygoutes were more frequent for CA. Allelic constitution was similar (A and B) for Hb, Tf and Alb while F and S were typed at CA locus. Gene A had higher frequency of occurrence at Tf and Alb loci while gene F and B was prevalent at CA and Hb locus, respectively. Average estimated genetic diversity (heterozygosity) across the genetic systems was 0.40 and moderate. Prevalence of genes F, A and B at their respective locus is suggestive of their relevancies to the survival and adaptability of the studied population to its natural habitat.

Key words: Protein polymorphism, electrophoresis, genetic diversity, heterozygosity, polymorphic.

Introduction

Efficient utilization, improvement and conservation of a species or breed are practically impossible in the absence of certain relevant background information of its unique attributes. Characterization of genetic resources of farm animals encompasses all activities associated with the identification, quantitative and qualitative description, and documentation of breed populations as well as its natural habitats and production systems to which they are or are not adapted

(Gizaw et al., 2011). Delgado et al. (2001) identified characterization of a livestock breed as the first approach to sustainable use of animal genetic resources. Contributing to the importance of characterization of farm animals, *Halima (2007)* posited that genetic characterization of the domestic animals is an integral component of the Food and Agriculture Organization's (FAO) global strategy for the management of farm animal genetic resources while *Gholizadeh et al. (2008)* stated that genetic characterization of populations/breeds allows the evaluation of genetic variability, a fundamental element in planning breeding strategies and genetic conservation plans.

Phenotypic diversity, morphological characters and indices which are easily assessed, having low cost and are easily measured have been widely used by researchers to characterize, discriminate and assess inherent diversities in farm animals. Nevertheless, in order to reduce the influence of environment on systematic information, biologists have borrowed approaches from protein chemists which mainly involve analysis of deoxyribonucleic acid (DNA) or of primary product (protein) from which codes are expressed so as to provide information on biological characters, status of individuals and populations within and among taxonomic units *(Hammed et al., 2011)*. Genetic characterization based on molecular assessment has been reported as the most common method to evaluate genetic diversity between and within livestock breeds, but requires high technology and is costly *(Wimmers et al., 2000; Romanov and Weigend, 2001; Hillel et al., 2003).*

Alternatively, the study of genetically-controlled biochemical polymorphisms of blood proteins has been used by researchers to characterize livestock breeds and populations and for the evaluation of genetic diversity existing in farm animals *(Oguntunji and Ayorinde, 2015; Akinyemi et al., 2014; Ige et al., 2013; Nyamsamba et al., 2003)*. In addition, the analysis of genetic markers based on protein variants detected by electrophoretic method has been a tool for studying genetic differentiation among populations, phylogenetic studies and has become equally important in biosystematics and evolutionary studies *(Nyamsamba et al., 2003). Egena and Alao (2014)* showed that biochemical study becomes imperative because of its importance in the improvement of farm animals, and the fact that some polymorphic alleles may be connected or linked with traits of economic importance due to pleiotropic effect, or general heterozygosity; and also possibly through complex interaction of additive and non-additive genes.

Guinea fowl is one of the indigenous poultry and also an integral part of rural poultry in Nigeria. A recent report indicated that it is the second most widely domesticated poultry after chicken *(NBS, 2012)* and is found mainly in northern Nigeria, while the few found in southern region were introduced through inter-

regional trade. This *galliforme* is also abundant in the wild in their natural habitat in grassland savanna of northern Nigeria.

In spite of the abundance and popularity of guinea fowl in northern Nigeria, its sizeable contribution to animal protein production and absence of known taboos and superstitions against its rearing, consumption and marketing; this bird has not received attention it deserves by researchers most especially in regions where it contributes to internal animal production and consumption. The neglect suffered is exemplified in sparse literature on this bird; and studies on its genetic attributes are limited. Furthermore, to the best knowledge of the authors, researches geared towards characterization using blood proteins or molecular markers and estimation of genetic diversity within and between Nigerian indigenous guinea fowl varieties are practically non-existent. The dearth of information on genetic resources present in the indigenous farm animals in developing countries has led to their under-utilization, replacement and dilution through crossbreeding and has underscored the significance of the characterization and conservation of the indigenous species *(Yakubu and Ugbo, 2013).*

In view of the foregoing, it is evident that characterization and conservation of diverse genetic attributes of the indigenous species are imperative and long overdue in order to maintain genetic biodiversity of the indigenous animals, enhance food security of the teeming population and boost economic empowerment in developing countries *(Oguntunji, 2013).* The present study was therefore conducted to characterize and estimate genetic diversity in Nigerian indigenous guinea fowls based on four blood proteins.

Materials and methods

Experimental animals

Thirty (30) adult pearl variety of guinea fowl were used for this study. The sample was randomly drawn from a large random mating population of guinea fowl of north-western part of Nigeria. They were reared primarily under a traditional free range system; whereby birds scavenged for their feed and water with little or no supplementation. Only apparently healthy birds were used for this study.

Electrophoretic procedures

The blood samples (3-5ml) were collected from the birds through venipuncture of the jugular veins into heparinsed tubes. The blood samples were then refrigerated in the ice packs and transferred to the Animal Breeding and Genetics Laboratory in the Department of Animal Science, University of Ibadan, Ibadan, Oyo State,

Nigeria for electrophoretic analysis. The electrophoretic procedures used were as described by (*Akinyemi et al., 2014; Akinyemi and Salako, 2012*).

Data analyses

The allelic frequency was estimated by simple gene counting method since all the observed variants were controlled by co-dominant alleles. Agreement of the observed genotype frequency with Hardy-Weinberg equilibrium (HWE) was tested with the chi-square test (X2).

Three genetic diversity parameters namely: Heterozygosity (H), effective number of allele (ne) and percentage polymorphic (%P) were used to assess genetic variability in the sample used in this study.
The unbiased estimate of mean heterozygosity *(Nei, 1978)* was estimated as:

Heterozygosity (H) = 1-Σx2í

Where:

X = the gene frequency of the íth allele in a locus

í= the number of tested loci

The effective number of allele (ne) per locus was estimated as:

1/1-H

Where:

H = heterozygosity *(Nyamsamba et al., 2003).*

Polymorphicity (%): a locus is polymorphic if the segregating genes are more than one and frequency of the rarest gene is at least 0.01% (Sanjalj et al., 2000).

The within population inbreeding coefficient (F) was estimated as 1- Ho/He *(Jean-Clauder, 2015).*

Where:

Ho = observed heterozygosity

He = expected heterozygosity

Results and discussion

Genotype and gene frequencies of blood proteins of adult Nigerian indigenous guinea fowls

Two iso-enzymatic variants controlled by a pair of allelic autosomal genes are detected at all the loci investigated (Table 1). Besides, the genotypic frequencies of Hb, Tf and Alb significantly ($P < 0.05$) deviated from the Hardy Weinberg equilibrium.

Table 1. Gene and genotype frequency distributions of blood proteins of adult Nigerian guinea fowl.

Locus	Genotypes	Observed	Expected	X2	Gene Frequency
Hb	AA	0	3.69		A: 0.35
	AB	21	13.65	8.71*	
	BB	9	12.68		B: 0.65
CA	FF	28	25.95		F: 0.93
	FS	0	3.91	6.35	
	SS	2	0.15		S: 0.07
Tf	AA	2	8.43		A: 0.53
	AB	28	14.95	22.90*	
	BB	0	6.63		B: 0.47
Alb	AA	2	8.43		A: 0.53
	AB	28	14.95	22.90*	
	BB	0	6.63		B: 0.47

*X2 significant at $P < 0.05$

Non-agreement of genotype frequencies with HWE is an indication that the loci under investigation have not been subjected to any of the systematic (selection, migration and mutation) and dispersive forces (genetic drift and inbreeding) *(Ramamoorthi et al., 2009).*

As far as authors are aware, precedent studies on biochemical characterization and estimation of genetic diversity of guinea fowl using blood protein systems are scarce. Unavailability of such study makes it impossible to compare the results. Nevertheless, the results were compared with the available reports on other indigenous poultry.

Haemoglobin

The two genotypes observed at the Hb locus in this study is lower than the three (HbAA, HbAB and HbBB) reported for Nigerian local chickens *(Yakubu and*

Aya, 2012), Muscovy ducks *(Oguntunji and Ayorinde, 2015),* chucker *(Alectoris chucker)* and pheasant *(Phasianus colchicus) (Ugur et al., 2006)*. Contrary to the prevalence of the heterozygouse genotype at this locus in guinea fowl, higher frequency of homozygous genotype HBBB has been reported for two ecotypes of Nigerian local chickens (Ige et al., 2013) while homozygouse HBAA was reported for Muscovy ducks *(Oguntunji and Ayorinde, 2015)* and three varieties of Nigerian indigenous chicken *(Yakubu and Aya, 2012).*
The higher frequency of gene Hb-B in guinea fowl agrees with the previous reports on mallard ducks *(Akinyemi et al., 2014)*, two Nigerian ecotypes of chicken *(Ige et al., 2013)* and four Chinese native breeds of chicken *(Okamoto et al., 2003).* Conversely, related studies on different indigenous poultry in Nigeria reported higher frequency of gene Hb-A *(Oguntunji and Ayorinde, 2015; Akinyemi et al., 2014; Yakubu and Aya, 2012; Salako and Ige, 2006)*. The non-agreement in gene and genotype frequencies of Hb in the population under study with some previous reports on indigenous poultry could probably be attributed to sample size or species differences in relevance of different genes and genotypes to their adaptability to their natural environments.

Schiliform and Folaranmi (1978) reported that different Hb allele types have selective advantage in different geographical areas. Higher frequency of Hb-B has been reported for breeds of sheep reared in drier northern part of Nigeria *(Akinyemi and Salako, 2012)* and sahelian countries of West Africa *(Missohou et al., 1999)*. The higher frequency of Hb-B in arid and semi-desert breeds of sheep in Nigeria and elsewhere lends credence to the assertion of *Schiliform and Folaranmi (1978)* that Hb-B confers adaptive advantage on carriers in arid region. Recent report of higher frequency (0.70) of Hb-B in Mallard duck *(Akinyemi et al., 2014)* commonly reared in drier savanna agro-ecological zones in Nigeria corroborates further this assertion. In view of the foregoing, it is postulated that higher frequency of Hb-B in the sampled population compared with high frequency of HB-A in other indigenous poultry species which are not limited to a particular geographical area is not just coincidental, but possibly an indicator of adaptive physiological modification in guinea fowl to survive in drier savanna region where they are mostly found in Nigeria. However, this assertion is subject to confirmation in future studies since the present sample size was not sufficient to draw conclusion.

Carbonic anhydrase

Comparison of the number of genes and genotypes reported in this study with previous reports on other indigenous poultry revealed disparity. Contrary to the two genotypes (CAFF and CASS) observed in this study, three CA variants of different compositions were reported for Muscovy ducks (CAFF, CASS and

CAMM) *(Oguntunji and Ayorinde, 2015)* and Nigerian local chickens (CAFF, CASS and CAFS) *(Ige et al., 2013).* However, in agreement with the report of this study, *Oguntunji and Ayorinde (2015)* reported higher frequency of CAFF in Muscovy ducks while *Ige et al. (2013)* documented prevalence of CASS in *Nigerian local chickens.*

The two genes segregating at the CA locus have their analogues in the earlier reports on Nigerian local chickens *(Ige et al., 2013)* and ducks *(Muscovy and Mallard) (Akinyemi et al., 2014). Conversely, Oguntunji and Ayorinde (2015)* reported three alleles (CA-F, CA-S and CA-M) at the same locus for Muscovy ducks. Similarly, the frequency obtained for CA-F in the present study is comparable to 0.913 reported for Muscovy duck *(Oguntunji and Ayorinde, 2015)* but higher than 0.675 to 0.763 reported for two Nigerian ecotypes of chicken *(Ige et al., 2013)* and 0.567 to 0.675 reported for Muscovy and Mallard ducks *(Akinyemi et al., 2014).* The striking aspect at this locus was that the prevalent genotype and gene were almost fixed and there was no heterozygote individual. The extremely higher frequency of CA-F is a pointer to its relevance to the yet-to-be-known physiological advantage it confers on this bird in its natural habitat.

Transferrin

Comparisons of the gene and genotype frequencies reported in this study are different from the reports of the related studies on indigenous poultry. In contrast to the two genotypes reported in this study; *Oguntunji and Ayorinde (2015)* reported six genotypes for Nigerian Muscovy ducks. In addition, the prevalence of heterozygouse genotype TfAB was contrary to TfBB and TfAC reported for Japanese indigenous fowls *(Tanabe et al., 2000)* and Nigerian local chickens *(Ige and Salako, 2014),* respectively.

The two co-dominant alleles segregating at this locus agrees with the two reported for Muscovy and Mallard ducks in Nigeria *(Akinyemi et al., 2014)* and three indigenous breeds of ducks in Indonesia *(Johari et al., 2013)* but lower compared to three and four reported for Chinese breeds of chicken *(Okamoto et al., 2003)* and Nigerian Muscovy ducks *(Oguntunji and Ayorinde, 2015),* respectively. The higher frequency of gene Tf-A agrees with the reports of *Johari et al. (2013)* on Mojosari breed of duck and *Akinyemi et al. (2014)* on Muscovy and Mallard ducks but contrary to the higher frequency of Tf-B reported for some Asian native breeds of chicken *(Okamoto et al., 2003; Tanabe et al., 2000),* two Indonesian local ducks *(Johari et al., 2013)* and Muscovy ducks in Nigeria *(Oguntunji and Ayorinde, 2015).*

Albumin

The two genotypes typed at this locus is lower compared to the three, four and seven observed in Japanese native fowl *(Tanabe, 2000)*, indigenous breeds of chicken in China *(Okamoto et al., 2003)* and Muscovy ducks in Nigeria *(Oguntunji and Ayorinde, 2015)*, respectively. The higher frequency of AlbCC and AlbBB in Muscovy ducks *(Oguntunji and Ayorinde, 2015)* and Asian indigenous chickens *(Tanabe et al., 2000)*, respectively were contrary to the higher frequency of AlbAB in this study.

The two co-dominant genes expressed at this locus was in agreement with the reports of *Akinyemi et al. (2014)* on Muscovy ducks and three breeds of Indonesian local duck *(Johari et al., 2013)* but at variance with the three reported for Asian indigenous breeds of chicken (Okamoto et al., 2003; Tanabe et al., 2000) and Mallard ducks *(Akinyemi et al., 2014)* and four reported for Muscovy ducks *(Oguntunji and Ayorinde, 2015)*. The higher frequency of gene Alb-A is consistent with the report of *Akinyemi et al. (2014)* on mallard duck.

Estimation of genetic diversity and local inbreeding co-efficient

Estimates of genetic diversity (H, ne, P) were presented in Table 2. The local inbreeding co-efficient (F) was -0.312.

Table 2: Estimates of genetic variability in Guinea fowl

Locus	Heterozygosity (He)	Effective number of allele (ne)	Percentage polymorphism (% P)
Hb	0.46	1.85	100
CA	0.13	1.15	100
Tf	0.50	2.00	100
Alb	0.50	2.00	100
Mean	0.40	1.75	100

Heterozygosity (H)

The average H reported for the loci under investigation was moderate and was comparable to 0.419, 0.41 and 0.450 reported for indigenous helmeted guinea fowl in Ghana using microsatellite markers *(Botchway, 2013)*, three varieties of Nigerian local chickens *(Yakubu and Aya, 2012)* and Nigerian Muscovy ducks *(Oguntunji and Ayorinde, 2015)*, respectively using blood protein markers.

The average estimated H obtained for the blood proteins in the present study was within the range (0.30 – 0.80) suggested for a marker to be useful in appraising genetic variation in a population *(Takenazi and Nei, 1996)*. The moderate heterozygosity in the population under study lends credence to the widely

believed assumption that gene pools of the locally adapted animals are rich and are reserviours of rare genes *(Oguntunji and Ayorinde, 2015).*

The lowest estimated H (0.130) reported for guinea fowl at CA locus could be linked to the absence of heterozygouse genotypes at this locus. Though two alleles were segregating at CA locus as observed for other three loci, however, its genotypic distribution revealed absence of heterozygosity, hence low H.

One possible underlying factor for the moderate heterozygosity in the present study is the low number of alleles segregating at the investigated loci. Though most blood protein genotypes were heterozygotes; they were controlled by just two alleles. A higher number of alleles segregating at different loci in a population are good indicator of genetic diversity and how rich the genome of the population is. In agreement with this assertion, syntheses of studies have shown that higher value of average heterozygosity within a breed could be attributed to the large number of allele detected at the tested loci (Kalinowski, 2002; Akinyemi et al. 2014). In addition, the small sample size could be another contributing factor to the moderate heterozygosity in the studied population. Small sample size limits the number of individuals covered and the number of genotypes and genes expressed at the different investigated loci of a population.

Effective number of allele (ne)

The mean 1.742 obtained in this study was comparable to 1.821 reported for Nigerian Muscovy ducks *(Oguntunji and Ayorinde, 2015)* using blood proteins but lower compared with the average value of 2.04 and 3.80 reported for indigenous guinea fowls in Ghana *(Botchway, 2013)* and four populations of guinea fowl in West Africa *(Kayang et al., 2010),* respectively using microsatellite markers. The higher ne in the referenced populations could be attributed to the fact that microsatellite markers used in those studies revealed higher number of alleles at different loci. Since this genetic variability parameter indicates the allelic richness of a population and its value is a function of number of alleles; consequently in most cases, higher number of allele segregating at different loci will generate higher ne and vice versa.

Percentage polymorphic loci (% P)

The complete polymorphism of the studied loci is consistent with the results of some previous studies on indigenous poultry using blood protein markers *(Oguntunji and Ayorinde, 2015; Akinyemi et al. 2014; Yakubu and Aya, 2012; Salako and Ige, 2006).* Polymorphicity in the studied population indicates absence of selection with respect to the investigated loci and reinforced the widely reported

diversity in the genome of indigenous livestock species in developing countries *(Oguntunji and Ayorinde, 2015).*

Local inbreeding coefficient (F)

This is a genetic index indicating the degree or level of inbreeding in a population. The F values calculated indicated further the potential reduction in heterozygosity due to non-random mating and may serve as an indication of inbreeding within the population *(Hartl, 1998)*. The negative F value reported in this study implies absence of inbreeding among members of the population and excess of heterozygoutes.

Furthermore, the reported low F might partially be attributed to the prevailing management system from which the sample used for this study was drawn. The extensive management system adopted by the farmers allows animals to scavenge, intermingle with animals from different genetic backgrounds and also encourage exchange of genetic materials; hence low inbreeding.

Conclusion

It is evident that majority of the genotypes typed was heterozygoutes at Hb, Tf and Alb loci. Since the base population from which the sample used for this study was random mating and had not undergone mild or intense selection for any trait; the prevalence of certain genes at their respective locus is suggestive of their importance to the adaptability and survival of this galliforme in its natural harsh tropical environment.

The reports of this study are limited due to the sample size and financial constraints. Future studies involving different breeds/varieties of guinea fowl, more sample size and application of microsatellite markers are recommended for further characterization and elucidation of the innate genetic diversity of the studied population.

Biohemijska karakterizacija nigerijske autohtone vrste biserke *(numida meleagris)*

Abel Olusegun Oguntunji, Kolawole Luke Ayorinde, Taiwo Olayemi Aremu

Rezime

Polimorfizam proteina u krvi se intenzivno koristi za karakterizaciju i procenu genetičke raznovrsnosti domaćih životinja. Ispitivanje biohemijske karakterizacije i procena genetske raznolikosti nigerijske autohtone biserke (Numida meleagris) je sprovedena pomoću četiri krvna proteina {hemoglobina (Hb), ugljene anhidraze (CA), transferina (Tf) i albumina (Alb)}. Celuloza acetat elektroforeza ukazuje da su svi proteinski markeri polimorfni, izražavajući dva ko-dominantna gena i dva genotipa na svojim respektivnim lokusima. Heterozigotni genotipovi su bili dominantni na HB, Tf i Alb lokusima, dok su homozigoni bili češći na CA. Alelna struktura je bila sličan (A i B) za HB, Tf i Alb, dok su F i S tipizirani na CA lokusu. Gen A ima veću frekvenciju pojavljivanja na Tf i Alb lokusima, dok su geni F i B bio pretežno na CA i Hb lokusima, respektivno. Prosečna ocenjena genetska raznolikost (heterozigotnost) preko genetskih sistema bila je 0,40 i umerena. Prevalencija gena F, A i B na svojim respektivnim lokusima ukazuje na njihovu relevantnost za opstanak i prilagodljivost ispitivane populacije u svom prirodnom staništu.

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Received 20 July 2016; accepted for publication 12 September 2016

Biotechnology in Animal Husbandry 32 (3), p 285-296 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637.04'637.4
DOI: 10.2298/BAH1603285M

RELATIONSHIP OF TEMPERATURE AND LENGTH OF STORAGE ON pH OF INTERNAL CONTENTS OF CHICKEN TABLE EGG IN HUMID TROPICS

Ayoola Mathew O.,* Alabi Olufemi M., Aderemi Foluke, Oguntunji Abel

Department of Animal sciences and Fisheries management,
Bowen University, P.M.B 284, Iwo, Osun state, Nigeria.
Corresponding author: Ayoola Mathew O, ayoolamatt@gmail.com
Original scientific paper

Abstract: All foods have limited shelf life which vary depending on the food and storage conditions. Table eggs are perishable food and storage temperature is an important factor that affects the shelf life. In tropical countries like Nigeria, eggs are usually preserved under ambient condition due to erratic power supply, which reduces the efficiency of refrigeration system. The aim of the present study was to examine the effects of storage periods, temperature and their relationship on the pH of chicken egg internal properties (yolk, albumen and whole egg). Fresh chicken table eggs were randomly allotted to three treatments of storage temperatures; refrigerator ($4^0C \pm 2$), laboratory ($32^0c \pm 4$), and poultry store room ($37^0C \pm 4$). Eggs were assigned to treatments in a completely randomized design, and each treatment was replicated thrice. The pH was measured daily for each storage temperature in all treatments. Storage temperature and periods had significant ($P<0.05$) effect on pH of measured parameters. The pH values increased with storage temperature and period of storage. The rate of pH increase was significantly ($P<0.05$) higher in ambient as compared to refrigerator temperature. In this study, only the refrigerator storage has pH values within the range for fresh table eggs. At storage period above three weeks, pH values increased beyond the range for fresh egg. It is validated that storage temperature and period affected egg shelf life, the rate of freshness reduced with increased temperature, thus, storage beyond three weeks of ambient temperature is not advisable in humid tropics.

Keywords: Humid tropics, chicken eggs, yolk, albumen, pH and temperature

Introduction

The eggs are a common food and one of the most versatile ingredients used in cooking, and are important in many branches of the modern food industry. Eggs

are laid by females of many different species, including birds, reptiles, amphibians and fish but the most often consumed by humans is the chicken egg. Chicken eggs provide a good source of nutrients for man of all ages. Chicken egg, whole and hard-boiled, contains 12.6 g/100 g protein, 10.6 g/100 g fat, 1.12 g / 100 g carbohydrate, and 647KJ (155Kcal) /100 g energy (*Eke et al., 2013)*. Egg yolks and whole eggs store significant amounts of protein and choline, and are widely used in cookery. Due to its high protein content, the United States Department of Agriculture categorized eggs as Meats within the Food Guide Pyramid. Despite the nutritional value of eggs, there are some potential health issues arising from egg quality, storage, and individual allergies (*Eke et al., 2013*).

The most important external and internal egg quality traits have been shown to be egg weight, egg shape, shell thickness, breaking strength, specific gravity, air cell, pH, albumen height, and weight, and yolk index (*Samli et al., 2005)*. Egg quality can be affected by the environmental conditions such as temperature and humidity of storage time, gaseous environment and storage time. Storage can modify some characteristics of the egg including loss of water, carbon dioxide and a subsequent increase in the pH (*Decuypere et al., 2001*).

Researchers have reported the application of coatings on eggs (*Rhim 2004; Pamarin et al., 2009*). These results can be justified since such coatings help to maintain the functional properties of food by decreasing moisture loss and gas transport (oxygen and carbon dioxide), hence the application of coating on eggs reduces weight loss and maintains internal measurement such as albumen and yolk (*Nadia et al., 2012*). Though oiling of eggs is very effective in slowing down reduction in albumen and yolk quality, it does not replace the need for cool storage *(Faris et al., 2011*). The internal quality of eggs starts to decline as soon as eggs are laid by hens (*Rovana and Usturoi, 2012*). The major difference between freshly laid eggs and stored eggs are linked with internal egg qualities (*Nadia et al., 2012*). Albumen quality, a standard measure of egg quality, is influenced by genetic and environmental factors such as temperature, time and humidity of storage (*Rovana and Usturol, 2012*).

The increased in rate of egg storage, weakens the vitelline layer (*Jin, et al., 2011*). Changes in albumen pH is an important indicator associated with altered of vitelline layer weight and reduction in protein and hexosamine content of egg (*Akyurek and Agma Okur, 2009*). During egg storage, the yolk becomes more susceptible to breaking (*Nadia et al., 2012*). The yolk absorbs water from albumen and increases in size thereby weakening the vitelline membrane. The flattening of the yolk is primarily due to increase in water content caused by osmotic migration from the albumen through the vitelline membrane. The decrease in vitelline layer strength observed during storage has been associated with loss of moisture from

egg through evaporation, influenced by temperature and storage environment. (From, 1967 in *Akyurek and Agma Okur, 2009*)

Studies have shown that storage of eggs in refrigerator at (4^0 C ± 2) will retain its nutritional value and wholesomeness for about five weeks (*Faris et al., 2011, Nadia et al., 2012*). However, in tropical countries like Nigeria, egg preservation is a serious problem. The common practice is to store under ambient condition due to inadequate refrigeration facilities, resulting from erratic power supply. To the best of our knowledge, little is known about relationship of storage temperature and storage length on pH of table eggs in humid tropics. Therefore, the aim of the present study was to examine effects and relationship of storage period and temperature on pH of some internal qualities of egg.

Materials and Methods

Experimental procedure

Five hundred and four (504) of freshly laid eggs were obtained from Isa brown hens at the poultry unit, teaching and research farm, Bowen University, Iwo, Nigeria. All the eggs were weighed using digital weighing scale (model: AX 1000) and the weight of each egg was recorded. The eggs were separated into 24 groups of 21 eggs each; poultry store room (37^0c ± 4), refrigerator (4^0c ± 2), and laboratory (32^0c ± 4), were evaluated over 8 weeks. Three eggs were selected randomly from each group daily to measure egg internal content parameters.

Chemical analysis

The yolk was separated from the albumen and both were distributed into three replicates of glass beakers. The pH of the albumen and the yolk were measured with a pH meter (Electronic Instrument Ltd). About 2.0 g of the sample was homogenised in 20.0 ml of de-ionised water in a beaker. The pH meter was first standardised using buffer solution of pH 4.01 and 9.20. The electrode was then rinsed with de-ionised water and dipped into the homogenate allowing sufficient time for stabilisation before taking reading. The yolk and albumen were then mixed thoroughly for each egg sample, and the pH reading was recorded as whole egg.

Statistical analysis

A two way analysis of variance (ANOVA) was performed using the fixed effect model. Bonferroni was used to test for the significance ($P<0.05$) of variance for all recorded and calculated data between different treatments, main effect of factors (storage temperature and storage length are considered) using model:

$$Y_{ijk} = \mu + T_i + S_j + e_{ijk}$$

Where Y_{ij} = Individual observation

μ = General mean

Ti = Fixed effect of storage temperature (i = 1…..3)

S_j = Fixed effect of storage length (j = 1……8)

e_{ijk} = Expected error

Coefficient of determination (R^2) and regression analysis model was used to investigate relationship existing between effect of temperature and storage length on egg (yolk, albumen and whole egg) pH.

The regression model used was of the form:

$$Y = a + b_1X_1 + b_2X_2 + e$$

Where,
Y = Dependent variable (pH)
a = Constant/intercept
b_1 = Regression coefficient of storage temperature
b_2 = Regression coefficient of storage length
X_1 = Storage temperature
X_2 = Storage length
e = Error term

All statistical analyses were carried out with SPSS (2001) version 16.

Results and Discussion

Effect of storage temperature

The results of the effects of storage temperatures on pH of yolk, albumen and whole egg are presented in Table 1. The study conducted over eight weeks period showed that storage temperature had significant ($P<0.05$) effect on the pH of yolk, albumen and whole egg. The pH values of fresh table egg internal properties measured before storage were yolk (6.05), albumen (7.10) and whole egg (7.21). The pH for yolk, albumen and whole egg had higher values with increase storage temperature over the study period. The yolk pH had the least

values significantly (P<0.05), while the albumen pH had the highest values as the storage temperature increased.

Table 1. Effect of temperature on pH of internal egg contents

	Treatments			
pH	Refrigerator (4^0c ± 2)	Laboratory (32^0c ± 4)	Poultry (37^0c ± 4)	SEM
Yolk	6.37^{c}	6.90^{b}	7.06^{a}	0.05
Albumen	7.72^{b}	9.01^{a}	9.09^{a}	0.11
Whole egg	7.57^{b}	8.65^{a}	8.80^{a}	0.15

^{abc} Means along the same column with different superscripts are significantly (P < 0.05) different using Bonferroni as post hoc analysis

Effect of storage length

As reported in Table 2, storage periods had significant (P<0.05) effect on pH of measured internal properties. The pH values increased significantly (P<0.05) with storage period in all measured internal egg properties. A rapid increase in albumen pH towards alkalinity scale was observed as compared to yolk after the 1^{st} week, and a slower rate of increase throughout the remainder of the storage period. The pH of whole egg for 1^{st} – 4^{th} week only differed significantly (P<0.05) as compared to 5^{th} – 8^{th} week. The egg stored under ambient conditions (32^0c and 37^0c) had higher pH values when compared to those that were refrigerated.

Table 2. Effect of storage period on pH of internal egg contents

	Period (Weeks)								
pH	1	2	3	4	5	6	7	8	SEM
Yolk	6.42^{e}	6.53^{de}	6.68^{cd}	6.73^{c}	6.84^{bc}	6.92^{b}	7.00^{ab}	7.11^{a}	0.04
Albumen	7.33^{b}	8.60^{a}	8.67^{a}	8.72^{a}	8.80^{a}	8.86^{a}	8.90^{a}	8.97^{a}	0.11
Whole egg	7.50^{b}	7.59^{b}	7.64^{b}	7.78^{b}	8.70^{a}	8.80^{a}	8.93^{a}	9.13^{a}	0.12

^{abc} Means along the same column with different superscripts are significantly (P < 0.05) different using Bonferroni as post hoc analysis

Regression analysis

Regression analysis of storage temperature and storage length on pH of internal properties of chicken table egg revealed significant (P < 0.05) relationship. The generated regression equation using standardize coefficients of the two factors on measured internal properties and their R^2 are:

Yolk, $Y = 5.855 + 0.793X_1 + 0.590X_2 + e$, $R^2 = 0.976$ ---------- (Equation 1)

Albumen, $Y = 6.782 + 0.750X_1 + 0.503X_2 + e$, $R^2 = 0.816$ ---------- (Equation 2)

Whole egg, $Y = 6.01 + 0.661X_1 + 0.621X_2 + e$, $R^2 = 0.822$ ---------- (Equation 3)

These results showed high positive correlation between storage temperature and length based on the high values for R^2.

The increase in yolk pH was not as high as the increase in albumen pH. The present results are in disagreement with those of previous researchers (*Samli et al., 2005; Akyurel and Okur, 2009*) who reported that increase in yolk pH was significantly affected by storage time, but not by temperature. However, these findings are in agreement with the results reported by Silversides and Villeneuve (1994), *Scot and Silversides (2000),* and *Lapao et al. (2009).* The rise in yolk pH of the eggs may be attributed to loss of carbon dioxide and moisture from the egg through the pores in the shell. This could be attributed to dilution of the egg yolk. As the storage temperature increases, the internal temperature of an egg increases, and leads to increase in yolk pH (*Jones 2006*). This effect of high storage temperature breakdown vitelline membrane and protein structure faster. As the membrane degenerates during increase storage period, water enters the yolk in the form of moisture and shell pores open for microorganism (*Ahn et al., 1999)*

The results for albumen were in agreement with findings reported by *Silversides and Villeneuve (1994).* Freshly laid eggs contain 1.44 to 2.05 mg CO_2/g of albumen (*Keener et al., 2001; Biladeau and Keener, 2009*) and have an albumen pH value of 7.6 to 8.7 (*Rhim et al., 2004;Waimaleongora-Ek et al., 2009; Ryu, et al., 2011).* In this study, the initial albumen pH was 7.10. During storage, only refrigerated storage had albumen pH range within the freshness category after eight weeks of storage, while loss in freshness started after 4weeks storage period. These results are consistent with the report of *Scott, and Silversides (2000),* and *Samli et al. (2005),* who reported significant increases in pH of albumen with increased storage time and temperature. In contrast, *Walsh et al. (1995)* reported that neither temperature nor storage time influenced albumen pH. The decrease in albumen pH may be due to the continuing breakdown of the constituents of the egg white and or a change in the bicarbonate buffer system due to loss of carbondioxide and moisture during storage at temperature above 4^0C (*Biladeau and Keener, 2009, Ryu et al., 2011).*

Related researches on relationship of storage temperature and length with egg pH are scarce to validate result obtained in this present study. As shown in equations (1….3), comparative evaluation of contribution of these two factors (temperature and period) to the deterioration of egg freshness through their

standardized coefficient indicated that storage temperature contributed more than storage period in loss of egg freshness using pH measurement as indicator in yolk, albumen and whole egg. The combined effect of these two factors (temperature and length of storage), had 97.6 %, 81.6%, and 82.2%, while Other factors that were not considered in this study contributed 2.4%, 18.4%, and 17.8% influence on deterioration of egg freshness in yolk, albumen and whole egg respectively based on R^2 values. The generated regression equation revealed positive relationship between storage temperature and period on egg pH (yolk, albumen and whole egg). The positive relationship implies that both storage temperature and period on pH of egg quality parameter go in the same direction, i.e. their increase induced increase in egg pH, hence, deterioration of egg freshness.

In fig (1…3), pH of yolk, albumen and whole egg increased with the storage length at all storage temperature in a nonlinear manner, which depicts that deterioration of internal egg parameters measured is a function of both storage temperature and length. These results are in agreement with results reported by *Samli et al. (2005), Akyurel and Okur (2009), Jin et al. (2011))* who found a similar relationship with temperature and storage length.

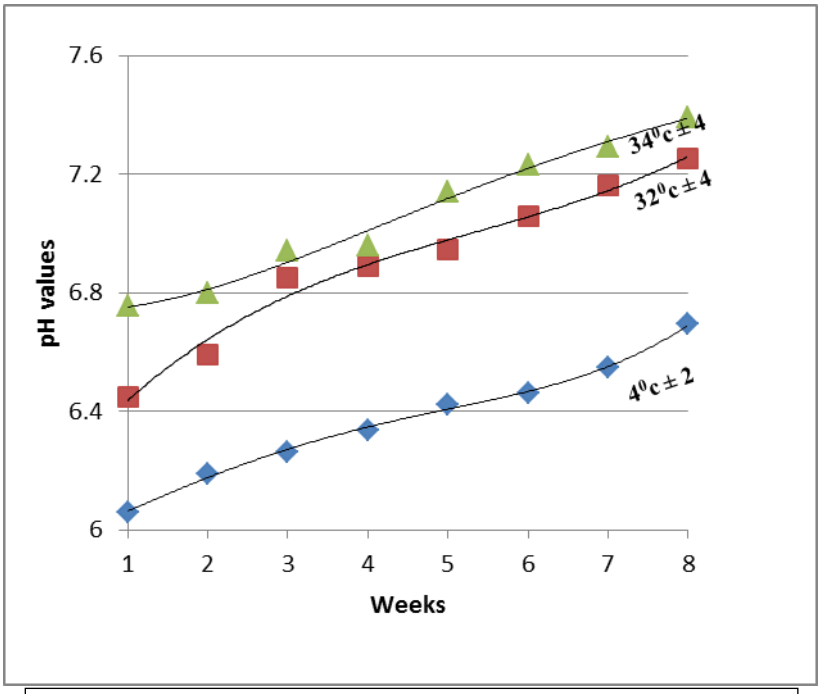

Figure 1. Relationship of storage temperature with length on Yolk pH

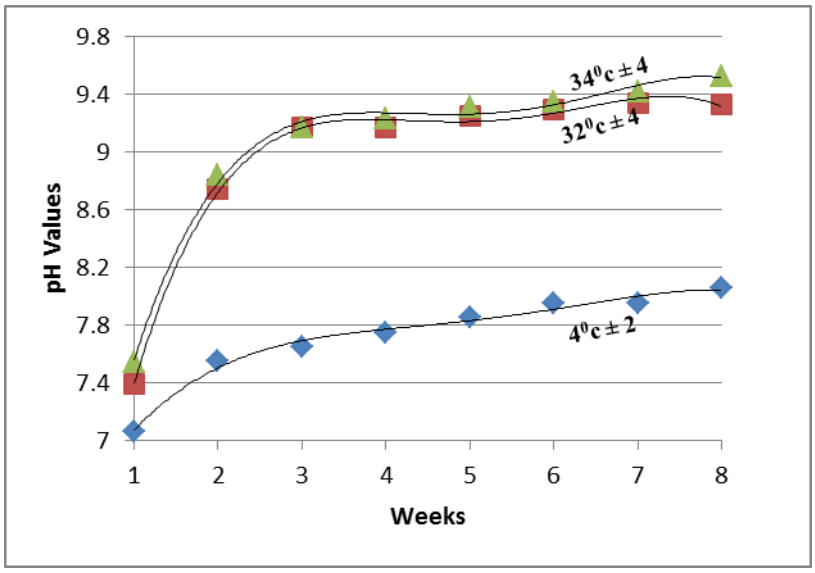

Figure 2. Relationship of storage temperature with length on albumen pH

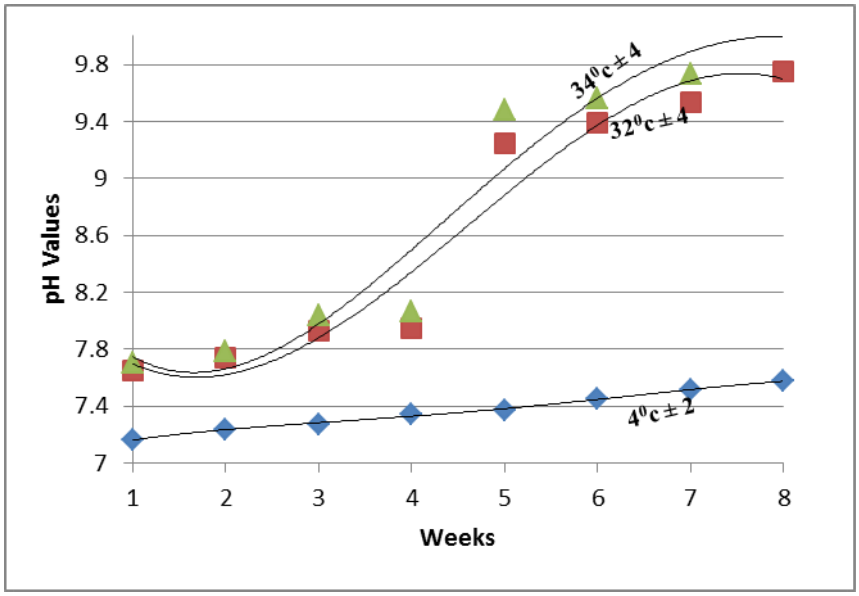

Figure 3. Relationship of storage temperature with length on whole egg pH

Staldelman and Cotteril (2007) stated that pH of fresh eggs should be 7.5 – 7.6, only storage temperature ($4^0c \pm 2$) with pH (7.57) is within this range at the end of the study. However, pH of whole egg increased beyond this range after three weeks. This result suggested that in the humid tropics, freshness of egg cannot be preserved for more than three weeks at ambient temperatures 32^oC and 37^0C.

Conclusion

It could be concluded that, both storage temperature and period play a significant role in maintaining egg freshness. However, storage temperature had more influence than storage period. Also, this study validated that refrigerator storage temperature is superior to ambient storage temperature for maintenance of egg freshness for table egg storage in humid tropics. Poultry farmers and egg users are advised to strictly consider storage temperature, in order to prevent economic and nutritional loss. Hence, it is recommended that chicken table egg should not be stored more than three weeks at ambient temperature to maintain egg freshness in humid tropics.

Odnos temperature i dužine skladištenja na pH unutrašnjeg sadržaja konzumnih jaja u vlažnim tropskim uslovima

Ayoola Mathew O., Alabi Olufemi M., Aderemi Foluke, Oguntunji Abel

Rezime

Sve namirnice imaju ograničen vek trajanja koji varira u zavisnosti od uslova skladištenja. Konzumna jaja su kvarljiva i temperatura skladištenja je važan faktor koji utiče na rok trajanja. U tropskim zemljama, kao što je Nigerija, jaja se obično skladište u uslovima sobne temperature zbog nesigurnog napajanja strujom, čime se smanjuje efikasnost sistema hlađenja. Cilj ove studije je bio da se ispita uticaj perioda skladištenja, temperature i njihovog odnosa na pH jaja (žumanceta, belanceta i celih jaja). Sveža kokošija jaja su nasumično podeljena u tri tretmana temperature skladištenja; hladnjak (4^0C ± 2), laboratorija (32^0c ± 4), i skladište za proizvode (37^0C ± 4). Jaja su dodeljivana u tretmane po potpuno slučajnom principu i svaki tretman je ponavljan tri puta. pH je merena svakodnevno za svaku od temperatura skladištenja u svim tretmanima. Temperatura i periodi skladištenja su imali značajan ($p<0,05$) uticaj na pH merenih parametara. Vrednosti pH povećavaju se sa temperaturom skladištenja i periodom skladištenja. Stopa rasta pH je bila znatno ($p<0,05$) veća na sobnoj temperaturi u odnosu na temperaturu frižidera. U ovoj studiji, samo jaja u tretmanu sa skladištenjem u frižideru imaju pH vrednosti unutar opsega za sveža konzumna jaja. U periodu skladištenja iznad tri nedelje, pH vrednosti su povećane izvan opsega za sveža jaja. Ispitivanje potvrdjuje da temperatura skladištenja i period skladištenja utiču na rok trajanja jaja, stopa svežine je smanjena sa povećanom temperaturom, samim tim, skladištenje duže od tri nedelje na sobnoj temperaturi nije preporučljivo u vlažnim tropskim krajevima.

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Received 18 July 2016; accepted for publication 27 August 2016

Biotechnology in Animal Husbandry 32 (3), p 297-309 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 633.15'631.82/86
DOI: 10.2298/BAH1603297S

CHEMICAL COMPOSITION AND YIELD OF MAIZE GREEN BIOMASS AS AFFECTED BY BACTERIAL AND MINERAL FERTILIZATION

Aleksandra Stanojković-Sebić[1], Dragutin A. Đukić[2], Leka Mandić[2], Violeta Mandić[3], Aleksandar Stanojković[3], Radmila Pivić[1]

[1]Institute of Soil Science, 11000 Belgrade, Serbia
[2]University of Kragujevac, Faculty of Agronomy, 32000 Čačak, Serbia
[3]Institute for Animal Husbandry, Belgrade – Zemun, 11080 Zemun, Serbia
Corresponding author: Aleksandra Stanojković-Sebić, astanojkovic@yahoo.com
Original scientific paper

Abstract: The purpose of the study was to evaluate the influence of an application of different rates of composite mineral fertilizers and their combination with bacterial inoculants (N-fixing *Klebsiella planticola* and *Enterobacter* spp.) on chemical composition and yield of the maize green biomass (without spikes) on acid Eutric Cambisol during the two growing seasons: 2006 and 2008. Unfertilized soil was used as a control. The contents of nitrogen, phosphorus, potassium and crude proteins in biomass samples were determined three times during the maize vegetation season, as follows: stage of intensive growth, milk-waxy maturity stage and full maturity stage. Measuring of the green biomass yield was carried out at the end of the vegetation. The results of the study showed that the use of high rates of composite mineral fertilizers and their combination with bacterial inoculants resulted in increased contents of nitrogen, phosphorus, potassium and crude proteins in the maize biomass during the both study years, which was noticeably observed in the stage of intensive growth. The highest increase in the biomass yield was obtained by the same mentioned treatments, although the combination of bacterial inoculants and lower rates of mineral fertilizers resulted in higher yields comparing to the application of lower rates of the pure mineral nutrients. The data suggest that the studied bacterial inoculants can be used in further investigations as the potential agents of new biofertilizers for improved maize production and other agriculture crops in animal nutrition.

Key words: maize green biomass, yield, chemical composition, composite mineral fertilizers, bacterial inoculants, Eutric Cambisol

Introduction

Along with wheat, maize (*Zea mays* L.) represents a major crop in agricultural production in Serbia, where its cultivation occupies an area of about 1.300.000 ha with an average yield of 5.00 to 6.00 t ha^{-1} *(Jocković et al., 2005)*. The great importance of maize stems primarily from the diversity of its use, yield potential, opportunities in achieving high yields of grain and silage and in conditions without irrigation, but also from the fact that it is the basic ingredient in livestock feed. According to the quantity of organic matter produced per hectare, together with sugar beet the maize occupies first place in agriculture production, surpassing all other cultivated plant species *(Latković, 2010)*. In livestock feed the whole maize plant or its parts can be used in ripe or green state. Grain is an important concentrated livestock feed, especially for fattening. The whole plant is used for making silage as a high quality food and provides more fodder units than any other plant *(Milosavljević et al., 2010)*.

Increasing the yield and improving the quality of maize crops have been the challenges for sustainable agriculture *(Yu-Kui et al., 2009; Abumhadi and Atanassov, 2010)*. The yield of maize, cultivated for different purposes, in addition to varietal characteristics, largely depends on the climate characteristics (rainfall and temperature regimes particularly during the summer seasons), tillage, chemical, physical and microbiological properties of the soil *(Jeličić et al., 1997; Protić et al., 2004; Mandić et al., 2016)*. Fertilization, among other factors, was one of the reasons that pushed crop production *(Salvagiotti et al., 2010)*, whereas the traits of the cumulative effect of fertilizers (the change of biological and chemical soil properties, the content of biogenic elements and heavy metals etc.) have often been disregarded. The plant production systems, type and rate of applied fertilizers and climate characteristics affect greatly on intensity of the N, P and K uptake by agricultural crops and their yield. Regardless of their major role in crop productivity and soil fertility, increased use of mineral fertilizers (particularly nitrogen) in agricultural production has however raised concerns, because the nitrogen surplus is at risk of leaving the plant-soil system and thereby causing environmental contamination *(Acosta-Martinez and Tabatabai, 2000; Alizadeh and Ghadeai, 2006)*. Consequently, sustainable agriculture in Serbia should not be only a steady and substantial increase in crop yields, but also the management and conservation of soil and water. The problems concerned can be overcome by partial replacement of these fertilizers by application of microbial inoculants, in order to inhibit or stimulate certain cellular processes, including mineralization ones, thus leading to the improvement of physical, chemical and biological soil properties *(Milošević et al., 2003; Pešaković et al., 2008)*.

Regarding the preceding comments, the main purpose of this research was to evaluate the influence of different rates of composite mineral NPK fertilizers

(15:15:15) and their combination with selected soil bacterial inoculants on chemical composition and yield of the maize green biomass cultivated on eutric cambisol type of soil.

Material and Methods

Study area

The investigation was conducted on Mladenovac experimental station of Institute of Soil Science, located 55 km south-east from Belgrade in Serbia, during 2006 and 2008. Mean monthly air temperatures and precipitation sums for the investigated period are presented in Figure 1. Year 2008 was warmer than 2006, due to a 2-3 °C higher temperature in May, June and August. This year was also lower in precipitation sum comparing to 2006. According to data in climate diagram, distribution of rainfall in 2006 was favorable for maize growing than in 2008 because of the drought periods preceding the rainy season, and during the summer months of June and July there were more than 100 mm of the rainfall.

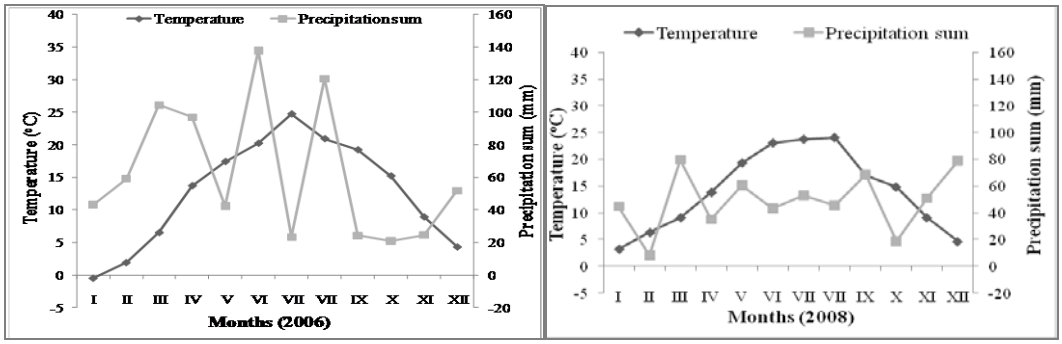

Figure 1. Climate diagram according to Walter for 2006 and 2008 for the study locality

Field trial

The soil type studied in present research was Eutric Cambisol *(WRB, 2014)*. The experiment was set up in a randomized block design on 9 × 6 m^2 plot size, with three replications, based on the following variants: control (*Ø*, non-fertilized soil); 60 kg ha^{-1} N and P_2O_5, and 40 kg K_2O ha^{-1} (N1); 120 kg ha^{-1} N, P_2O_5 and K_2O (N2); *Enterobacter* sp. strains + 60 kg ha^{-1} N and P_2O_5, and 40 kg K_2O ha^{-1}

(ES+N1); *Enterobacter* sp. strains + 120 kg ha^{-1} N, P_2O_5 and K_2O (ES+N2); *Klebsiella planticola* + 60 kg ha^{-1} N and P_2O_5, and 40 kg K_2O ha^{-1} (KP+N1); *Klebsiella planticola* + 120 kg ha^{-1} N, P_2O_5 and K_2O (KP+N2). Maize (hybrid ZP-341, FAO 300) in 2006 and 2008, was used as a test plant in the trial.

Mineral fertilization and soil bacterial inoculation

Composite NPK mineral fertilizer in relation 15:15:15 was applied in the trial. Nitrogen (N) fertilizer was applied in the form of urea with 46% N, phosphorus (P) – in the form of monoammonium phosphate (MAP) with 52% P_2O_5 and 11% N, and potassium (K) – as a 40% potassium salt (KCl). The established amounts of mineral fertilizer have been applied in the spring of 2006 and 2008, before sowing the maize.

The pure culture of an associative N-fixing bacterium *Klebsiella planticola* (strain TSHA-91) was obtained from the stock culture of the Microbiology Laboratory of Faculty of Agronomy (Čačak, Serbia) and cultivated on the slanting nutrient medium for 24 h at 28°C ± 1. Chemical composition of the medium was as follows: peptone 1 - 1.20 g; K_2HPO_4 - 0.50 g; KH_2PO_4 - 0.30 g; $MgSO_4$ - 0.10 g; $CaCl_2$ - 0.03 g; sucrose - 6.00 g; $(NH_4)_2SO_4$ - 0.14 g; yeast extract - 0.10 g; agar - 16.00 g; distilled deionized water – 1.00 dm^3; pH 7.3. The pure culture of associative N-fixing *Enterobacter* strains KG-75 and KG-76 were obtained from the stock culture of the Microbiology Laboratory of the Center for Small Grains (Kragujevac, Serbia), where they have been isolated from the rhizosphere of wheat. These strains were cultivated for 48 h at 28°C ± 1 on the slanting nutrient medium (MPA, Torlak, Belgrade) with the following chemical composition: peptone 1 – 15.00 g; meat extract - 3.00 g; NaCl - 5.00 g; K_2HPO_4 - 0.30 g; agar - 18.00 g; distilled deionized water – 1.00 dm^3; pH 7.3.

The pure liquid inoculum of *K. planticola* (100-300 x 10^7 cells per 1.0 cm^3 of inoculum) in amount of 18.00 dm^3, as well as the pure liquid inoculum of *Enterobacter* strains (100-180 x 10^7 cells per 1.0 cm^3 of inoculum) in the same amount, were made using fermentors with suitable nutrient broth and incubated with aeration for 48 h at 28°C ± 1.

The bacterial inoculation of the soil was carried out using plastic haversack sprinkler with 300.00 $cm^3 m^{-2}$ of diluted liquid bacterial inoculum, previously made by adding 32.00 dm^3 of the tap water in 18.00 dm^3 of the pure bacterial liquid inoculum. The inoculation was performed when the maize was in the stage of 2-3 formed leaves.

The method of mineral fertilization and soil bacterial inoculation used in this study was previously described *(Stanojković et al., 2012)*.

Soil preparation and analysis

The samples of soil were air-dried, crushed and passed through a sieve with a diameter of ≤ 2 mm. The preliminary analysis of the study soil included the following chemical parameters: soil acidity (pH in H_2O and 1M KCl, v/v - soil:H_2O=1:5, soil:1M KCl=1:5) was analyzed potentiometrically, using glass electrode *(SRPS ISO 10390, 2007)*; total nitrogen (N) was analyzed on elemental CNS analyzer Vario EL III *(Nelson and Sommers, 1996)*; available phosphorus (P_2O_5) and potassium (K_2O) were analyzed by Al-method according to Egner-Riehm *(Riehm, 1958)*, where K_2O was determined by flame emission photometry and P_2O_5 by spectrophotometer after color development with ammonium molybdate and stannous chloride; humus content was determined using Tiurin's method, modified by Simakov *(Ostrowska et al., 1991)*.

Plant analysis

The maize biomass without spikes was taken in three stages of the plant: intensive growth (vegetation stage I), milk-waxy maturity stage (vegetation stage II) and full maturity stage (vegetation stage III). The samples of the plant material was then weighed before and after drying at 105°C. For all the plant samples from all the variants studied the chemical analyses of the maize biomass were done. The contents of phosphorus (P) and potassium (K) were determined by so called "wet" combustion, i.e. they were heated to boiling with the mixture of concentrated sulfuric (H_2SO_4) and perchloric ($HClO_4$) acids. In the obtained solution, P was determined by spectrophotometer with molybdate, and K – by flame emission photometry *(Jakovljević et al., 1985)*. The content of nitrogen (N) was analyzed using elemental CNS analyzer, Vario model EL III *(Nelson and Sommers, 1996)*, while the content of crude proteins was calculated on the basis of N content according to *Licitra et al. (1996)*, using the following formula: crude proteins (%) = N (%) x 6.25 (factor for conversion of nitrogen content to crude protein).

Maize harvest was performed manually from each plot in the full maturity stage, when the dry matter was 20-25% during the first decade of October in 2006 and 2008. Plants from each plot were cut on height 20 cm at harvest time and biomass yield was measured. The yield was converted into t ha^{-1}.

Data analysis

The obtained data on soil properties were presented as arithmetic means of three replicates, standard deviation values and intervals. The effects of different fertilization variants on all the variables tested were evaluated using Analysis of

Variance (SPSS 20.0, Chicago, USA), followed by Duncan's Multiple Range Test (DMRT). Significant differences between means were tested by the LSD test at P = 0.05 and P = 0.01.

Results and Discussions

Chemical properties of the study soil

The main chemical characteristics of the study soil are presented in Table 1. According to the reference values *(Šestić et al., 1969)*, the soil is characterized by acid reaction, high available potassium and medium available phosphorus, humus and total nitrogen supply.

Table 1. Main chemical characteristics of the studied Eutric Cambisol

Chemical parameter	Value (means ± standard deviation)	Intervals
pH in H_2O	4.90±0.03	4.87-4.92
pH in 1M KCl	4.06±0.05	4.00-4.10
Total N (%)	0.136±0.005	0.132-0.141
Humus (%)	2.19±0.01	2.18-2.19
Available P_2O_5 (mg $100g^{-1}$)	15.73±0.31	15.51-16.09
Available K_2O (mg $100g^{-1}$)	25.30±0.30	25.08-25.65

Effect of applied fertilizers on the chemical composition of green biomass

By analyzing the dynamics of accumulation of nitrogen, phosphorus, potassium and proteins in maize biomass during 2006 and 2008 (Tables 2 and 3) it was determined that the biomass chemical composition depended on the fertilization variant used, as well as the vegetation period of maize studied.

Table 2. Effect of the fertilization variants on chemical composition of the maize biomass during 2006

Variant	Vegetation stage	Total N (%)	Crude proteins (%)	P_2O_5 (%)	K_2O (%)
Ø	I	2.799±0.001g	17.491±0.002g	1.142±0.002f	2.413±0.002g
	II	0.295±0.001g	1.840±0.001g	0.888±0.002e	1.344±0.002g
	III	0.153±0.003f	0.958±0.005g	0.765±0.002f	1.205±0.005g
N1	I	3.903±0.002f	24.386±0.002f	1.262±0.002e	2.533±0.003f
	II	0.554±0.002f	3.457±0.002f	1.005±0.003d	1.943±0.002f
	III	0.378±0.001d	2.370±0.001e	0.882±0.003e	1.712±0.011f
N2	I	4.964±0.002c	31.022±0.005c	1.453±0.003c	3.040±0.008c
	II	0.770±0.001c	4.807±0.002c	1.188±0.029b	2.716±0.006c
	III	0.711±0.002c	4.456±0.005c	1.071±0.002c	2.553±0.003c
KP+N1	I	4.422±0.002d	27.622±0.003d	1.326±0.002d	2.917±0.001d
	II	0.671±0.002d	4.202±0.001d	1.071±0.002c	2.150±0.003e
	III	0.378±0.003d	2.383±0.003d	0.948±0.002d	1.942±0.002d
KP+N2	I	5.439±0.002b	33.993±0.004b	1.689±0.008a	3.283±0.003a
	II	0.913±0.002b	5.713±0.002b	1.437±0.002a	2.860±0.002a
	III	0.778±0.002b	4.589±0.003b	1.241±0.002b	2.609±0.008a
ES+N1	I	4.111±0.001e	25.699±0.002e	1.264±0.002e	2.854±0.001e
	II	0.592±0.002e	3.713±0.003e	1.006±0.007d	2.155±0.002d
	III	0.333±0.003e	2.090±0.001f	0.881±0.002e	1.886±0.005e
ES+N2	I	5.563±0.001a	34.776±0.002a	1.673±0.001b	3.265±0.002b
	II	1.3033±0.002a	8.138±0.002a	1.422±0.003a	2.847±0.002b
	III	0.856±0.002a	5.357±0.002a	1.291±0.002a	2.591±0.002b
P value	I	***	***	***	***
LSD (0.05)		0.0026	0.005	0.006	0.006
LSD (0.01)		0.0037	0.007	0.008	0.009
P value	II	***	***	***	***
LSD (0.05)		0.003	0.003	0.019	0.005
LSD (0.01)		0.004	0.004	0.027	0.006
P value	III	***	***	***	***
LSD (0.05)		0.005	0.006	0.003	0.010
LSD (0.01)		0.007	0.008	0.004	0.014

LSD indicates least significant differences at P = 0.05 and P = 0.01; *** indicates statistical significant differences at the P<0.05, P<0.01 and P<0.001 levels, respectively; DMRT was used to compare different variants at P≤ 0.05, where values followed by the same letter in a column are not significantly different.

Table 3. Effect of the fertilization variants on chemical composition of the maize biomass during 2008

Variant	Vegetation stage	Total N (%)	Crude proteins (%)	P_2O_5 (%)	K_2O (%)
Ø	I	2.828±0.002g	17.676±0.021g	1.165±0.004f	2.433±0.006f
	II	0.505±0.009g	3.211±0.010g	0.913±0.003e	1.366±0.005f
	III	0.429±0.025f	2.755±0.001g	0.782±0.004f	1.228±0.002g
N1	I	4.366±0.005f	27.295±0.005f	1.285±0.001e	2.555±0.005e
	II	0.613±0.015f	3.736±0.032f	1.032±0.001d	1.964±0.004e
	III	0.495±0.003e	3.072±0.002f	0.902±0.002e	1.741±0.003f
N2	I	6.173±0.006c	38.555±0.051c	1.477±0.012c	3.065±0.003b
	II	1.131±0.001c	7.066±0.004c	1.225±0.002b	2.745±0.004c
	III	0.716±0.014c	4.534±0.002c	1.095±0.001c	2.577±0.002c
KP+N1	I	5.524±0.022e	34.446±0.005e	1.353±0.006d	2.944±0.003c
	II	0.896±0.004d	5.552±0.045d	1.096±0.002c	2.175±0.004d
	III	0.587±0.004d	3.686±0.002d	0.968±0.002d	1.967±0.003d
KP+N2	I	6.943±0.002a	43.395±0.010a	1.716±0.002a	3.306±0.002a
	II	1.435±0.003a	8.970±0.026a	1.350±0.026a	2.886±0.003a
	III	1.025±0.001a	6.416±0.001a	1.136±0.003a	2.636±0.004a
ES+N1	I	6.001±0.002d	37.537±0.032d	1.288±0.001e	2.856±0.040d
	II	0.733±0.002e	4.595±0.003e	1.033±0.003d	2.176±0.003d
	III	0.533±0.028e	3.416±0.001e	0.904±0.004e	1.910±0.010e
ES+N2	I	6.632±0.001b	41.476±0.032b	1.693±0.003b	3.288±0.003a
	II	1.3033±0.002b	8.133±0.003b	1.345±0.002a	2.872±0.001b
	III	0.946±0.040b	6.063±0.004b	1.118±0.002b	2.616±0.002b
P value	I	***	***	***	***
LSD (0.05)		0.015	0.048	0.009	0.027
LSD (0.01)		0.021	0.066	0.013	0.037
P value	II	***	***	***	***
LSD (0.05)		0.012	0.041	0.018	0.005
LSD (0.01)		0.0017	0.057	0.025	0.006
P value	III	***	***	***	***
LSD (0.05)		0.037	0.004	0.005	0.008
LSD (0.01)		0.052	0.005	0.006	0.010

LSD indicates least significant differences at P = 0.05 and P = 0.01; *** indicates statistical significant differences at the P<0.05, P<0.01 and P<0.001 levels, respectively; DMRT was used to compare different variants at P≤ 0.05), where values followed by the same letter in a column are not significantly different.

Application of high rates of mineral NPK fertilizers and their combination with bacterial inoculants has caused a significant increase in the share of nitrogen, phosphorus, potassium and crude proteins in the maize biomass compared to the other tested variants. This trend was noticeably observed in the stage of maize

intensive growth, the vegetation period in which the accumulation of nutrients is the most intensive *(Čurić, 1982)*.
Hence, the excess of microbiologicaly fixed nitrogen, with higher amounts of mineral nitrogen, influenced positively on the accumulation of the stated elements and compounds in the study plant material, which is in accordance with previous researches *(Pandey et al., 1998; Dalla Santa et al., 2004)*. According to these studies, microbial inoculation of seeds, combined with different rates of mineral NPK fertilizers, significantly increases both the content of nitrogen and phosphorus in plants.

Effect of applied fertilizers on the yield of green biomass

The analysis of the yield of maize green biomass (based on Duncan's test) showed highly significant yield differences between the applied fertilization treatments (Table 4). The highest increase in yield was obtained by combined application of bacterial inoculants used and high rates of mineral NPK fertilizers for both study years. In addition, it should be noted that with combined usage of bacterial inoculants and low rates of mineral NPK fertilizers were obtained higher yields comparing to the application of lower rates of the pure mineral NPK nutrients. Similar results were obtained in the previous study *(Dalla Santa et al., 2004)*, in which it was determined significantly higher maize yield in treatments that were treated with microbial fertilizer and high rates of mineral nitrogen (150 kg ha^{-1}). Other authors *(El-Sirafy et al., 2006)* also found a significant interaction effect of nitrogen fertilizers and microbial inoculation on crops yield compared to the unfertilized variants.

Table 4. The effect of the fertilization variants on the yield of maize biomass in the study years

Fertilization variant	Maize biomass yield (t ha^{-1})	
	Year 2006	Year 2008
Ø	4073±63g	3373±19g
N1	5140±61f	4464±25f
N2	13242±70c	12074±78c
KP+N1	7083±34d	5954±51d
KP+N2	16736±44a	12215±48b
ES+N1	5885±46e	5485±66e
ES+N2	16454±51b	12721±34a
P value	***	***
LSD (0.05)	94.38	87.66
LSD (0.01)	130.99	121.67

LSD indicates least significant differences at P = 0.05 and P = 0.01; *** indicates statistical significant differences at the P<0.05, P<0.01 and P<0.001 levels, respectively; DMRT was used to compare different variants at P≤ 0.05), where values followed by the same letter in a column are not significantly different.

The character of the applied fertilizers effects on the yield of maize biomass also depended on the weather conditions specific to each year of study. Specifically, the yield of maize, for most of the variants, was noticeably lower in 2008 than in 2006 (Table 4), which is likely due to unfavorable weather conditions during the maize growing period in 2008. This is consistent with some previous results *(Josipović et al., 2005; Maklenović et al., 2009)*, which point out at high correlation relationship between temperature and precipitation and yield of maize. In addition, in 2008 was also observed noticeably less interactive effects of microbiological and lower rates of mineral fertilizers in relation to their effects in 2006.

Conclusion

The present study demonstrated the significant positive effects of combined application of bacterial inoculants used and high and low rates of the composite mineral fertilizers on the yield of maize green biomass (without spikes) for both study years. The same results were obtained regarding the effects of the mentioned applied combinations on the contents of nitrogen, phosphorus, potassium and crude proteins in the maize biomass, which was noticeably observed in the stage of the maize intensive growth. These data suggest that the studied bacterial inoculants (*Klebsiella planticola* and *Enterobacter* spp.) can be used in further investigations as the potential agents of new biofertilizers for improved maize production and other agriculture crops in animal nutrition.

Acknowledgements

This research was carried out with the support of Ministry of Education, Science and Technological Development of the Republic of Serbia and was financed by the Project TR-37006.

Procena uticaja bakterijske i mineralne fertilizacije na hemijski sastav i prinos zelene biomase kukuruza

Aleksandra Stanojković-Sebić, Dragutin A. Đukić, Leka Mandić, Violeta Mandić, Aleksandar Stanojković, Radmila Pivić

Rezime

Cilj ovog istraživanja je bio da se proceni uticaj primene različitih doza kompleksnih mineralnih đubriva i njihovih kombinacija sa bakterijskim

inokulantima (azotofiksirajuće bakterije *Klebsiella planticola* i *Enterobacter* spp.) na hemijski sastav i prinos zelene biomase kukuruza na kiselom eutričnom kambisolu tokom dve vegetacione sezone: 2006 i 2008. Neđubreno zemljište je služilo kao kontrola. Sadržaj azota, fosfora, kalijuma i sirovih proteina u uzorcima biomase su određivani tri puta tokom vegetativne sezone kukuruze, i to: u fazi intenzivnog porasta, fazi mlečno-voštane zrelosti i fazi pune zrelosti. Merenje prinosa zelene biomase obavljeno je krajem vegetacije. Rezultati istraživanja su pokazali da je primena visokih doza kompleksnih mineralnih đubriva i njihova kombinacija sa bakterijskim inokulantima uticala na povećanje sadržaja azota, fosfora, kalijuma i sirovih proteina u biomasi kukuruza tokom obe godine istraživanja, što je naročito izraženo u fazi njegovog intenzivnog porasta. Najveći porast prinosa biomase je dobijen na istim navedenim varijantama, a isto tako je i primena kombinacije bakterijskih inokulanata i manjih doza mineralnih đubriva rezultirala većim prinosima u odnosu na primenu manjih doza čistih mineralnih hraniva. Dobijeni podaci ukazuju da se ispitivani bakterijski inokulanti mogu koristiti u daljim istraživanjima kao potencijalni agenti novih biofertilizatora u cilju poboljšanja proizvodnje kukuruza i drugih poljoprivrednih kultura u ishrani životinja.

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Received 4 August 2016; accepted for publication 10 September 2016

Biotechnology in Animal Husbandry 32 (3), p 311-320 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 633.3
DOI: 10.2298/BAH1603311B

COMPARATIVE STUDIES OF ANNUAL LEGUMES

Tatyana Bozhanska[1*], Tsvetoslav Mihovski[1], Galina Naydenova[2], Daniela Knotová[3], Jan Pelikán[4]

[1]Research Institute of Mountain Stockbreeding and Agriculture, Troyan 5600, Bulgaria
[2]Experimental Station on Soybean, Pavlikeni 5200, Bulgaria
[3] Agricultural Research, Ltd. Troubsko 664 41, Czech Republic
[4] Research Institute for Fodder Crops, Ltd.
*corresponding author: tbozhanska@abv.bg
Original scientific paper

Abstract: The aim of present study was to get comparative data on forage productivity and quality of forage of Checz cultivars of legumes in the conditions of Northern Bulgaria and respectively to select species and genotypes with the potential for successful introduction in the structure of forage production in Bulgaria. Five species of forage crops were observed and the respective cultivars: Egyptian clover (*Trifolium alexandrinum L.),* cv. Faraon*;* crimson clover *(Trifolium incarnatum L.),* cv. Kardinal; annual bird's-foot-trefoil *(Lotus ornithopoides L.)* cv. Junak; black medick *(Medicago lupulina L.)* cv. Ekola and white melilot *(Melilotus albus L.),* cv. Adela. The studied legumes differed significantly in their fodder productivity. They are ranked in the following order of DM yield: white melilot - black medick - annual bird's-foot-trefoil - Egyptian clover - crimson clover. The productivity and participation of Egyptian clover and crimson clover in grasslands varied significantly in years. The biomass of crimson clover had the highest content of crude protein (15.24%) and the lowest of crude fiber (21.69%) and no digestible components. According to the comprehensive evaluation of data on productivity and forage quality of studied annual legumes, black medick could be defined as the species with the highest potential for cultivation in the conditions of the Central Northern Bulgaria. It is characterised by high productivity of green mass and dry matter, it has regrowing ability, it is distinguished by a high content of crude protein (14.92%) and crude fat (4.66%), optimal content of neutral and acid detergent fibers (34.67 and 24.99%) and with high levels of hemicellulose content (9.68%). Energy value of forage of that species, assessed by means of feed unit of milk (FUM) and growth (FUG) was assessed as very high (FUM – 0.69/kg DM and FUG – 0.63/kg DM).

Key words: annual legumes, yield, fodder quality

Introduction

The importance of annual legume as forage crops is significantly less than perennial ones. However, in many cases because of specific agro-ecological conditions or requirement of the sustainable agriculture, forage production for ruminants is successfully based on annual species (*Simić and Vučković*, 2014). In Bulgaria, only peas and vetch are used as a annual legumes (*Kirilov*, 2016). The introduction of new practices for increasing the seasonal productivity and forage quality of temporary swards by annual legume requires studies on plant material of different origin. Most of the studies, which have already been conducted, include species, which have a greater significance as fodders for regions with Mediterranean climate (*Goranova et al., 2007; Vasileva et al., 2011; Vasileva and Vasilev, 2012, Naydenova et al., 2014*). It was found that only some of these legumes show good adaptability to the conditions of Northern Bulgaria (*Vasilev, 2006, 2009; Mitev et al., 2013*).

The aim of present study was to get comparative data on productivity and quality of Checz cultivars of annual legumes in the conditions of Northern Bulgaria and to select species and genotypes with the potential for successful introduction in the production structure of forage crops in Bulgaria.

Material and methods

The experiment was carried out at the Experimental Station on Soybean – Pavlikeni, during the period 2013-2014. The region is characterized by temperate continental climate and altitude of 114 m. Soil type is leached chernozem, with an average reserve of phosphorus and nitrogen and a good reserve of potassium. According to rainfall density for the period of April/October, the year of legumes establishment was relatively drier, with a rainfall amount closer to the normal for the region (423 mm, compared to 367 mm, average for 50 year period) - Fig.1. There was more humidity and more favourable distribution of rainfalls in 2014, with a rainfall amount in the vegetation period of 579 mm.

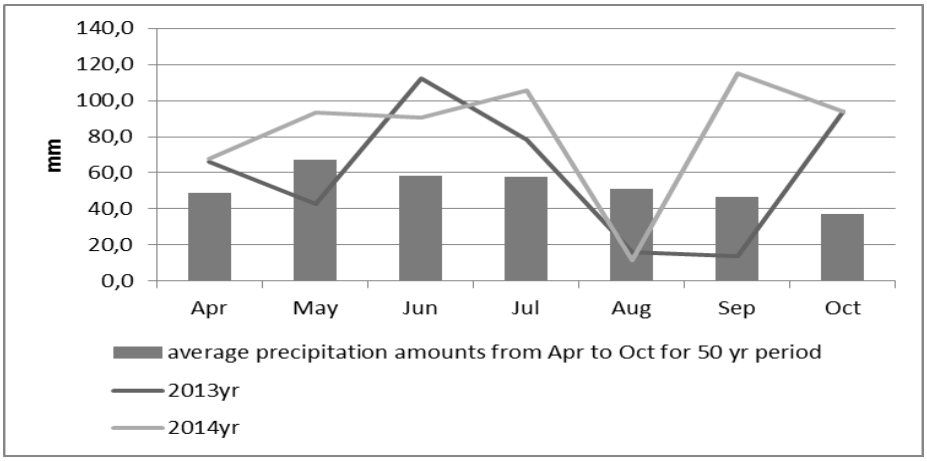

Figure 1. Rainfall amount (mm) for the experimental period

Five species were included in the experiment: Egyptian clover (*Trifolium alexandrinum* L.), cv. Faraon; crimson clover (*Trifolium incarnatum* L.), cv. Kardinal; annual bird's-foot-trefoil (*Lotus ornithopoides* L.) cv. Junak; black medick (*Medicago lupulina* L.) cv. Ekola and white melilot (*Melilotus albus* L.), cv. Adela. The species were studied in pure crop. The randomized block method was used, the number of replications was 4 and the area of the plot was $5m^2$. Mineral fertilization was not applied. Sowing was in rows, with 15 cm space between the rows and sowing rate was 1000 $g.s.m^{-2}$. Sowing dates were 12. April 2013 and 14. March 2014. Mowing was conducted at the stage of budding to early flowering of species at a height of 3-4 cm. The following characteristics were observed: plant growth and development, sward botanical composition, yield of green mass and dry matter (t ha^{-1}), regrowing ability, and forage quality. The following indicators for the forage quality have been studied: crude protein (CP,%) according to Kjeldahl; crude fiber (CF,%) according to Weende analysis, crude fat (Cft,%) by extraction in an extractor of Soxhlet type, Ash (%) - ashing in a muffle furnace at 550 °C, humidity content (%) - the sample was dried under a temperature of 105 °C till it reached a constant weight, calcium (Ca,%) - according to (*AOAC*, 2007), phosphorus (P,%) according to (*AOAC*, 2007) spectophotometer (Agilent 8453 UV – visible Spectroscopy System), measuring within the sphere 425 ηm, NEF=100 (CP,%+ CFr,%+ CF,%+ Ash,%+ Humidity,%). The fiber composition of cell walls was determined as a percentage of dry matter, which included: neutral detergent fibers (NDF,%), acid detergent

fiber (ADF,%) and acid detergent lignin (ADL,%) according to the *Goering and Van Soest* (1970) analysis, polysaccharides hemicellulose (NDF-ADF) and cellulose (ADF-ADL). The degree of lignification was expressed as a percentage share of ADL/NDF. The potential energy nutritional value of fodder was assessed according to the Bulgarian system as FUM and FUG (*Todorov*, 1995). Equations were used for legumes according to experimental values of CP, CF and NFE and were precalculated according to the coefficients of digestibility. The coefficient for the energy exchange (q) expresses the share of the exchangeable energy (EE) of the net energy (NE), calculated as a part of unit with the equation: q = EE/NE.

For the statistical processing of yield data was used two-factor analysis of variance and multiple comparison of yields in years by means of least significant differences (LSD). The statistic program STATGRAPHICS PLUS was used.

Results and discussion

White melilot exceeded all species included in the study in green and dry matter yield, with the average values for the experimental period of 29.6 and 7.3 t ha^{-1} (Table 1) respectively. This was also the earliest maturing species, and its productivity varied slightly in years, which determined its good adaptability to the region of testing. It achieved harvest maturity stage in the first half of June, as it regrew quickly and formed a second regrowth up to the middle of July. It participated with 89 and 97% in the biomass composition in the first regrowth, respectively in the first and second experimental year. It is important to note that the economic value of high yield of white melilot could be low because of the bitter taste and specific smell of forage caused by alkaloid coumarin (*Duke*, 1981; *Sanderson et al*., 1986). In the contemporary selection of grasses is required to be cultivated low-coumarin varieties of that species, which are less productive (*Meyer*, 2005).

High average green mass yields - 21.3 t ha^{-1}, 20.7 t ha^{-1} and dry matter – 5.8 t ha^{-1}, 5.4 t ha^{-1} were gathered with black medick and annual bird's-foot-trefoil. In both experimental years, these species had great presence in fodder mass (>60-70%, Table 1), which is a prerequisite for high competitiveness and adaptability. The dry matter productivity of Checz cultivar of black medick was significantly higher than Bulgarian population of that species (3.3-3.6 t ha^{-1}) grown also in the conditions of the Central

Northern Bulgaria (*Naydenova et al.,* 2014) and harvested in the same phenophase.

In the first experimental year, the values of green and dry matter yield, as well as the participation of crimson clover in fodder biomass were significantly higher than in the second year, 21.5 t ha^{-1}, 4.05 t ha^{-1}, 73 % and 11.2 t ha^{-1}, 2.5 t ha^{-1}, 22 %, respectively. Crimson clover, in the spring growth, was equal in productivity with black medick and the annual bird's-foot-trefoil, but it did not regrow after cutting. In conditions of the spring sowing only a part of plants developed generative stems and could be considered that the studied genotype of that species had a winter type of development and its productivity should be studied under conditions of pre-winter sowing. Crimson clover was distinguished by the highest degree of weed infestation – over 52% average for the period.

The productivity and participation of Egyptian clover in biomass also varied significantly by years. In the first year that species had the smallest percentage share in the sward (49%), as well as the lowest productivity of green and dry mass (7.8 t ha^{-1} and 1.5 t ha^{-1}). In the second experimental year, when also the rainfall amount was higher, the presence of Egyptian clover in sward significantly increased (78%) and respectively the obtained yields of green and dry matter yield were very high (26.4 t ha^{-1} and 7.8 tha^{-1}). Variability in values of the main indicators, which determine the economic significance of this crop, is a sign for its slighter adaptability to soil and climate conditions for the region of study.

Table 1. Fresh and dry matter yield and participation of annual legume in fodder biomass

Species	FMY (2013) t ha^{-1}	FMY (2014) t ha^{-1}	Mean t $ha^{-1}yr^{-1}$	DMY (2013) t ha^{-1}	DMY (2014) t ha^{-1}	Mean t $ha^{-1}yr^{-1}$	% in fodder mass (2013)	% in fodder mass (2014)
T. alexandrinum	7.8^{b}	26.4^{a}	17.1	1.5^{c}	7.8^{a}	4.7	49	78
T. incarnatum	22.2^{b}	11.3^{b}	16.8	4.2^{b}	2.5^{b}	3.4	73	22
L. ornithopoides	25.9^{ab}	15.4^{b}	20.7	6.2^{a}	4.5^{b}	5.4	77	75
M. lupulina	25.4^{ab}	17.2^{b}	21.3	7.4^{a}	4.1^{b}	5.8	70	58
M. albus	31.0^{a}	28.1^{a}	29.6	7.1^{a}	7.4^{a}	7.3	89	97
$LSD_{0.05}$	7.5	8.3		1.8	2.3			

*values of annual productivity by years, followed by equal letters do not differ significantly at P<0.05
FMY - fresh matter yield; DMY - dry matter yield

Significant differences were found among studied species for protein and fiber content (Table 2). The biomass of crimson clover had the highest

crude protein content (15.24% of dry matter) and the lowest level of crude fiber (21.68%) for a two year period. This species had also the lowest mean content of no digestible components in the forage (Table 3) - lignin (3.08%) and cellulose (17.73%). The decreased content of fractions of the structural fiber components of the cell walls which was observed in crimson clover (NDF – 31.16%, ADF – 20.81% and ADL – 3.08%) could be explained by the fact that forage mass of this species consisted predominantly by leaves.

Black medick was closer to crimson clover in content of crude protein (14.92% of DM) and hemicellulose (9.68%). The content and ratio of NDF (34.67%) and ADL (5.01%) in the forage of Black medick can be defined as optimal (*Oba and Allen*, 1999). This species had the highest content of crude fat - 4.66%.The annual bird's-foot-trefoil was caharacterized by the lowest content of crude protein (10.07%), neutral detergent fibers (30.55%) and hemicellulose (6.43%), as well as with the highest level of lignification (17.09%). Those values suggest low levels of intake and low digestibility of biomass of that species. In comparison to common bird's-foot-trefoil, grown in the foothill conditions of the Northern Bulgaria (*Churkova*, 2012; 2013), annual bird's-foot-trefoil has a significantly lower content of NDF and ADF and does not differ in relation to content of macroelements.

Significant variation depending on legume species was observed in relation to content of mineral substances, and the content of macroelements of calcium and phosphorus, as well as according to values of their ratio in the forage. The fodder of the annual bird's-foot-trefoil had the highest percentage content of mineral substances (15.63%) and calcium (2.64%). White melilot significantly gave in to the other species in relation to mineral concentration (5.28).The ratio Ca:P was close to the optimal 2:1 (NRC, 2000) only for Egyptian clover (3.79:1) and exceeded the determined one as critical 6:1 for the white melilot (7.41:1) and for bird's-foot trefoil (7.65:1).

Variation among studied species in relation to coefficient of energy exchange (q) could be specified as low - from 0.42 to 0.46 (Table 4). The exchangeable energy (7.34 MJ/kg DM) for crimson clover took the greatest share of the net energy (15.81 MJ/kg DM). The highest energy value of forage, measured as feed unit of milk (FUM) and growth (FUG) was found for crimson clover, black medick and white melilot, and the lowest - for Egyptian clover (Table 4).

Table 2. Chemical composition of annual legume- average for a period of two years (% DM)

Species	CP	CF	Cft	Ash	Ca	P	Ca:P†
T. alexandrinum	11.18	33.44	3.02	12.24	2.14	0.565	3.79
T. incarnatum	15.24	21.68	3.61	11.85	2.34	0.420	5.57
L. ornithopoides	10.07	25.26	4.61	15.63	2.64	0.345	7.65
M. lupulina	14.92	24.92	4.66	10.29	1.94	0.321	6.04
M. albus	11.76	32.59	3.16	5.28	1.84	0.248	7.41

CP – crude protein; CF – crude fibers; Cft – crude fats; Ash – mineral composition; Ca – calcium content; P – phosphorus content

†-relationship is not expressed as a percentage

Table 3. Content of fiber components of cell walls in annual legumes- average for a period of two years (% DM)

Species	NDF	ADF	ADL	Hemicellulose	Cellulose	Degree of lignification
T. alexandrinum	42.86	30.95	6.13	11.91	24.82	14.30
T. incarnatum	31.16	20.81	3.08	10.35	17.73	9.88
L. ornithopoides	30.55	24.12	5.22	6.43	18.90	17.09
M. lupulina	34.67	24.99	5.01	9.68	19.98	14.45
M. albus	33.60	24.15	3.05	9.45	21.10	9.08

NDF – neutral detergent fiber; ADF – acid detergent fiber; ADL – acid detergent lignin

Table 4. Energy nutritional value of annual legume- average for a period of two years

Species	NE	EE	q	FUM	FUG
T. alexandrinum	15.93	6.71	0.42	0.61	0.55
T. incarnatum	15.81	7.34	0.46	0.69	0.63
L. ornithopoides	15.20	6.79	0.45	0.63	0.57
M. lupulina	16.47	7.42	0.45	0.69	0.63
M. albus	17.11	7.48	0.44	0.69	0.63

NE – net energy (MJ/kg CB); EE – exchangeable energy (MJ/kg CB); FUM – feed unit of milk (kg DM); FUG – feed unit of growth (kg CB); q – coefficient of energy exchange

Conclusion

According to the comprehensive evaluation of data on productivity and forage quality of studied annual legumes, black medick cultivar 'Ecola' could be defined as the species and genotype with the highest potential for cultivation in the conditions of the Central Northern Bulgaria. It is

characterised by high productivity of green mass and dry matter, it has regrowing ability, it is distinguished by a high content of crude protein and crude fat, optimal content of neutral and acid detergent fibers and high levels of hemicellulose content. Energy value of forage of that species, assessed by means of feed unit of milk (FUM) and growth (FUG) was assessed as very high.

Acknowledgement: These results were obtained thanks to a (partial) institutional support enabling a long-term conceptional development of the research organisation and from research project NAZV QJ1310100.

Komparativno ispitivanje jednogodišnjih mahunarki

Tatyana Bozhanska, Tsvetoslav Mihovski, Galina Naydenova, Daniela Knotová, Jan Pelikán

Rezime

Cilj ovog istraživanja je bilo dobijanje komparativnih podataka o produktivnosti i kvalitetu krmnog bilja čeških sorti jednogodišnjih mahunarki u uslovima severne Bugarske i, respektivno, odabir vrste i genotipova sa potencijalom za uspešno uvođenje u strukturu proizvodnje krme u Bugarskoj. Pet vrsta krmnog bilja je bilo uključeno u istraživanje, i to sledeće sorte: egipatska detelina (*Trifolium alexandrinum* L), sorta Faraon; inkarnatska detelina (*Trifolium incarnatum* L), sorta Kardinal; jednogodišnji žuti zvezdan (*Lotus ornithopoides* L.) sorta Junak; hmeljasta vilja (*Medicago lupulina* L.) sorta Ekola i beli kokotac (*Melilotus albus* L.) sorta Adela. Ispitivane mahunarke značajno su se razlikovale u produktivnosti. One su rangirane po sledećem redosledu prinosa SM: beli kokotac – hmeljasta vija - jednogodišnji žuti zvezdan - egipatska detelina - inkarnatska detelina. Produktivnost i učešće egipatske deteline i inkarnatske deteline u travnjaku značajno varira u godinama. Biomasa inkarnatske deteline je imala najveći sadržaj sirovih proteina (15,24%), a najmanji sirovog vlakna (21,69%). Prema sveobuhvatne proceni podataka o produktivnosti i kvalitetu krme ispitivanih godišnjih mahunarki, hmeljasta vija bi se mogla definisati kao vrsta sa najvećim potencijalom za gajenje u travnjacima u uslovima centralne severne Bugarske. Ona se odlikuje visokom produktivnošću zelene mase i suve materije, ima sposobnost ponovnog rasta, ona se odlikuje visokim sadržajem sirovih proteina (14,92%) i sirove masti (4,66%), optimalnim sadržaem neutralnih i kiselih deterdžent vlakana (34,67 i 24.99%) i visokim nivoom sadržaja hemiceluloze (9,68%). Energetska

vrednost krme ove vrste, vrednovana korišćenjem hranidbene jedinice mleka (Feed Unit of Milk -FUM) i rasta (Feed unit of growth - FUG) je ocenjena kao veoma visoka (FUM – 0,69/kg SM i FUG – 0,63/kg, SM).

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Received 16 August 2016; accepted for publication 20 September 2016

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[1]Institute for Animal Husbandry, Belgrade – Zemun, 11080 Zemun, Serbia
[2]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia
Corresponding author: Milan M.Petrović, milpet99@gmail.com
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
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"Modern Trends in Livestock Production"
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