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CONTENTS

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DIAGNOSIS OF SUBCLINICAL KETOSIS IN DAIRY COWS

Radojica Djoković[2], Zoran Ilić[1], Vladimir Kurćubić[2], Miloš Petrović[2], Marko Cincović[3], Milan P. Petrović[4], Violeta Caro Perović[4]

[1]University of Priština, Faculty of Agriculture, Department of Animal Science, JeleneAnžujske bb, Lešak, Serbia
[2]University of Kragujevac, Faculty of Agronomy, Department of Animal Science, Cara Dušana 34, Čačak, Serbia
[3]University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, TrgDositejaObradovića 8, Novi Sad,
Serbia
[4]Institute for Animal Husbandry 11080 Belgrade-Zemun
Corresponding author: radojicadjokovic@gmail.com
Review paper

Abstract: Ketosis is a common disease in high producing dairy cows during the early lactation period. Subclinical ketosis (SCK) and periparturient diseases considerably account for economic and welfare losses in dairy cows. Subclinical ketosis poses an increased risk of production-related diseases such as clinical ketosis, displaced abomasum, retained placenta, lameness, mastitis and metritis. Production efficiency decreases (lower milk production, poor fertility, and increased culling rates), which results in economic losses. Increased concentrations of circulating ketone bodies, predominantly β-hydroxybutyrate (BHB), without the presence of clinical signs of ketosis are considered as SCK. It is characterized by increased levels of ketone bodies in the blood, urine and milk. The gold standard test for ketosis is blood BHB. This ketone body is more stable in blood than acetone or acetoacetate. The most commonly used cut-points for subclinical ketosis are 1.2 mmol/L or 1.4 mmol/L for BHB in the blood. Clinical ketosis generally involves much higher levels of BHB, about 3.0 mmol/L or more. Usually, detection of SCK is carried out by testing ketone body concentrations in blood, urine and milk. A variety of laboratory and cowside tests are available for monitoring ketosis in dairy herds.Butno cowside test has perfect sensitivity and specificity compared to blood BHB as the gold standard test. The aim of this review is to overview diagnostic tests for SCK in dairy cows, including laboratory and cowside tests.

Keywords: dairy cows, subclinical ketosis, laboratory tests, cowside tests

Introduction

Production diseases i.e. diseases associated with improper nutrition or management are common in dairy cows*(Ospina et al., 2010; Brunner et al., 2019).* Subclinical ketosis (SCK) is an important production disease of dairy cows and continues to cause significant economic losses to the dairy industry. Ketone body levels in blood, urine and milk can be monitored to detect SCK in cows, and to increase their chances of successful lactation *(Geishauser et al., 2001; Seifi et al., 2011; Zhang et al., 2012).* Ketosis results in decreased milk production, impaired fertility and increased frequency of other diseases. Most cases occur in the first 6 weeks to 2 months after calving. As the course of the disease is often subclinical, early detection is very important. SCK causes greater losses than clinical ketosis because it occurs more frequently and often cannot be detected by farmers *(Duffield, 2000; Geishauser et al., 2000; Oetzel, 2004, 2007; Brunneret al., 2019).*

Herds with ketosis problems in early lactation cows also tend to have increased incidence of displaced abomasum (>8%) and increased herd removals in the first 60 days in milk (>8%).The costs associated with SCK in affected cows are substantial, and include the loss of milk yield (up to about 7%). An additional major cost associated with high incidence of subclinical and clinical ketosis is the increased risk for numerous other health disorders, such as displaced abomasum, mastitis, metritis, lameness and reduced reproductive efficiency *(Cook et al., 2001; Oetzel, 2007; Duffield et al.,2003; Seifi et al., 2011; Brunner et al., 2019).*

Across the world, SCK prevalence was 24.1%, ranging from 8.3% to 40.1% *(Brunner et al., 2019).* The prevalence of subclinical ketosis in ten European countries was on average 21.8% (ranging from 11.2 to 36.6%), and clinical ketosis was 3.7% (0.4 to 11.1%). The prevalence of subclinical ketosis in Serbia was up to 19.5% in 42 herds *(Suthar et al., 2013).*

On average, 40% of cows have SCK at least once during lactation, while clinical ketosis affects on average 5% of cows*(Oetzel, 2004; Suthar et al., 2013).*However, some reports have indicated that the incidence of SCK may affect 40% of cows, with the incidence rate varying widely among farms, and may be as high as 80% on individual farms *(Jenkins et al., 2015; Brunner et al., 2019).*

The gold standard test for ketosis is blood BHB. This ketone body is more stable in blood than acetone or acetoacetate *(Tyopponen and Kauppinen, 1980).* The most commonly used cut-point for SCK is ≥ 1.2mmol/l of blood BHB *(Geishauseret al., 1998; Dufffield, 2000; Duffield and Bagg, 2002; Zhang et al., 2012; Djokovic et al., 2013).* Early lactation cows with blood BHB concentrations above this cut-point are at threefold greater risk to develop displaced abomasum or clinical ketosis, and cows with blood BHB concentrations above 2.0 mmol/L are at risk for reduced milk yield *(Duffield, 2000).* Some studies use a slightly higher cut-point 1.4mmol/L of blood BHB for defining SCK *(Geishauseret al., 2000; Carrier*

et al., 2004; Oetzel, 2004, Iwersenet al., 2009). The exact cut-point chosen usually has a minor effect on the interpretation of herd-based results. Clinical ketosis generally involves much higher levels of BHB, about 3.0 mmol/L or more *(Oetzel, 2007)*.

A variety of laboratory and cowside tests are available for monitoring ketosis in dairy herds. Diagnosing ketosis in a herd requires a completely different diagnostic approach than diagnosing ketosis in an individual cow. Comparing blood BHB results from a small number of cows to normal ranges is not appropriate. Herd-based testing is performed by subsampling 12 or more dairy cows, representative of the animals at risk for ketosis (about 5 to 50 days in milk),followed by the evaluation of the proportion of cows above the cut-point of 1.4 mmol/L. The alarm level for the proportion of cows above this cut-point shows an average ketosis prevalence of about 15% *(Duffield and Bagg, 2002)*. The other author suggests using 10% as the alarm level for herd-based ketosis testing *(Oetzel, 2004)*.

Milk is a very suitable sample for the determination of ketosis as it can easily be collected by farm personnel compared to blood and urine samples. In cases of SCK, the content of BHB in milk is increased but concentrations are lower than in the blood *(Samiei et al., 2010)*. BHB concentration in milk can be measured in the field by using the semi-quantitative colorimetric dipstick test. The cut-off value is 100 to 200 μmol/L and higher values indicate ketosis *(Dirksen and Breitner, 1993; Eicher, 2004; Oetzel, 2007)*.

This review aims to overview diagnostic tests for SCK in dairy cows, including laboratory and cowside tests.

Types of ketosis in dairy herds

Herd ketosis problems can be categorized into three general types of ketosis (Table 1). Each type is of different etiology and therefore requires a different prevention and diagnosis strategy. There is an overlap between the categories, and herds may have a combination of the types. These classification types are largely adapted from Swedish authors *(Holtenius and Holtenius, 1996)* and have been described in detail *(Herdt, 2000)*.

Table 1. Summary of types of ketosis observed in dairy herds (modified from *OETZEL, 2007*)

Outcome	Type I	Type II	Type III. Butyric Acid Silage Ketosis
Highest risk period	3 to 6 weeks after calving	1 to 2 weeks after calving	Very high or high
Description	Spontaneous, underfeeding	Fat cows, fatty liver	Normal or High
Blood BHB	Very high	High	Variable
Blood glucose	Low	Low (may be high initially)	Variable
Blood insulin	Low	Low (may be high initially)	Variable
Body condition	Probably thin	Often fat (or lost fat)	Variable
Fate of NEFA	Ketone bodies	Liver triglycerides initially, then ketone bodies	Variable
Liver gluconeogenesis	High	Low	Variable
Liver pathology	None	Fatty liver	Variable
Prognosis	None	Poor	Good
Key diagnostic test	Post-fresh BHB	Pre-fresh NEFA	Silage analysis
Key intervention	Post-fresh management and nutrition	Pre-fresh management and nutrition	Destroy, dilute or divert the silage

Diagnosis of ketosis

Laboratory tests

Enzyme catalysis method: The enzyme catalysis method is a traditional test for blood serum BHB determination in cows. The test kit requires the use of an ultraviolet spectrophotometer or biochemistry analyzer and can be used to determine blood serum BHB values in humans and animals (*Zhang et al., 2012*). This is the gold standard for the detection of ketosis in dairy cows. The most commonly used blood BHB cut-points for SCK are ≥1.2 mmol/L *(Geishauseret al., 1998; Dufffield, 2000; Duffield and Bagg 2002; Zhang et al., 2012; Djokovic et al., 2013)* or ≥1.4 mmol/L *(Geishauseret al., 2000; Carrier et al., 2004; Oetzel, 2004;Iwersenet al., 2009)* or only ≥ 1.0 mmol/L *(Whitaker, 1997; Kinoshita et al., 2010; Ospina et al., 2010; Jozek et al., 2017; Djokovic et al., 2018a).* BHB concentrations in blood and milk sera can be measured using a biochemical analyzer *(Samiei et al., 2010).* A statistically significant correlation ($r=0.705$, $p<0.01$) was determined between BHB concentrations in blood and milk sera of cows. The best sensitivity (94%) and specificity (74%) were observed for BHB measurements in milk, with the optimal cut-point for BHB in milk of ≥ 80 μmol/L,

(AUC=0.91±0.03; p<0.001), for the detection of SCK in dairy cows (by the receiver operatingcharacteristic analysis method - ROCA) *(Jozek et al., 2017; Djokovic et al., 2018b).*

Fourier transform infrared (FTIR) spectrometry method: As a diagnostic method for screening diary cows for SCK by determining milk acetone concentration using FTIR spectrometry *(Hansen, 1999; Heurer et al., 2001)*, FTIR spectrometry is fast, inexpensive and easy to implement on a large scale. FTIR spectrometry was used to measure the concentration of BHB and acetone in milk to detect SCK. The sensitivity and specificity of this method is 70% and 95%, respectively, with 25 to 27% false positives and 6 to 7% false negatives, when using cut-points of 0.15mmol/L for acetone and 0.1mmol/L for BHB *(De Roosetet et al., 2007)*. When testing BHB and acetone concentrations in milk by FTIR, higher sensitivity (80%) and specificity (71%) were obtained in the detection of SCK compared to blood BHB, with the cut-point of ≥1.2 mmol/L *(Van Knegseletet et al., 2010)*. According to previous research, the FTIR test based on milk acetone and BHB values is valuable in the detection of dairy cows for SCK *(Zhang et al., 2012)*.

Fluorometric determination of BHB levels: The fluorometric determination of BHB values in milk and blood plasma based on an enzymatic method has been described *(Williamson et al., 1962, 1974; Larsen and Nielsen, 2005)*. This fluorometric method correlated closely with results obtained by the traditional spectrophotometric method (r=0.987, p<0.001) *(Larsen and Nielsen, 2005)*. The advantages of the fluorometric determination of BHB are that detection results are not affected by the hemolysis of blood samples, and that whole milk samples do not need pre-treatment. This method is excellent for large numbers of samples, especially for large-scale in-line sampling of milk *(Larsen and Nielsen, 2005; Zhang et al., 2012)*.

Gas liquid chromatography (GLC) to test acetone levels in blood and milk: GLC method using N-propanol as an internal standard is valuable for the determination of acetone and BHB in milk and blood serums *(Enjalbertcet al., 2001; Zhang et al., 2012)*.The best cut-point for blood acetone was 175 μmol/l, with the sensitivity of 91.7% and specificity of 68.3%, and for milk acetone 160 μmol/L, with 90% sensitivity and 57.4% specificity, compared to the gold standard ketosis test (blood BHB), cut-point >1.2 mmol/L. The cut-point level for acetone in milk was 400 μmol/L or higher for ketotic cows *(Cook et al., 2001)*, and the same cut-point level was used in detecting acetone in milk by the qualitative and quantitative salicylaldehyde test for SCK in dairy cows *(Venkateswarlu and Choudhuri, 2001)*.

Nuclear magnetic resonance (NMR) spectroscopy and gas chromatography-mass spectrometry (GC-MS) to test acetone and BHB values: High-resolution NMR spectroscopyand GC-MS were used to determine acetone and BHB values in the blood *(Kleinet al., 2010; Zhang et al., 2012)*. In this

experiment, acetone and BHBwere only used as indicators of energy metabolism during the transitional period in dairy cows, and the diagnosis effect ofSCK was not investigated *(Kleinet al., 2010)*.

Cowside tests for ketosis

Different types of cowside tests are available for monitoring ketosis in dairy herds. However, none of the cowside tests have perfect sensitivity and specificity compared to blood BHB. Sensitivity is true positive results compared to the gold standard ketosis test (blood BHB) and specificity is true negative results compared to the gold standard ketosis test (blood BHB). Therefore, the gold standard ketosis test (blood BHB) is the most accurate for herd monitoring, and is particularly suitable for investigating herds with ketosis. Cowside ketosis tests have the advantages of lower cost, less labor and immediate results, when compared to blood BHB testing. This makes them particularly useful for making (or excluding) a clinical diagnosis of ketosis in individual sick cows. However, testing herds for ketosis requires a very different testing strategy compared to diagnostic decision-making for individual sick cows (*Oetzel, 2007*).

Cowside urine tests for ketosis: Urine can be evaluated for cowside ketosis testing. However, it is much more difficult to collect a urine sample than a cowside milk sample. This is an important practical limitation on farms, which greatly increases labor costs for testing. Urine acetoacetate can be evaluated quantitatively by nitroprusside tablets (Acetest, Bayer Corp. Diagnostics Division, Elkhart, IN). This test has excellent sensitivity but poor specificity *(Nielen et al., 1994; Carrier et al.,2004; Oetzel, 2004)*. This makes it a useful test for evaluating individual sick cows, but not very useful for herd-based monitoring *(Osborne et al., 2002; Oetzel, 2007)*.

Cowside milk (Nitroprusside powder) tests for ketosis: Cowside milk tests have huge advantages over urine cowside tests for ease of collection and for certainty that all eligible cows can be tested. However, milk tests are generally not as sensitive as urine tests in detecting ketosis. Nitroprusside powders (Utrecht powder, Keto Check powder) can be used to qualitatively test milk acetoacetate. However, these tests generally have very poor sensitivity (Table 2) for ketosis compared to blood BHB and cannot be recommended as tests for herd-based monitoring. They have some, but very limited value as cowside tests for diagnostic decisions for individual cows *(Osborne et al., 2002; Eicher, 2004; Oetzel, 2007)*.

Table 2. Sensitivity and specificity of cowside milk nitroprusside powders compared to blood BHB (cut-point of ≥ 1.2mmol/L or ≥1.4 mmol/L)(modified from *OETZEL, 2007*)

Test type/study	BHB Cut-point	Herds tested	% ketosis	Total samples	TP	FN	FP	TN	Sensitivity	Specificity
Utrecht powder										
Nielen et al., (1994)	≥1.4mmol/L	18	10.3%	185	17	2€	7	159	89%	96%
Geishauser et al., 1998	≥1.2mmol/L	25	16.4%	529	37	50	0	442	43%	100%
KetoCheck powder (≥ trace)										
Geishauser et al., (1998)	≥ 1.2mmol/L	25	16.4%	529	24	63	0	442	28%	100%
Carrier et al., (2004)	≥ 1.4mmol/L	1	7.5%	878	28	38	9	803	42%	99%
Bioketone powder(≥ trace)										
Geishauser et al.(1998)	≥ 1.2 mmol/L	25	16.4%	529	24	63	0	442	28%	100%

Legend: BHB – beta-hydroxybutyric acid, mmol/L; Ketosis – blood BHB ≥1.2 mmol/L or ≥1.4mmol/L; TP – true positives, FN –false negatives; FP – false positives; TN – true negatives.

Cowside milk (BHB) tests for ketosis: The most promising cowside milk ketone test used is a semi-quantitative milk BHB test strip manufactured by Sanwa Kagaku Kenkyusho Co., Ltd. (Nagoya, Japan). This test strip is marketed under various names (KetoTest, Ketolac BHB, and Sanketo paper) in different parts of the world.Results of numerous studies evaluating the sensitivity and specificity of the milk BHB test strip compared to blood BHB results are presented in Table 3.When used at the cut-point of ≥100 μmol/L, this test is about 83% sensitive and 82% specific. For individual cow testing, the cut-point of ≥50 μmol/L provides better sensitivity (89%) but has a false positive rate of 69%. Increasing the cut-point to ≥200 μmol/L reduces test sensitivity to 54% (Table 3). At this higher cut-point, the test is of little value for diagnosing ketosis in individual sick cows but has potential use for herd-based evaluations. The best cut-point for herd monitoring when using the milk BHB strip appears to be ≥200 μmol/L. At this cut-point, the prevalence of positive test results is similar to true prevalence, allowing the same alarm level for ketosis prevalence (10%) to be used for both tests. Unfortunately, milk BHB test strip prevalence changes little as true prevalence increases, making the test practically useful only for identifying herds with a very high prevalence of ketosis*(Oetzel, 2007)*.

Table 3. Sensitivity and specificity of cowside milk BHB test compared to blood BHB (cut-point of ≥1.4 mmol/L)
(modified from *OETZEL, 2007*)

Test type/Study	Herds Tested	% Ketosis	Total Samples	TP	FN	FP	TN	Sensitivity	Specificity
Milk BHBA strip (≥ 50 µmol/L)									
Geishauser et al. (2000)	21	11.9%	469	51	5	182	231	91%	56%
Carrier et al. (2004)	1	7.6%	883	59	8	100	716	88%	88%
Oetzel,2004	17	17.2%	221	34	4	36	147	89%	80%
Pooled data (by cow)	39	10.2%	1573	144	318	318	1094	89%	77%
Milk BHB strip (≥100 µmol/L)									
Jorritsma et al.(1998)	8	8.4%	190	14	2	31	143	88	82%
Geishauser et al.(2000)	21	11.9%	469	45	11	99	314	80	76%
Carrier et al. 2004)	1	16.5%	248	39	2	65	142	95	69%
Duffield et al.(2003)	5	27.2%	235	52	12	64	107	81	63%
Carrier et al.(2004)	1	7.6%	883	50	17	54	762	75	93%
Oetzel,(2004)	17	17.12%	221	33	5	32	151	87	83%
Pooled data (by cow)	53	12.6%	2246	233	49	345	1619	83	82%
Milk BHB strip (≥200µmol/L)									
Jorritsma et al., 1998)	8	8.4%	190	14	2	31	143	88%	82%
Geishauser et al.(2000)	21	11.9%	469	45	11	99	314	80%	76%
Duffield et al.(2003)	5	27.2%	235	52	12	64	107	81%	63%
Carrier et al.(2004)	1	7.6%	883	50	17	54	762	75%	93%
Oetzel, (2004)	17	17.2%	221	17	21	5	178	45%	97%
Pooled data (by cow)	52	12.1%	1998	129	112	100	1657	54%	94%

Legend: BHB – beta-hydroxybutyric acid, mmol/L; Ketosis – blood BHB ≥1.4mmol/L; TP – true positives, FN – false negatives; FP – false positives; TN – true negatives.

Cowside blood tests for ketosis: A cowside blood BHB test system using a human instrument marketed for diabetic patients (Precision Xtra™ blood glucose and ketone monitoring system, Abbott Laboratories, Abbott Park, IL). The glucometer/ketometer system is very easy to use cowside. A strip is inserted in the meter, less than a drop of blood is added to the end of the strip, and results are displayed in about 15 seconds. The strips do not require calibration prior to use. It is necessary to collect a small amount of blood from the tail vein using a small needle (25 gauge) and a small syringe (1 mL) to use with the glucometer/ketometer. The preliminary results with the glucometer/ketometer system are very encouraging. The system is more accurate as a ketometer (for BHB) than as a glucometer (glucose). Sensitivity and specificity for BHB appear to be outstanding (over 95%), *(Heuwieser et al., 2007; Oetzel, 2007; Konkol et al., 2009; Voyvoda and Erdogan, 2010).*

Other tests for the detection of SCK in dairy cows

Fat and protein in milk: Dairy cows suffer from negative energy balance (NEB) during the first two weeks of lactation, a high mobilization of lipids from body fat reserves, ketogenesis and hypoglycaemia *(Elitoket al., 2010; Djokovic et al., 2014, 2015).* A portion of the free fattyacids that are mobilized are directly incorporated into milk fat, resulting in an increase in milk fat percentage. By contrast, milk protein percentage will slightly decrease in these cows due to a reduction in energy supply. Fat to protein ratio (FPR)in milk is used to monitor the prevalence of SCK in a herd *(Eicher, 2004; Richard, 2004; Gantneret al., 2009; Jenkins et al., 2015).*

AFPR greater than 1.5 indicates SCK, whereas a FPR lower than 1.1 indicatesrumen acidosis*(Cejna and Chladek, 2005).* Using a blood BHB level of 1.2 mmol/L or higher as a cut-point concentration for SCK, both the test-day fat percentage and the test-day protein percentage were significantly associated with SCK *(Duffield et al., 1997).* The specificity of FPR for the detection of SCK was lower (77-81%) than cowside milk (BHB) tests (KetoLac BHB test with a cut-point of 200 µmol/L of BHB in milk) and cowside urine tests (KetoStix test), (97 - 99%) *(Krogh et al., 2011). Jenkins et al. (2015)* reported high sensitivity of FPR > 1.42 or lower (> 1.35 or > 1.25) for SCK, and found that these cut-points could be used as a screening test. FPR is a good measure of SCK at the whole herd level, but it is not sensitive enough for the diagnosis of SCK in individual cows *(Zhang et al., 2012).*

Nonesterified fatty acid (NEFA): The optimalcut-point for NEFA in blood serum for SCK, by the ROCA, was >0.26 mmol/L, with 82.54% sensitivity and 91.89% specificity, compared to BHB in the blood with a cut-point of >1.2 mmol/L as the gold standard test *(Aslet al., 2011).*

Blood biochemical indicators of ketosis*:* Based on blood biochemical indicators, SCK in cows may be diagnosed in the following values of these indicators (BHB >1.2 mmol/L, glucose <2.5 mmol/L, NEFA>0.26 mmol/L and TG <0.12 mmol/L) and blood values of NEFA >0.7 mmol/L and AST activity above 100 IU/L, which is indicative of hepatic lipidosis*(Oetzel, 2004; Gonzalez et al.,2011; Djokovic et al., 2013, 2018a).*

Conclusions

Measurement of blood BHB values in serum or plasma is the gold standard test for the diagnosis of SCK. The most commonly used cut-points for SCK are ≥ 1.2 mmol/L or ≥1.4 mmol/L for BHB in the blood. The cowside blood BHB test using a hand-held meter (ketometer) has higher levels (above 95%) of sensitivity and specificity than other cowside tests, and can replace laboratory blood BHB testing. Thecowside milk (BHB) test (a Ketolac BHB test strip with a cut-point of ≥200 μmol/L of BHB in milk) is a potentially useful tool for routine herd monitoring for SCK in early lactation dairy cows.

Dijagnoza subkliničke ketoze kod mlečnihkrava

Radojica Djoković, Zoran Ilić, Vladimir Kurćubić, Miloš Petrović, Marko Cincović, Milan P. Petrović, Violeta Caro Petrović

Rezime

Standardnizlatni test za dijagnozu subkliničke ketoze kod mlečnih krava jeste merenje koncentracije beta-hidoksibuterne kiseline (BHB) u krvnom serumu ili krvnojplazmi. Najčešće korišćene granične vrednosti za dijagnozu subkliničke ketoze jesu vrednosti za BHB u krvi veće od 1.2 mmol/L ili veće od 1.4 mmol/L. Cowside blood test zaodređivanjekoncentracije BHB u krvi jeste jednostavni ručni uređaj (ketometar) i može se upotrebljavati na farmi, a ovaj test ima visoki procenat (više od 95%) senzitivosti i specifičnosti u odnosu na druge cowside testove i može zameniti laboratorijska ispitivanja za testiranje BHB u krvi u dijagnozi subkliničke ketoze. Cowside milk BHB test, odnosno ketolac BHB test trakice sa graničnim vrednostima većim od 200 μmol/L za BHB u mleku je supotencijalno korisno sredstvo za rutinski pregled stada mlečnih krava na početku laktacije za dijagnozu subkliničke ketoze.

Ključne reči: mlečne krave, subklinička ketoza, laboratorijski testovi, cowside testovi.

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References

ASL A.N., NAZAFI S., GHASRODASHTIA.R., OLYAEE A. (2011): Prevalence of subclinical ketosis in dairy cattle in the Southwestern Iran and detection of cut off point for NEFA and glucose concentrations for diagnosis of subclinical ketosis. Preventive of Veterinary Medicine, 100: 38-43.

BRUNNER N., GROEGER S., RAPOSO J.C., BRUCKMAIER R.M., GROSS J.J. (2019): Prevalence of subclinical ketosis and production diseases in dairy cows in Central and South America, Africa, Asia, Australia, New Zealand, and Eastern Europe.Translation Animal Science, 3, 19–27.

CARRIER J., STEWARD S., GODDEN S., FETROW J., RAPNICKI P. (2004): Evaluation and use of three cowside tests for detection of subclinical ketosis in early postpartum cows. Journal of Dairy Science, 87, 3725-3735.

CEJNA V., CHLADEK G. (2005): The importance of monitoring changes in milk fat to milk protein ratio in Holstein cows during lactation. Journal of Central Europe Agriculture, 6, 539-546.

COOKN. B, WARD W.R., DOBSON H. (2001): Concentrations of ketones in milk in early lactation, and reproductive performance of dairy cows. Veterinary Record, 148, 769-772.

DE ROOS A.P.W., VAN DEN BIJGAARTH J., HRRLYK J., DE JONG G. (2007): Screening for subclinical ketosis in dairy cattle by Fourier transform infrared spectrometry. Journal of Dairy Science, 90, 1761-1766.

DIRKSEN G., BREITNER W. (1993): A new quick test for semiquantitative determination of betahydroxybutyric acid in bovine milk. Journal of Veterinary Medicine A, 40, 779-784.

DJOKOVIC R., KURCUBIC V, ILIC Z, CINCOVIC M., FRATRIC N., STANIMIROVIC Z., PETROVIC M.D., PETROVIC M.P. (2013): Evaluation of the metabolic status of Simmental dairy cows in early and mid lactation.Animal Science Papers and Reports, 31, 101-110.

DJOKOVIC R.,CINCOVIC M.,KURCUBIC V.,PETROVIC M.,LALOVIC M.,JASOVIC B., STANIMIROVIC Z. (2014): Endocrine and metabolic status of dairy cows during transition period. Thailand Journal of Veterinary Medicine, 44, 59-66.

DJOKOVIC R., CINCOVIC M., BELIC B., TOHOLJ B.,DAVIDOV I.,HRISTOVSKA T. (2015): Relationship between blood metabolic hormones,

metabolites and energy balance in Simmental dairy cows during peripartum period and lactation. Pakistan Veterinary Journal, 35, 163-167.
DJOKOVIC R., CINCOVIC M., ILIC Z., KURCUBIC V., FRATRIC N., PETROVIC M., ANDJELIC B. (2018a): Diagnosis of metabolic disorders in transitional dairy cows based on changes in characteristic blood biochemical indicators. p. 304,The 30th World Buiatrics Congress, August 28 to September 1, Sapporo, Japan.
DJOKOVIC R., JOZEK J., CINCOVIC M., NEMEC M., BELIC B., KLINKOM M., STARIC J. (2018b): Beta-hydroxybutyrate in milk as screening test for subclinical ketosis in dairy cows.p. 127. The 30th World Buiatrics Congress, August 28 to September 1, Sapporo, Japan.
DUFFIELD T., KELTON D.F., LLESLIE K.E., LISSEMORE K.D., LUMSDEN J.H. (1997): Use of test day milk fat and milk protein to detect subclinical ketosis in dairy cattle in Ontario. Canadian Veterinary Journal, 38, 713-718.
DUFFIELD T. (2000): Subclinical ketosis in lactating dairy cattle.Veterinary Clinics of North America: Food Animal Practice,16, 231-253.
DUFFIELD T., LEBLANC S., R. BAGG R., LESLIE K., TEN HAG J., DICK P. (2003): Effect of a monensin controlled release capsule on metabolic parameters in transition dairy cows. Journal of Dairy Science, 86, 1171-1176.
DUFFIELD T., BAGG R. (2002): Herd level indicators for the prediction of high-risk dairy herds for subclinical ketosis, 175-176 In: Proceeding of American Association of Bovine Practitioner, Rome, GA.
EICHER R. (2004): Evaluation of the metabolic and nutritional situation in dairy herds: Diagnostic use of milk components. 23rd World Buiatrics Congress, Québec, Canada.
ELITOK B., SOLAK M., KABU M., ELITOK O.M., SOYLEMEZ Z., FISTIK T. (2010): Clinical, haematological, serum biochemical and cytogenetic study in cows with primary ketosis. Pakistan Veterinary Journal, 30, 150-154.
ENJALBERT F., NICOT M.C., BAYOURTHE C., MONCULON R. (2001): Ketone bodies in milk and blood of dairy cows: Relationship between concentrations and utilization for detection of subclinical ketosis. Journal of Dairy Science, 84, 583-589.
GANTNERV., POTOCNIK K., JOVANOVIC S. (2009): Test-day records as a tool for subclinical ketosis detection. ActaVeterinariaBeograd, 59, 185-191.
GEISHAUSER T., LESLIE K., KELTON K., DUFFIELD T. (2001): Monitoring for subclinical ketosis in dairy herds. Compendium, S65-S71.
GEISHAUSER T., LESLIE K., KELTON D., DUFFIELD T. (1998): Evaluation of five cowside tests for use with milk to detect subclinical ketosis in dairy cows. Journal of Dairy Science, 81, 438-443.
GEISHAUSER T., LESLIE K., TEN HAG,J., BASHIRI A. (2000): Evaluation of eight cow-side ketone tests in milk for detection of subclinical ketosis in dairy cows. Journal of Dairy Science, 83, 296-299.

GONZALEZ F.D., R.MUINOR., PEREIRA V., CAMPOS R. (2011): Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. Journal of Veterinary Science, 12, 251–255.
HANSEN P.W. (1999): Screening of dairy cows for ketosis by use of infrared spectroscopy and multivariate calibration. Journal of Dairy Science, 82, 2005-2010.
HEURER C., LUINGE H.J., LUTZ E.T.G., SCHUKKEN Y.H., VAN DER MAAS J.H., WILMINK H. NOORDHUIZEN J.P.T.M. (2001): Determination of acetone in cow milk by fourier transform infrared spectroscopy for the detection of subclinical ketosis. Journal of Dairy Science, 84, 575-582.
HERDT T.H. (2000): Ruminant adaptation to negative energy balance: Influences on the etiology of ketosis and fatty liver. Journal of Dairy Science, 16, 215-230.
HOLTENIUS, P., HOLTENIUS K. (1996): New aspects of ketone bodies in energy metabolism of dairy cows: A review. Journal of Veterinary Medicine A, 43, 579-587.
HEUWIESER W., FALKENBERG U., IWERSEN M., VOIGTSBERGRERR., PADBERG W. (2007): Evaluation and use of an automated human β-hydroxybuturate (BHBA) test for cowside detection of subclinicalketosis in dairy cattle. Proceedings of the Fortieth Annual Conference, American Association of Bovine Practitioners, Vancouver, British Columbia, Canada, pp: 253-254.
IWERSEN M., FALKENBER U., VOIGTSBERGER R., FORDERUNG D., HEUWIESER W. (2009): Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. Journal of Dairy Science, 92, 2618-2624.
JENKINS T.N., PENA G., RISCO C., BARBOSA C.C., VIERIRA NETO A., KLIBS N.G. (2015): Utility of inline milk fat and protein ratio to diagnose subclinical ketosis and to assign propylene glycol treatment in lactating dairy cows. Canadian Veterinary Journal, 56, 850–854.
JORRITSMA R., BALDEE S.J.C., SCHUKKEN Y.H., WENSING T., WENTIG G.H. (1998): Evaluation of a milk test for detection of subclinical ketosis. Veterinary Quarterly, 20, 108-110.
JOZEK J., CINCOVIC M., NEMEC M., BELIC B., DJOKOVIC R., KLINKOM M., STARIC J. (2017): Beta-hydroxybutyrate in milk as screening test for subclinical ketosis in dairy cows. Polish Journal of Veterinary Science, 20, 507-512.
KINOSHITA A., WOLFC., ZEYNER A. (2010): Studies on the incidence of hyperketonemia with and without hyperbilirubinaemia in cows in Mecklenburg-Vorpommern (in Germany) in the course of the year. Tieraerztliche Praxis, 38, 7-15.
KLEIN M.S., ALMSTETTER M.F., SCHLAMBERGER G., NURNBERGER N., DETTMER K., OEFNER P.J., MEYER H.H.D., WIEDMAN S., GRONWALD W. (2010): Nuclear magnetic resonance and mass spectrometry-based milk

metabolomics in dairy cows during early and late lactation. Journal of Dairy Science, 93, 1539-1550.
KONKOL K., GODDEN S., RAPNICKI P., OVERTON M., ROLLIN E. (2009): Validation of a rapid cowside test for the measurement of blood betahydroxybutyrate in fresh cows. Minnesota Dairy Health Conference, pp: 86-87.
KROGH M.A., TOFT N., ENEVOLDSEN C. (2011): Latent class evaluation of a milk test, a urine test, and the fat-to-protein percentage ratio in milk to diagnose ketosis in dairy cows. Journal of Dairy Science, 94, 2360-2367.
LARSEN T., NIELSEN N. (2005): Fluorometric determination of β-hydroxybutyrate in milk and blood plasma. Journal of Dairy Science, 88, 2004-2009.
NIELEN M., AART M.G.A., JONKER A.G.M., WESING T., SCHUKKEN Y.H. (1994): Evaluation of two cowside tests for the detection of subclinical ketosis in dairy cows. Canadian Veterinary Journal, 35, 229-232.
OETZEL G.R. (2004): Monitoring and testing dairy herds for metabolic disease. Veterinary Clinics of North America: Food Animal Practice, 20, 651-674.
OETZEL G.R. (2007): Herd level ketosis - Diagnosis and risk factors. Preconference Seminar 7C: Dairy Herd Problem Investigation Strategies: Transition Cow Troubleshooting, American association of bovine practitioners 40th Annual Conference, pp. 67-91, September 19, Vancouver, BC, Canada
OSPINA P.A., NYDAM D.V., STOKOLT.,OVERTON T.R. (2010): Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. Journal of Dairy Science, 93, 546-554.
OSBORNE T.M., LESLIE K.E., DUFFIELD T., PETERSSON C.S., TEN HAG J., OKADA Y. (2002): Evaluation of Keto-Test in urine and milk for the detection of subclinical ketosis in periparturient Holstein dairy cattle, 188-189 In: Proceedings of the 35th Conference of the American Association of Bovine Practitioners, Rome, GA.
RICHARD W. (2004): Milk composition as an indicator of nutrition and health. The Breeding, 11, 26-7.
SAMIEI A., LIANG J.B., GHORMANI G.R., HIROOKA H., YAAKUB H., TABATABAEI M. (2010): An evaluation of beta-hydroxybutyrate in milk and blood for prediction of subclinical ketosis in dairy cows. Polish Journal of Veterinary Science, 13, 349-356.
SEIFI H.A., LE BLANC S.J., LESLIE K.E., DUFFIELDT.F. (2011): Metabolic predictors of post-partum disease and culling risk in dairy cattle. Veterinary Journal, 188, 216-220.
SUTHAR V.S., CANALAS-RAPOSO J., DENIZ A., HEUWIESER W. (2013): Prevalence of subclinical ketosis and relationship with postpartum diseases in European dairy cows. Journal of Dairy Science, 96, 2925-2938.

TYOPPONEN J., KAUPPINEN K. (1980): The stability and automatic determination of ketone bodies in blood samples taken in field conditions. ActaVeterinariaScandinavica, 21, 55-61.
WILLIAMSON D.H., MELLANBY J., KREB H.A. (1962): Enzymic determination of D(-)-β-hydroxybutyric acid and acetoacetic acid in blood. Biochemistry Journal, 82, 90-96.
WHITAKER D.A. (1997): Interpretation of metabolic profiles in dairy cows.Cattle Practitioner 5, 57- 60.
VAN KNEGSEL A.T.M., VAN DER DRIFT S.G.A., HORNEMAN M., DE ROOS A.P.W., KEMP B. GRAAT E.A.M. (2010): Ketone body concentration in milkdetermined by fourier transform infrared spectroscopy: Value for thedetection of hyperketonemia in dairy cows. Journal Dairy Science, 93, 3065-3069.
VENKATEWARLU K., CHOUDHURI P.C. (2001): Detection of acetone inmilk: Qualitative and quantitative salicylaldehyde test for diagnosisof subclinical ketosis in dairy cows. Indian Veterinary Journal, 78, 335-336.
VOYVODA H., ERDOGAN H. (2010): Use of a hand-held meter for detectingsubclinical ketosis in dairy cows. Research Veterinary Science, 89, 344-351.
ZHANG Z.,LIU G., WANG H., LI.X.,WANG Z. (2012): Detection of subclinical ketosis in dairy cows. Pakistan Veterinary Journal, 32, 156-160.

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FSHR (EXON 10) GENE POLYMORPHISMS AND ITS ASSOCIATION WITH FERTILITY TRAIT IN EGYPTIAN OSSIMI SHEEP

Salah Abdel-Rahman[1*], Yehia Mustafa[2], Hagar Abd Errasool[2], Hanim Heikal[3], Ayaat Elmaghraby[1]

[1]Department of Nucleic Acid Research, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt
[2]Department of Genetics, Faculty of Agriculture, Alexandria University, Egypt
[3]Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Sadat City University, Egypt
*Corresponding author: salahmaa@gmail.com
Original scientific paper

Abstract: For the association between of Follicle stimulating hormone receptor (*FSHR*) gene (partial part of exon 10) polymorphisms and litter size trait in Egyptian Ossimi sheep, polymerase chain reaction-single stranded conformational polymorphism (PCR-SSCP) and DNA sequencing techniques were developed. Fifty female Ossimi sheep reared under Egyptian conditions were selected according to their litter size. DNA from blood samples of these animals was isolated to amplify 250-bp of the *FSHR* gene influencing litter size production trait in sheep. Based on litter size, 50 animals were selected from the highest to the lowest litter size productivity during three seasons. PCR-SSCP analysis of the *FSHR* gene (250-bp) showed two various genotypes AA and AB with frequencies 0.64 and 0.36, respectively. The frequencies of the *A* and *B* alleles were 0.82 and 0.18, respectively. PCR fragment of *FSHR* gene (191-bp) was sequenced only in the high and low litter size productivity animals (GenBank accession numbers from MG973191 to MG973207, sequentially). The result indicated that 6SNPs (G/71, G/72, G/77, A/110, A/111, A/191) in high fertile animals, while, 10 SNPs (T/1, C/2, T/14, A/69, A/70, A/71, A/74, G/74, A/75, A/136) have found in low fertile animals. Statistically, AA and AB genotypes have no significant differences ($p > 0.05$) on litter size trait in Ossimi sheep. *FSHR* (exon 10) locus was moderate polymorphic (PIC= 0.25) and it can be used for high litter size productivity in Ossimi sheep as a marker-assisted selection (MAS).

Key words: Ossimi sheep, Fertility, *FSHR* gene polymorphisms, PCR-SSCP, DNA sequencing

Introduction

Sheep (*Ovis aries*) have positive economic benefits for human. Where, this kind of animals is preferable as a result of low maintenance cost, adaptation in many environments in the world and resourcefulness for many products such as meat, milk, wool and hides (*Abulyazid et al., 2011*). In Egypt, sheep meat production is more important than fiber production (6% of the total animal meat production). Ossimi sheep (under study) is an Egyptian Nile Valley breed, which is one of the three major Egyptian sheep breeds besides Barki and Rahmani which represent 65% of the total population of sheep in Egypt (*ICARDA annual report, 2007*). Ossimi sheep is a most popular and environmentally adaptable in Lower Egypt. In addition, it is a carpet wool breed (*Mason, 1996*). The mean of Ossimi Kidding rate production under Egyptian condition is 1.22 (lamb/ewe) (*Elshenawy, 1995*).

Improvement of fertility trait in sheep has become desiring interest for breeders, where moderate increases in litter size can equal outsized gains in profit (*Abraham and Thomas, 2012*). This can be achieved by improving the genetic worth of the stock by proper selection methods for improving reproduction rate and production efficiency. Therefore research community constantly searches some affecting genes that can act as marker influencing fertility in animals by marker assisted selection (*Mishra, 2014*). Fertility trait can be improved using marker assisted selection (MAS) which make improvement by detecting variations of fertility or fecundity genes linked with high and low fertile animals (*Abdoli et al., 2016*).

Many genes affect fertility in sheep such as Boroola gene, growth differentiation factor 9 gene and follicle stimulating hormone receptor gene. This study is concerned with *Ovis arias's* FSHR gene which located in chromosome 3 (3:75470485-75470694) in sheep (n=54). Sequence version Oar_v3.1 in *www.ensemble.com* has determined that chromosome 3 has 1994 coding genes and 523 noncoding genes, one of these coding genes is FSHR gene (size= 2088bp) consists of 10 exons coded into FSHR protein (656 amino acids). FSHR gene has a large number of variants which differentiated into two categories missense and synonymous. FSH (Follicle stimulating hormone) secreted by an anterior pituitary regulates gonadal functions in male and female, it is under regulation of gonadotropin releasing hormone (GNRH) as well which activates its receptor in granulosa cells (GCs) in the ovary.

FSHR is a member of the family of G-coupled protein activated FSH which secreted by anterior pituitary regulates gonadal functions in males and females, as well as, it is under regulation of gonadotropin releasing hormone (GNRH) (*Richards and Midglay, 1976*). Thus, the level and the target of hormone response are controlled by mechanisms that determine FSHR levels and cell

specific expression, which are supported by transcription of its genes (*George et al., 2011; Dias et al., 2002; Bogerd, 2007*).

Exon 10 of FSHR gene is large and encodes the C-terminal part of the extra cellular domain (ECD) (hinge region), the transmembrane domain (TMD) and the intracellular domain of the receptor (*Fan & Hendrickson 2005; Jiang et al., 2012*).The objectives of the present study are to detect SNPs (mutations) in a partial region of exon 10 of FSHR gene in a high fertile Egyptian sheep breed (Ossimi sheep) using PCR-SSCP and sequencing techniques. Also, to investigate the relation between the FSHR gene and fertility trait in Ossimi sheep breed under Egyptian conditions.

Materials and Methods

Animals. Blood samples were collected from the jugular vein of 50Ossimi ewes (5ml/ewe) puncture into tubes containing an anticoagulant disodium EDTA. The samples have stored at -20°C until needed for DNA isolation.

DNA isolation. Genomic DNA was isolated from whole blood samples using a commercially available kit (EZ-10 Spin Column Genomic DNA kit for Blood samples, Bio Basic, Canada). Isolated genomic DNA was separated on agarose gel electrophoresis using 1% (w/v) agarose in 0.5X TBE buffer. The gel was photographed using the gel documentation system (Syngene, UK) to visual genomic DNA band.

PCR amplification and genotyping of FSHR gene. 250-bp fragment of exon10 of FSHR gene in 50Ossimi sheep was amplified by PCR using forward (5'-ATCACGCTGGAAAGATGGCATACC-3') and reverse (5'-ACATTGAGCACAAGGAGGGAC-3') primers (*Li et al., 2010*). PCR was performed in a reaction volume of 25µl using 200ng of genomic DNA of each sample, 25 pmol of each primer and 2X Taq DNA polymerase Mix (Bioline, UK). Thermal cycling (Peltier-based Thermocycler, Long Gene) was carried out by initial denaturation at 94°C for 5 min, followed by 30 cycles each at 94°C for 1 min, annealing temperature at 60°C for 30 sec, polymerization temperature at 72°C for 30 sec and final extension at 72°C for 10 min, then the samples were held at 4°C. The amplified DNA fragments were separated on 2% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by Gel Documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada).

Single stranded conformational polymorphism (SSCP). Aliquots of 5µlPCR products were mixed with denaturating solution (98% formamide, 0.025%xylene cyanol, 0.025% bromophenol blue and 10 mM EDTA) and incubated at 98°C for 10 min and then chilled on ice rapidly. Denaturated DNA was loaded on10% PAGE gel (10X 10 CM) in 1X TBE buffer and constant voltage 65V for 5hours.

For staining DNA bands and visualizing, the gel was stained with ethidiumbromide and photographed by using gel documentation system.

Sequencing. DNA sequencing for purified FSHR amplicon had performed by Genetic Analyzer (Applied Biosystems, Hitachi, Japan). Where, the highest eight (2, 4, 5, 6, 7, 13, 16 and 17) and the nine of lowest (21, 22, 23, 24, 25, 26, 28, 40 and 41) litter size animals were sequenced (one direction, forward) at Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt.

Statistical analysis. For litter size, each ewe has three seasons' records (winter, spring and next winter). Random selection of three parities ewes was done and documented in Sakha farm, Agricultural Research Station, Kafer Elshiekh, Egypt.

Polymorphisminformation content (homogeneity).PIC (polymorphism information content) is a measurement that gives an indication how much the locus is highly, moderate or poorly mutated by knowing the alleles frequencies of this locus: PIC= 1- Σ (p_{ij}) x 2, where, pij is the frequency of different ijth allele of studied locus.The calculation of PIC was processed by PIC Calculator (*www.liverpool.ac.uk*).

Q-Q Plot.We put the assumption of litter size values of the 50 animals are normal distributed, so we should test this assumption by run the Q-Q plot using (*IBM SPSS Statistics, Version 22, 2013*). A Q-Q plot is a scatterplot created by plotting two sets of quantities against one another, one of it is litter size values of tested animals and normal disrupted expected value that the program put it. If both sets of quantities came from the same distribution, we should see the points forming a line that's roughly straight.

Independent t-test.The Independent samples t-test compares two genotypes means to determine whether they are significantly different or not. The two independent samples (AA and AB genotypes) are assumed to be drawn from populations with unequal variances (i.e., $\sigma_1^2 \neq \sigma_2^2$), the test statistic *t* is computed as:

$$t=\frac{\bar{X}_1-\bar{X}_2}{\sqrt{\frac{s_1^2}{n_1}+\frac{s_2^2}{n_2}}}$$

Where: $\bar{X}_1$= Mean of first genotype AA; $\bar{X}_2$= Mean of second genotype AB; n_1= Sample size (*i.e.* number of observations) of AA genotype; n_2= Sample size (i.e., number of observations) of AB genotype; s_1= Standard deviation of AA genotype; s_2= Standard deviation of AB genotype,

Least mean squares analysis.Least mean square test was used to examine the relationship between different genotypes and the litter size. The least squares means were used for multiple comparisons in litter size among the different genotypes. Analysis was performed using the general linear model procedure of (*IBM SPSS Statistics, Version 22, 2013*).The litter size trait of Ossimi ewes was analyzed using the following fixed effects model:

$$y_{ijklm}=m+A_i+KS_j+P_k+G_l+e_{ijklm}$$

Where: *yijklm*= phenotypic value of litter size; *m*= population mean; A*i*= fixed effect of the*i*th age of ewe (*i*= 3, 4, 5, 6, 7, 9, 13); *KSj*= fixed effect of the *j*th kidding season (*j*= 1, 2, 3); *Pk*= fixed effect of the *k*th parity (*k* = 1, 2, 3); *Gl*= fixed effect of the *l*th genotype (*l* = 1, 2, 3); *eijklm*= random residual effect of each observation.

Results and Discussion

PCR-SSCP of FSHR gene. A partial part of exon 10 of FSHR gene have amplified by PCR and yielded 250-bp in length (Figure1) and screened for polymorphism by PCR-SSCP. The analysis of PCR-SSCP showed only two genotypes AA and AB (Figure 2). Genotype AA (homozygous) was in thirty-two animals, while AB genotype (heterozygous) found in eighteen animals. The calculated frequencies of AA and AB genotypes were 0.64 and 0.36, respectively, and the frequencies of the A and B alleles were 0.82and 0.18, respectively (Table 1). The results indicated that the Ossimi sheep with AA or AB genotypes have no statistically significant differences ($p\leq 0.05$) on litter size in these animals (50 Ossimi sheep). The PIC and heterozygosity show indications how much this locus mutated. So, by entering data of allele frequencies (A and B) of studied locus of partial part of exon 10 of FSHR gene in PIC calculator (*www.liverpool.ac.uk*) the heterozygosity and PIC are 0.295 and 0.25, respectively. In general, PIC is an ideal index to evaluate polymorphism on the fragment of the gene, as follows: PIC> 0.50 (high polymorphism), 0.25 <PIC< 0.50 (moderate polymorphism) and PIC< 0.25 (low polymorphism). In our results, PIC indicates that polymorphism in this fragment (exon 10 of FSHR gene) has moderate polymorphism and suitable to be candidate gene marker for fertility trait breeding. On the other hand, the heterozygosity of this locus indicates high heterozygosis and genetic diversity in Ossimi sheep; hence, it can be selected as a genetic breeding marker.

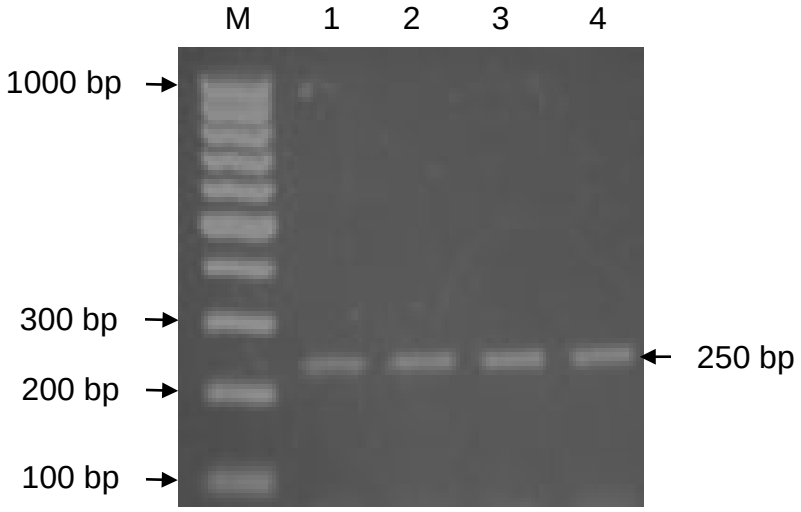

Figure 1. PCR products (250-bp) generated by the FSHR gene (part of exon10) primers. Where, lane M is a molecular weight marker (100 bp) and lanes 1-4 are female Ossimi sheep (as an example)

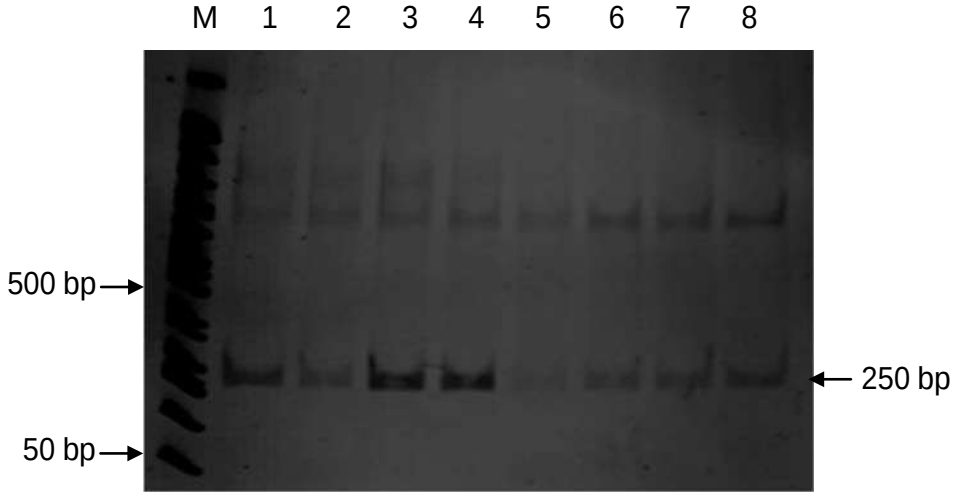

Figure 2. PCR-SSCP analysis of FSHR gene (250-bp). Lanes 1-4 represent AB genotype and lanes 5-8 represent AA genotype. Lane M is DNA marker (50-bp)

Independent t-test. An independent t-test was conducted to compare differences on litter size mean in AA and AB genotypes by knowing the significance. According to this test AA genotype and AB genotype (M= 1.24, SD= 0.33) and (M= 1.2, SD= 0.28); t (125)= 0.685, p= 0.495. This result suggested that no

significance differences between two genotypes mean and genotype does not have an effect on litter size.

Least square analysis. A least mean square test has performed based on data to determine if there was a significance relationship between genotypes, age, parity and season on litter size. The litter size of studied 50 animals of Ossimi sheep was significantly affected by age and not influenced by the other factors. The t-statistics for the slope was significant at the 0.05 critical alpha level (Table 1), t (149)= -0.07, p= 0.9. So, there was a negative significant relationship between Genotypes and litter size. Furthermore, only 4% of the variability of litter size could be explained by these tested factors.

Table 1. Genotypic and allelic frequencies of *FSHR* gene, least mean squares and estimated standard errors of litter size in Ossimi sheep

Gene	Genotypes & alleles	Number of animals	Genotype and allele frequency	PIC	Heterozygosity	Litter size ± SE
FSHR	AA	32	0.64	0.25	0.295	0.74 ± 0.67^{a}
	AB	18	0.36			1.4 ± 0.36^{a}
	A	-	0.82			-
	B	-	0.18			-

Least squares means followed by the same letter means no significant differences at P< 0.05.

Sequencing. For SNP's detection, the fragment 250-bp of FSHR gene was sequenced, aligned and accessioned (Figure 3). Where, the eight highest twins production animals (2, 4, 5, 6, 7, 13, 16 and 17) and the nine lowest (21, 22, 23, 24, 25, 26, 28, 40 and 41) were sequenced and aligned (https://www.genome.jp/tools-bin/clustalw)and compared with reference sequence (*Ovis aries* FSHR gene (transcript ID) ENSOART00000004728.1). These 17 sequences were accessioned (MG973191-MG973207) by www.ncbi.nlm.nih.gov (191-bp). Table 2 shows the SNPs in the sequenced fragment (191-bp) in these animals which highlighted in green color for high fertile animals (2, 4 and 16), orange color for low fertile animals (24, 25, 26, 28 and 41).

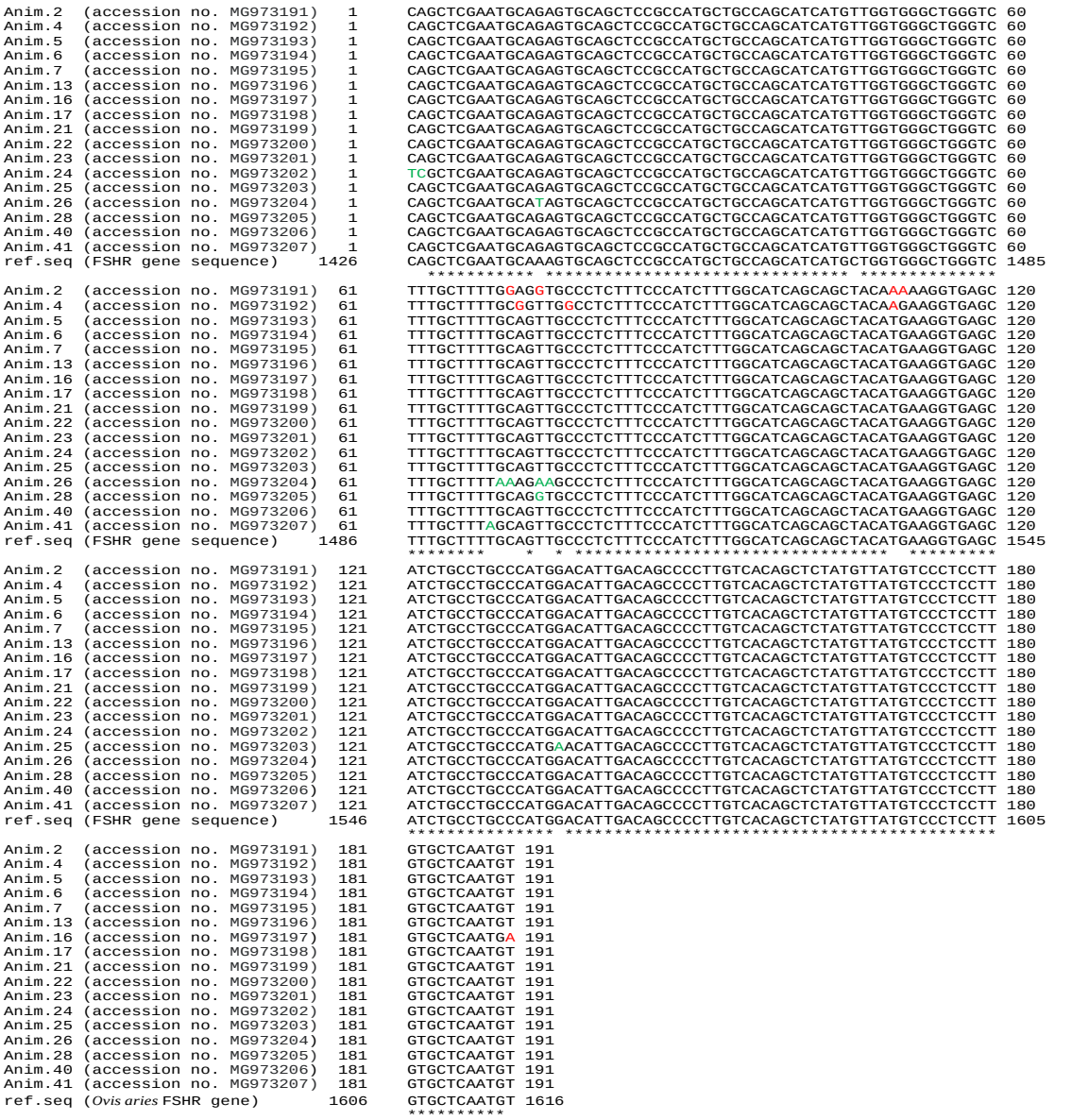

Figure 3. DNA sequence alignment of *FSHR* gene (191-bp) among the 17 female Ossimi sheep and *Ovis aries* FSHR gene (transcript ID) ENSOART00000004728.1. The asterisks represent the similarity

Table 2. Genotypes and nucleotide sequence variation of the eight animals ordered from high to low litter size

Animal no.	Litter size	Genotype	Nucleotide sequence variations													
			Amplicon nucleotide no.													
			1	2	14	69	70	71	72	74	75	77	110	111	136	191
2	2	AA	C	A	G	T	G	**G**	A	T	T	C	**A**	**A**	G	T
4	1.6	AA	C	A	G	T	G	C	**G**	T	T	**G**	**A**	G	G	T
16	1.3	AB	C	A	G	T	G	C	A	T	T	C	T	G	G	**A**
24	1	AA	**T**	**C**	G	T	G	C	A	T	T	C	T	G	G	T
25	1	AB	C	A	G	T	G	C	A	T	T	C	T	G	**A**	T
26	1	AA	C	A	**T**	T	**A**	**A**	A	**A**	**A**	C	T	G	G	T
28	1	AA	C	A	G	T	G	C	A	**G**	T	C	T	G	G	T
41	1	AB	C	A	G	**A**	G	C	A	T	T	C	T	G	G	T
Gene nucleotide no.			1426	1427	1439	1494	1495	1496	1497	1499	1500	1502	1535	1536	1561	1616

Results of virtual (in silco) translated FSHR gene showed13 amino acids variants in FSHR peptide which may be occurred, as a result of SNP's, in high (2, 4 and 16) and low (24, 25, 26, 28 and 41) litter size production animals by Mega software version 7 (*Kumar et al., 2016*). One amino acid variant is synonymous and the other 12 amino acids variants are non-synonymous (Table 3).

Table 3. Reference codon, mutated codon, amino acids variation and synonymous/non-synonymous variants

Animal no.	Genotype	Base number	Reference codon	Mutated codon	Peptide amino acid no.	Amino acid	Synonymous or Non-synonymous
2	AA	71	GCA	GGA	460	Ala/Gly	Non-syn
		110, 111	ATG	AAA	473	Met/Lys	Non-syn
4	AA	72	GCA	GCG	460	Ala/Ala	Synonymous
		77	GCC	GGC	462	Ala/Gly	Non-syn
		110	ATG	AAG	473	Met/Lys	Non-syn
16	AB	191	GTC	GAC	500	Val/Asp	Non-syn
24	AA	1, 2	CAG	TCG	437	Glu/Ser	Non-syn
25	AB	136	GAC	AAC	482	Asp/Asn	Non-syn
26	AA	14	AAA	ATA	441	Lys/Ile	Non-syn
		70,71	GCA	AAA	460	Ala/ Lys	Non-syn
		74,75	GTT	GAA	461	Val/ Glu	Non-syn
28	AA	74	GTT	GGT	461	Val/ Glu	Non-syn
41	AB	69	TTT	TTA	459	Phy/Leu	Non-syn

In this study, fifty Ossimi sheep animals were divided into two groups according to their litter size (1.2 lamb/ewe); high fertile group (litter size= 2, 1.6 or 1.3) and low fertile group (litter size= 1).For understanding the relationship between polymorphism in FSHR gene locus (part of exon 10) and fertility trait in Ossimi sheep, SSCP and sequencing techniques were used, and then this relationship was statistically analyzed. However, we found then this locus is moderate polymorphic (PIC=0.25) and has two alleles (A and B) and two genotypes (AA and AB) in all animals. AA genotype frequency was 0.64 and AB genotype was 0.36, hence, A allele frequency was 0.82 and B allele was 0.18.

For 191-bp FSHR fragment sequencing, we found 6 SNPs in high fertile animals (G/71, G/72, G/77, A/110, A/111, A/191). While in low fertile animals, we found 10 SNPs (T/1, C/2, T/14, A/69, A/70, A/71, A/74, G/74, A/75, A/136). Statistically, AA and AB genotypes have no significant differences ($p>0.05$) in litter size, as well as, regression statistics considered that 10% of genotype differences could describe the differences of litter size in this sample (fifty animals). The results have a valuable meaning in choosing this polymorphic locus of FSHR as a candidate gene for research and breeding programs of fertility trait. These results preliminary showed that FSHR gene is a major gene that influences the prolificacy of experimental animals or a molecular marker in close linkage with prolificacy trait. Consequently, FSHR gene has considered as a possible candidate gene for increasing litter size in Ossimi sheep.

In the previous related studies, FSHR gene polymorphisms and its association with litter size in animals classified to two categories according to regulatory and their translated regions of FSHR gene. An example for this, the 5' regulatory region of FSHR gene had a significant effect on fertility trait and proofed as a marker-assisted selection (MAS) in the improvement programs. Where, *Chu et al.(2012)*found that in 5' regulatory region of FSHR gene 2 mutations (-681 C/T and -629 C/T) in Hu sheep and 3 mutations (-200 G/A, -197 G/A and -98 T/C) in small tail Han sheep. In the same study, the author found that the heterozygous Small tail Han sheep (EG and EF) had 0.89 lambs more than the homozygous. In another study on sheep, *Wang et al*.,(*2015*) found that the CC genotype of FSHR gene in small tailed and Han sheep had lamb production more than those the TC and TT genotypes with 0.52 ($p< 0.01$) and 0.72 ($p< 0.01$), respectively.

The study in goat, Xiangdong Black, NanJiang Brown and Guizhou Black litter size was affected significantly by FSHR gene ($p<0.05$) only in Guizhou Black goat and BB genotype was significantly higher than AA and AB genotypes (*Zhu et al., 2007*). On the other hand, in these three goat breeds, the author found the same five SNPs (-93C/A, -80 G/C,-63 C/A, -56C/G and -55 T/C) of 5' regulatory region of FSHR gene which were not necessarily affect significantly on litter size. In another study in three goat breeds (Jining Grey, Beor and Inner Mongolia Cashmere), *Guo et al*.(*2013*) found three genotypes CC, CD and DD. Only in Jining Grey goat, the author noticed that the CC genotype had 0.46 and 1.3 kids more than the other CD and DD genotypes, respectively. While CD genotype had 0.57 kids more than DD genotype. On the other hand, the two transversions in 70T/A and 130G/C positions of amplified sequenced region of FSHR gene, are found in DD and CC genotypes in the three previous goat breeds. In Yunling Black and Boer goat breeds, *Cui et al., (2009)* found four mutations in the coding region of FSHR gene 486C/A (162Arg/Ser), 1042C/G (348Pro/Ala), 1930T/A (644 Phe/Ile) and 2036T/C (679Thr/Ile). In Yunling Black goat only, the author recognized that the FSHR mRNA and protein expression levels were significantly

and positively correlate with fecundity, as well as reduction levels may be associated with the fewer observed oocytes and fewer follicles.

In cows (Bostaurus and Bosindicus), *Marson et al., (2008)* evaluated the effect of polymorphisms in exon 10 of FSHR gene using PCR-RFLP (*Alu*I restriction enzyme) and the results showed three genotypes GG, GC and CC. Genotype GC revealed higher pregnancy rate (66%) than the two other genotypes (GG and CC). According to the author, has observed no significant effect of the three genotypes (GG, GC and CC) in both Bostaurus and Bosindicus beef populations. In another research, *Yang et al., (2010)* found a mutation (-278 G/A) and three genotypes (CC, CD and DD) in Chinese Holstien cows. Genotype CC had a significant increase in the total number of ova and transferable embryos than the other two genotypes, which had an absence of super ovulation response. Also, *Sharifiazdi et al., (2018)* emphasized that this mutation (-278 G/A) may affect some reproductive variables in Holstein dairy cows.

Lastly in Erhualian and Yorkshire sows, *Zhang et al., (2002)* detected a transition mutation (566 C/T) in exon 7 of FSHR gene. Consequently, a prediction of Ala/Val substitution has done at residue 189 in ECD and this polymorphism significantly associated with the total born number and number born alive. However, our results with the other previous mentioned studies confirm that the FSHR gene in Ossimi sheep will be a strong candidate gene for further applications in marker-assisted selection (MAS) and breeding programs for fertility trait improvement.

Conclusion

PCR-SSCP genotyping and DNA sequencing techniques were developed to study the association between FSHR gene polymorphism and fertility trait in fifty Ossimi sheep. The results indicated two genotypes (AA and AB) which have no significant differences ($p > 0.05$) on litter size trait, but this locus (partial part of exon 10) was moderate polymorphic (PIC= 0.25). Using DNA sequencing, in the high fertile animals 6 single nucleotide polymorphisms (SNP's) at 6 different positions have observed G/71, G/72, G/77, A/110, A/111, A/191. While in the low fertile animals, 10 SNPs (T/1, C/2, T/14, A/69, A/70, A/71, A/74, G/74, A/75, A/136) were observed. Thus, these findings can be used as marker-assisted selection (MAS) for high fertility trait in Ossimi sheep reared under Egyptian conditions.

Polimorfizmi gena FSHR (ekson 10) i njegova povezanost sa osobinama plodnosti ovaca rase osimi u Egiptu

Salah Abdel-Rahman, Yehia Mustafa, Hagar Abd Errasool, Hanim Heikal, Ayaat Elmaghraby

Rezime

Za povezanost između polimorfizama gena receptora za stimulaciju folikula (Follicle stimulating hormone receptor - *FSHR*) (delimični deo egzona 10) i osobine veličine legla ovaca egipatske osimi rase, razvijene su tehnike lančana reakcija polimeraza - jednocevni konformacioni polimorfizam (PCR-SSCP) i tehnike sekvenciranja DNK. Pedeset ženskih grla rase osimi uzgajanih u egipatskim uslovima odabrano je prema veličini njihovog legla. DNK iz uzoraka krvi ovih životinja je izolovana da amplifikuje 250 bp gena FSHR gena koji utiču na proizvodnu osobinu veličina legla kod ovaca. Na osnovu veličine legla, 50 životinja je izabrano od najvećih do najnižih vrednosti veličine legla tokom tri sezone. PCR-SSCP analiza *FSHR* gena (250-bp) pokazala je dva različita genotipa AA i AB sa frekvencijama 0,64 i 0,36, respektivno. Frekvencije *A* i *B* alela su bile 0,82 i 0,18, respektivno. PCR fragment *FSHR* gena (191-bp) je sekvencioniran samo u životinjama sa visokom i niskom produktivnošću veličine legla (GenBank pristupni brojevi od MG973191 do MG973207, sekvencijalno). Rezultat je pokazao da je 6SNPs (G/71, G/72, G/77, A/110, A/111, A/191) utvrđen kod visoko plodnih životinja, dok je 10 SNPs (T/1, C/2, T/14, A/69, A/70, A/71, A/74, G/74, A/75, A/136) utvrđen kod nisko plodnih grla. Statistički, genotipovi AA i AB nemaju značajnih razlika ($p > 0,05$) na osobini veličine legla ovaca rase osimi. Lokus *FSHR* (ekson 10) bio je umereno polimorfan (PIC = 0,25) i može se koristiti za visoku produktivnost veličine legla osimi ovaca kao metoda selekcije uz pomoć markera (MAS).

Ključne reči: Osimi ovce, plodnost, polimorfizmi gena *FSHR*, PCR-SSCP, sekvenciranje DNK

References

ABDOLI R., ZAMANI P., MIRHOSEINI S.Z., GHAVI HOSSEIN-ZADEH N., NADRI S. (2016): A review on prolificacy genes in sheep. Reproduction in Domestic Animals, 51, 631-637.

ABRAHAM A., THOMAS N. (2012): Role of fecundity genes in prolificacy of small ruminants. Journal of Indian Veterinary Association, 10, 34-37.

ABULYAZID I., ABDALLA M.S., SHARADA H.M., HASSANIN W.F. (2011): Prolificacy detection in Egyptian sheep using RFLP-specific PCR. Egyptian Academic Journal of Biological Sciences, 3, 1–4.

BOGERD J. (2007): Ligand-selective determinants in gonadotropin receptors. Molecular and Cellular Endocrinology, 260–262, 144–152.
CHU M. X., GUO X. H., FENG C. J., LI Y., HUANG D.W., FENG T., CAO G. L., FANG L., DI R., TANG Q. Q., MA Y. H., LI K. (2012): Polymorphism of 5′ regulatory region of ovine *FSHR* gene and its association with litter size in Small Tail Han sheep. Molecular Biology Report, 39, 3721–3725.
CUI H. X., ZHAO S. M., CHENG M. L., GUO L., YE R. Q., LIU W. Q., GAO S. Z. (2009): Cloning and expression levels of genes relating to the ovulation rate of the Yunling black goat. Biology of Reproduction, 80, 219–226.
DIAS J. A., COHEN B. D., LINDAU-SHEPARD B., NECHAMEN C. A., PETERSON A. J., SCHMIDT A. (2002): Molecular, structural, and cellular biology of follitropin and follitropin receptor. Vitamins and Hormones, 64, 249–322.
ELSHENNAWY M. (1995): Sheep development program in Egypt, Strategies for sheep and goat breeding. Zaragoza, 27-32. http://om.ciheam.org/article.php?IDPDF=9 6605538.
FAN Q.R., HENDRICKSON W.A. (2005): Structure of human follicle-stimulating hormone in complex with its receptor. Nature, 433, 269–277.
GEORGE J.W., DILLE E.A., HECKERT L.L. (2011): Current concepts of follicle-stimulating hormone receptor gene regulation. Biology of Reproduction, 84, 7-17.
GUO X., YANG L., MINGXING C., FENG C., DI R., LIU Q., FENG T., CAO G., HUANG D., FANG L., TANG Q., MA Y., LI K. (2013): Polymorphism of 5′regulatory region of caprine *FSHR* gene and its association with litter size in Jining Grey goat. Turkish Journalof Veterinary and Animal Sciences, 37, 497-503.
IBM Corp. Released (2013): IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.
ICARDA. ICARDA Annual Report 2006 (2007): International Center for Agricultural Research in the Dry Areas. Aleppo, Syria; p.130.
JIANG X., LIU H., CHEN X., CHEN P.H., FISCHER D., SRIRAMAN V., YU H.N., ARKINSTALL S., HE X. (2012): Structure of follicle-stimulating hormone in complex with the entire ectodomain of its receptor. Proceeding of The National Academy of Sciences, 109, 12491–12496.
KUMAR S., STECHER G., TAMURA K. (2016): MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33, 1870-1874.
LI Y.J., ZHANG L., SHANG L.Q., WANG H.F., ZOU H., ZHANG H., JI D.J. (2010): Genetic polymorphisms at three loci of PRLR and FSHR Gene correlate with litter size in Chinese Haimen goat. Journal of Animal and Veterinary Advances, 9, 2835-2838.
MARSON E.P., FERRAZ J.B., MEIRELLES F.V., BALIEIRO J.C., ELER J.P. (2008): Effects of polymorphisms of LHR and FSHR genes on sexual precocity in

a *Bostaurus*× *Bosindicus*beef composite population. Genetics and Molecular Research Journal, 7, 243–251.
MASON I.L. (1996): A World Dictionary of Livestock Breeds, Types and Varieties. Fourth Edition. C.A.B International, 273.
MISHRA C. (2014): Genetic basis of prolificacy in sheep. International Journal of Livestock Research, 4, 46-57.
RICHARDS J.S., MIDGLEY A.R. (1976): Protein hormone action: a key to understanding ovarian follicular and luteal cell development. Biology of Reproduction, 14, 82–94.
SHARIFIYAZDI H., ABDOLAH M., ZAHRA G. (2018): Characterization of polymorphism in the FSH receptor gene and its impact on some reproductive indices in dairy cows. Animal Reproduction Science, 188, 45-50.
WANG W., SHIJIA L., FADI L., XIANGYU P., CHONG L., XIAOXUE Z., YOUJI M., YONGFU L., RUI X., TINGFU L. (2015): Polymorphisms of the Ovine BMPR-IB, BMP-15 and FSHR and Their Associations with Litter Size in Two Chinese Indigenous Sheep Breeds. International Journal of Molecular Sciences, 16, 11385-11397.
YANG W.C., LI S.J., TANG K.Q., HUA G.H., ZHANG C.Y., YU J.N., HAN L., YANG L.G. (2010): Polymorphisms in the 50 upstream region of the FSH receptor gene, and their association with superovulation traits in Chinese Holstein cows. Animal Reproduction Science, 119, 172–177.
ZHANG S.J., XIONG Y.Z., DENG C.Y., ZHENG R., JIANG S.W., XIAO S.M., XIA Y., XU J.X., LIU X.H., WANG C.F., YUAN Z., GONG S.Y. (2002): Study on follicle stimulating hormone receptor as candidate gene for litter size in pigs. Journal of Huazhong Agricultural University, 21, 506–508.
ZHU J., YANG S.L., OUYANG X.X., LIU H.X., HU S.G. (2007): Relationship between polymorphism of follicle stimulating hormone receptor gene and litter size traits in goats. Grassfeeding Livestock 1, 12–15.

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SOME QUANTITATIVE GENETIC TRAITS IN VIETNAMESE INDIGENOUS NOI CHICKEN FROM 0 TO 28 DAYS OLD

Do Vo Anh Khoa[1,2,3], Nguyen Thi Hong Tuoi [1,3], Nguyen Thao Nguyen [1,3], Nguyen Thi Dieu Thuy[4], Shin Okamoto[5], Kataro Kawabe[5], Takeshi Shimogigri[5]

[1]Can Tho University, Vietnam
[2]Southwest University of Science and Technology, China
[3]Animal Husbandry Association of Vietnam, Vietnam
[4]Institute of Biotechnology, Vietnam
[5]Kagoshima University, Japan
Corresponding author: dvakhoa@ctu.edu.vn, Tel.: +84-918026653
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Abstract: The aim of this study is to characterize some quantitative traits of Noi chicken, one of the Vietnamese famous native breeds for meat quality and fighting, at the stage of 0-28 days old. Therefore, 742 chicks were used to measure, record, analyze and evaluate on these traits. As results, there was significant difference in observation traits among stages of age (P=0.000). Interesting, the wings (+64.88%) and keel lengths (+58.83%) rapidly developed within the first week of life, followed by the most of other dimensions, especially breast diameter (+31.85%), thigh diameter (+71.17%) in the second week of age. An increase in the size of the measurements was due to development of the skeleton at observing time points along the experiment. However, there was strong development of muscle tissue in the second week of life as rate of the breast and thigh diameter was greatest. Noi chicks consumed amount of feed of 16.54 g/bird/day and gained a weight of 6.98 g/bird/day. Thus, their feed conversion ratio was 2.37 at the stage of 0-28 days old. Perhaps a direct positive relationship between observed traits and age were randomly established according biological characteristics of animal. This work provided initial benchmarks of Noi chicks for further studies.

Key words: indigenous Noi chicken breed, measurements, quantitative traits

Introduction

Noi, one of famous local chicken breeds, are playing an important role in increase of farmer's income in rural area of Vietnam. Although Noi is known as an excellent breed with good meat quality, adaptability and natural disease resistant, there is not any evidence for these. Similarly to some countries in the world, Vietnam also increases in demands of native chicken products, in which Noi chicken seems first choices for Southern consumers. At householders in the Mekong delta, Noi is popularly raised in many different ways (backyard, semi-scavenging, intensive/semi-intensive conditions, etc.) for commercial objectives as well as householders own consumption. Until now, a few of studies worked on Noi chickens indicated that *(i)* most of males had the black-red mixed feather color (~42% of population), while females were in brown commonly (~55.6% of population). Most of their shanks were in yellow (42.5-46.4%). Body weight of adult male and female was 2.89 kg/bird and 1.77 kg/bird, respectively (*Ngu et al., 2016*); *(ii)* Earlier studies also indicated that if Noi was raised as a backyard chicken at householders, laying on hers first egg was later than other Vietnamese local chicken breeds. A hen produced around 48.35 eggs/year with an average weight of 48.87 g/egg (Cv% = 3.68) and a yolk percentage of 37.77% (*Quyen and Son, 2008*); *(iii)* Polymorphisms on some genes such as GH, GHR, GHSR, insulin gene (*Khoa et al., 2013*) PRL, VIP, VIPR-1, BMPR-IB, MTNR1C, NPY, DRD2, and IGF-I (*Vu and Ngu, 2016*) were found in Noi chickens; and *(iv)* Recently, genotypes at the NPY/*Dra*I, VIPR-1/*Taq*I and VIPR-1/*Hha*I mutation points significant associated with total egg production of laying Noi chickens of 20 laying weeks were also found (*Ngu et al., 2015*). Generally, although there are many previous studies, most of them focused on traits for genetics and egg performance in Noi chickens. Therefore, growth, feed intake, feed conversion ratio and some measurements in Noi chicks from birth to 28 days old will be characterized in this study.

Materials and methods

Experimental management

A total of 742 chicks generated from the Noi resource population in two previous studies (*Vu and Ngu, 2016; Ngu et al., 2015*) was allotted to 6 private cages with a density of 0.3 m^2/ bird in an opened housing system. One month before the arrival of the chicks, the house was washed, cleaned and sprayed by water, detergent, antiseptic and lime.

The infrared light and thermometer were used to keep a stable warm temperature (around 30^{o}C) for chicks during experiment time. *Ad libitum* feeding was offered to

chicks according to instruction of GreenFeed Vietnam Join stock Company. It is declared that GF1312 feed for backyard chicks is made by main feed stuff ingredients such as soybean cake, fish meal, rice bran, broken rice, corn, wheat bran, cassava mi, amino acids, vitamins and minerals (Table 1). Along the experiment, clean water mixed with ProBAC (containing *Bacillus subtilis, Bacillus licheniformis, Bacillus clausii and Bacillus coagulans* with concentration of $\geq 10^{11}$CFU/g, 1g ProBAC for 5 litters of water) was fully provided for chicks. Supplementation of multivitamins was done at time points of high temperature or after vaccinating with Gumboro and IBV vaccines at the fifth and fourteenth day of age, respectively.

Measured parameters

Body weight, average daily gain, feed intake, feed conversion ratio, beak length, skull length, skull width, neck length, back length, wings length, thigh length, tarsus length, keel length, breast diameter, and thigh diameter (*Francesch et al., 2011; Ojedapo, 2013*) were collected on all experimental chickens at every week of age.

Table 1. Nutrient component of the experimental feed

Items	0-30 days old (GF1312)
Crude protein (min), %	20
Humidity (max), %	14
Crude fiber (max), 5	5
Methionine and cysteine (min), %	0.75
Metabolism energy (min), kcal/kg	2,900
Calcium, %	0.8-1.2
Phosphorus, %	0.6-1.0
Total lysine (min), %	-

Statistical analysis

Chicks were divided into three different groups of hatching weight ($\mu \pm \sigma$) or various stages of age before using R software (ver. 3.4.2) for analyzing statistics. Measurements at various time points were analyzed by descriptive statistics. General Linear Model was applied to analyze effects of groups of hatching weight or stages of age on observed traits.

Results and discussion

In this study, it was demonstrated that all measurements were increased due to aging of chicks (Table 2). These increments were significantly found among

different stages of age (P=0.000) (Table 3). Wings length (64.88%) and keel length (58.83%) developed very early with the highest rate in the first week of life, followed by developing rate of beat length (14.03%), skull length (7.71%), skull width (9.47%), neck length (24.07%), back length (25.29%), thigh length (25.95%), shank length (24.87%), breast diameter (31.85%), thigh diameter (71.17%) at the second week compared with other weeks. No highest measurement was found at the third week of rearing period. Based on our observation, at the end of the second week old, some of chicks could fly 1-2 m far and 0.2-0.4 m high although the first few feathers on their wings had just formed. The early development of wings length and feathers helps the chicks fly and move faster at the beginning of feeding as natural competition among them. This also helps raiser easily to assess health status of herd. At the fourth weeks of age, black length (113.77 vs. 140.1 mm), shank length (25.89 vs 106.9 mm), and keel length (68.38 vs 83.41 mm) of Noi chicks showed lower than those of Marshall ones (*Ojedapo, 2013*) because of difference on daily gain and body weight (225.56 vs 785.63 g/bird) between breeds, respectively. However, it was in contrast for thigh length (89.7 vs 105.04) and breast girth (72.1 vs 115.4) between breeds while their hatching weights were approximate (35.00 vs 34.8 g/bird, respectively) together. This may be due to genetic, feed consumption and weight gain crossing between breeds. Clearly, Marshall chicks' consumption (125.07 vs 152.70 g/bird/day) and gain (51.36 vs 85.80 g/bird/day) was respectively closed to Cobb (*Amao et al., 2015*) but far from Noi chicks. Perhaps, this is the special stage for development of a large skeleton as a basic foundation for development of muscle later in Marshall chicks. More addition, according to *Molenaar et al. (2008)* hatchling length seems to be a better parameter to predict subsequent chick performance, excluding FCR, than hatchling weight.

It is also known that Aseel is also one of famous fighting chicken breeds in India. Recently, *Rajkumar et al. (2017)* reported that at 28 days old, Aseel' shank length was 43.5 mm higher than Noi' one (25.89 mm) in this study. *Rajkumar et al. (2017)* implied that because of fighting purpose, Aseel cocks were selected with longer and stronger shanks and legs either naturally or by farmers. Noi chicks in this study were not selected for those.

In the rural area of Vietnam, besides development of industrial poultry farms investigated by companies, most of the households are dependent on indigenous chickens for their livelihoods and their own consumption. Unfortunately, large amount of genetic potential of the indigenous chicken breeds has not been fully recognized, realized, and utilized. Probably, data linkage among research programs was not good for further objectives. Many studies on the quantitative genetic traits (morphology, productivity and economic efficiency, etc.) in indigenous chickens were done well, but the collected data have no breadth, depth, uniformity and completeness. Therefore, it is not easy to summarize all of them in a set and some

data are still absent in Table 5. Comparing the obtained data in this study with the other data in previous studies in population of Vietnamese native breeds at the first month of age could be fixed for every trait.

A linear increase of the average daily gain, feed intake and feed conversion ratio by aging was found statistically significant in this study (P=0.000) (Table 4). This is consistent with the biological characteristics for growth and development in chickens at early stages of age. During the experimental time, each chick gained 6.98 g/bird and consumed 16.54 g of feed/bird. That means the chick converted 2.37kg of feed into 1 kg of live weight. It demonstrated that feed conversion ratio in Noi chicks was lower than that of other indigenous ones of Vietnam such as Tau Vang chick (*Khoa & Thong, 2013*) and Long Cam chick (*Mui et al., 2012*), Thai native chicken of Thailand (*Jaturasitha et al., 2002*), or other indigenous breeds of Ethiopia such as Tilili, Gellilia, Debre-Elliasm, Mello-Hamusit, Gassay, Guangua, Mecha, and RIR (*Hassen et al., 2006*), but higher than Nhieu Ngon chick of Vietnam (*Thinh et al., 2016*) (Table 5). Although Noi chicks were superior to other indigenous breeds, their feed conversion ratio was 1.5 times higher than that of Abor Acres chicks (*Jaturasitha et al., 2002*) (Table 5). Differences in weight gain, feed consumption and feed conversion ratio among chicks may be due to influence of breeds, nutrition management, nursing conditions, etc. through underdeveloped and developing countries. Furthermore, it was shown that the hatching weight of Abor Acres (44.7 g/bird) was 1.18 to 1.73 times higher than that of other native breeds (ranged from 25.9 to 38.0 g/bird) and 1.28 times compared with Noi chicks. Hatching weight may be influenced by the size, weight and quality of the eggs which they are not easy quickly to improve by genetic and nutritional means.

Table 2. Descriptive statistics for body weight and measurements

Traits	At birth	7 days old	14 days old	21 days olds	28 days old
BW	34.83±5.03	57.79±8.48	104.68±17.37	153.77±29.72	225.56±44.89
Range	20.00-50.00	34.00-88.00	50.00-172.00	59.00-315.00	80.00-370.00
CV%	0.14	0.15	0.17	0.19	0.20
BL	12.86±0.55	14.48±0.65	16.49±0.79	18.50±1.13	20.88±1.06
Range	11.45-15.07	11.30-16.41	13.43-19.97	15.16-21.80	16.54-27.72
CV%	0.04	0.04	0.05	0.06	0.05
SL	17.52±0.65	18.23±0.56	19.59±0.66	20.77±0.78	22.26±0.86
Range	14.29-19.01	14.98-19.90	14.56-21.18	18.24-23.85	19.17-24.97
CV%	0.04	0.03	0.03	0.04	0.04
SW	15.98±0.60	17.04±0.76	18.59±0.83	19.77±0.80	21.49±0.99
Range	13.68-17.94	14.62-18.90	14.96-20.26	17.06-22.26	18.00-29.25
CV%	0.04	0.04	0.04	0.04	0.05
NL	41.57±3.81	45.61±3.67	56.69±5.58	66.74±5.74	76.30±5.89
Range	30.00-50.00	30.00-57.00	45.00-100.00	55.00-95.00	57.00-95.00
CV%	0.09	0.08	0.10	0.09	0.08
BaL	57.09±3.88	66.68±3.82	83.41±5.74	99.70±7.55	113.77±7.72
Range	46.00-69.00	55.00-80.00	67.00-100.00	80.00-120.00	80.00-135.00
CV%	0.07	0.06	0.07	0.08	0.07
WL	118.26±7.12	154.70±9.11	197.85±14.01	229.14±16.57	262.47±18.33
Range	87.00-140.00	115.00-175.00	100.00-240.00	150.00-280.00	190.00-315.00
CV%	0.06	0.06	0.07	0.07	0.07
TL	51.49±3.30	59.27±3.91	74.60±5.37	87.94±7.14	105.04±8.67
Range	41.00-63.00	46.00-70.00	55.00-90.00	63.00-115.00	72.00-175.00
CV%	0.06	0.07	0.07	0.08	0.08
ShL	14.64±1.86	15.41±0.95	19.18±1.61	22.32±2.28	25.89±2.07
Range	10.00-23.00	13.00-19.00	13.00-24.00	12.00-28.00	20.00-36.00
CV%	0.13	0.06	0.08	0.10	0.08
KL	20.49±2.29	32.30±4.97	46.90±4.65	58.32±5.21	68.38±5.84
Range	14.00-27.00	20.00-60.00	30.00-66.00	39.00-75.00	45.00-86.00
CV%	0.11	0.15	0.10	0.09	0.09
BD	75.05±5.05	87.03±6.52	114.32±7.02	133.53±9.19	155.40±11.01
Range	56.00-85.00	65.00-103.00	89.00-132.00	90.00-150.00	106.00-188.00
CV%	0.07	0.07	0.06	0.07	0.07
TD	18.25±1.93	21.12±1.40	36.03±3.34	43.78±4.66	49.79±5.10
Range	13.00-22.00	15.00-24.00	23.00-41.00	24.00-55.00	35.00-66.00
CV%	0.11	0.07	0.09	0.11	0.10

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

Table 3. Increase in the lengths of Noi chickens at different stages of age

Traits	0-7	0-14	0-21	0-28	7-14	7-21	7-28	14-21	14-28	21-28	P
BL	1.64±0.10	3.67±0.28	5.71±0.66	8.10±0.64	2.03±0.17	4.08±0.56	6.46±0.54	2.04±0.44	4.43±0.41	2.39±0.26	
%	12.76^{b}±0.48	28.53^{f}±1.35	44.30^{g}±3.24	62.91^{h}±2.54	14.03^{c}±0.84	28.03^{e}±2.65	44.56^{g}±2.00	12.29^{a}±2.07	26.81^{d}±1.35	12.97^{b}±1.68	0.000
SL	0.73±0.11	2.14±0.12	3.32±0.22	4.82±0.64	1.41±0.12	2.59±0.30	4.09±0.64	1.18±0.23	2.69±0.64	1.54±0.62	
%	4.21^{a}±0.82	12.20^{d}±0.77	18.94^{g}±0.93	27.57^{i}±3.85	7.71^{c}±0.48	14.17^{f}±1.29	22.45^{h}±3.50	6.04^{b}±1.30	13.74^{e}±3.41	7.45^{c}±3.08	0.000
SW	1.07±0.21	2.68±0.31	3.87±0.27	5.59±0.53	1.61±0.14	2.80±0.16	4.53±0.43	1.19±0.15	2.92±0.38	1.72±0.34	
%	6.64^{a}±1.15	16.70^{e}±1.49	24.18^{g}±0.85	34.94^{h}±2.25	9.47^{c}±0.89	26.63^{g}±2.86	6.44^{a}±0.94	6.44^{a}±0.94	15.70^{d}±1.82	8.70^{b}±1.45	0.000
NL	4.32±1.25	15.09±1.98	25.41±2.44	35.09±3.01	10.86±2.23	21.17±3.08	30.85±3.16	10.31±1.89	19.99±1.91	9.68±1.61	
%	10.35^{a}±3.21	36.73^{e}±6.78	61.84^{g}±9.84	61.84^{g}±9.84	24.07^{d}±5.92	46.90^{f}±9.06	68.27^{h}±10.57	18.41^{c}±4.06	35.63^{e}±5.14	14.62^{b}±2.83	0.000
BaL	9.77±0.62	26.54±2.38	42.95±4.32	57.08±4.56	16.77±2.32	33.18±4.32	47.31±4.52	16.42±2.45	30.54±2.67	14.16±1.29	
%	17.20^{b}±1.65	46.79^{f}±6.03	75.77^{h}±10.70	100.64^{i}±12.37	25.29^{d}±4.19	50.05^{g}±8.02	71.31^{h}±9.13	19.84^{c}±3.71	36.86^{e}±4.80	14.26^{a}±1.91	0.000
WL	36.82±3.26	80.06±7.97	111.58±10.04	145.05±12.11	43.59±3.92	74.77±7.92	108.24±9.98	31.52±5.84	64.99±7.09	33.47±3.40	
%	64.88^{e}±7.93	141.09^{g}±18.54	196.77^{h}±25.69	255.74^{i}±31.77	28.16^{b}±3.97	48.61^{d}±6.88	70.35^{f}±9.01	16.10^{a}±3.75	33.15^{c}±5.57	14.71^{a}±2.02	0.000
TL	7.93±1.10	23.22±2.31	36.63±3.98	53.82±6.06	15.29±1.69	28.70±3.36	53.82±6.06	13.41±1.94	30.60±4.27	17.19±3.17	
%	15.50^{a}±2.68	45.37^{f}±6.22	71.61^{h}±10.46	105.20^{i}±15.92	25.95^{d}±3.70	48.74^{g}±7.37	91.39^{f}±13.71	18.13^{b}±3.29	41.35^{e}±7.48	19.73^{c}±4.59	0.000
ShL	1.08±0.47	4.80±0.53	7.98±1.17	11.56±0.99	3.80±0.78	6.98±1.45	10.54±1.35	3.18±0.85	6.75±0.86	3.58±0.82	
%	7.82^{a}±2.78	33.39^{d}±5.86	55.52^{g}±11.58	80.30^{i}±13.11	24.87^{c}±6.15	45.68^{f}±11.15	68.98^{h}±11.84	16.81^{b}±5.12	35.59^{e}±6.54	16.23^{b}±4.43	0.000
KL	11.79±2.55	26.50±2.49	38.06±3.18	48.13±3.89	14.71±0.85	26.26±1.33	36.34±1.90	11.55±1.13	21.63±1.68	10.07±0.98	
%	58.83^{d}±16.54	131.66^{g}±23.38	189.07^{h}±32.98	239.05^{i}±40.98	46.72^{c}±8.08	83.55^{e}±15.46	115.59^{f}±21.40	25.00^{b}±4.39	46.76^{c}±7.46	17.46^{a}±2.73	0.000
BD	12.06±1.88	39.59±2.48	58.92±4.73	80.84±6.59	27.53±2.07	46.85±3.54	68.77±5.23	19.33±2.94	41.24±4.73	21.92±2.77	
%	16.21^{a}±3.13	53.10^{d}±5.98	79.09^{e}±10.28	108.53^{f}±14.39	31.85^{b}±3.70	54.28^{d}± 6.96	79.70^{e}±10.50	17.05^{a}±3.23	36.36^{c}±5.78	16.56^{a}± 2.85	0.000
TD	2.87±0.77	17.78±1.74	25.53±2.82	31.54±3.23	14.91±2.10	22.66±3.34	28.67±3.78	7.75±2.01	13.76±2.33	6.01±0.99	
%	15.83^{a}±4.33	98.91^{e}±16.26	142.18^{g}±26.22	175.67^{h}±31.86	71.17^{d}±12.55	108.26^{f}±20.67	136.96^{g}±24.61	21.96^{b}±7.40	38.90^{c}±9.99	13.97^{a}±3.24	0.000

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

Table 4. Descriptive statistics for average daily gain, feed intake, and feed conversion ratio at different stages of age

	0-7	0-14	0-21	0-28	7-14	7-21	7-28	14-21	14-28	21-28	P
ADG	3.67^{a}±0.17	5.14^{b}±0.08	5.93^{c}±0.07	6.98^{d}±0.14	6.62^{d}±0.33	7.06^{de}±0.18	8.08^{f}±0.18	7.50^{e}±0.33	8.82^{g}±0.31	10.13^{h}±0.55	0.000
Range	3.46-3.83	5.08-5.24	5.86-6.01	6.82-7.14	6.33-7.03	6.94-7.29	7.86-8.25	7.38-7.57	8.54-9.21	9.70-10.84	
CV%	4.69	1.50	1.14	2.05	4.93	2.54	2.26	1.29	3.53	5.45	
FI	6.70^{a}±0.24	10.36^{b}±0.41	13.23^{c}±0.62	16.54^{d}±0.80	14.02^{c}±0.61	16.50^{d}±0.80	19.82^{e}±0.99	18.98^{e}±1.28	22.72^{f}±1.27	26.46^{g}±1.41	0.000
Range	6.39-6.87	9.89-10.80	12.45-13.73	15.50-17.08	13.39-14.75	15.48-17.16	18.54-20.49	17.56-20.42	21.11-23.78	24.66-27.57	
CV%	3.62	3.93	4.65	4.87	4.39	4.87	5.01	6.74	5.58	5.32	
FCR	1.87^{a}±0.09	2.01^{ab}±0.09	2.23^{c}±0.08	2.37^{cd}±0.08	2.19^{bc}±0.17	2.34^{cd}±0.09	2.45^{de}±0.07	2.59^{ef}±0.09	2.58^{ef}±0.12	2.71^{f}±0.14	0.000
Range	1.79-1.99	1.94-2.13	2.12-2.29	2.27-2.45	1.98-2.33	2.23-2.43	2.36-2.51	2.50-2.70	2.47-2.73	2.54-2.84	
CV%	4.98	4.41	3.78	3.38	7.70	3.74	2.94	3.53	4.71	5.07	

ADG: average daily gain (g/bird/day); FI: feed intake (g/bird/day); FCR: feed conversion ratio.

Table 5. Summary of body weight, average daily gain, feed intake and feed conversion ratio of local chicken breeds in some countries

Local chicken breed	BW0	BW28	ADG	FI	FCR	Source
Vietnam						
- Noi	34.83	225.56	6.98	16.54	2.37	This study
- Tau Vang	38.00	319.90	10.07	24.80	2.48	Khoa & Thong, 2013
- Long Cam	28.78	125.76	3.46	15.01	2.42	Mui et al., 2012
- Nhieu Ngon	27.98	270.60	8.67	-	-	Thinh et al., 2016
- Ri	29.28	231.39	7.22	-	-	Mui & Dang, 2016
- H'Mong	26.80	263.25	8.84	18.21	2.05	Phuong et al., 2017
India						
- Aseel	28.9	142.4	-	-	-	Rajkumar et al., 2017
Thailand						
- Thai	30.91	213.27	5.78	24.15	4.12	Jaturasitha et al., 2002
Pakistan						
- Lakha	30.83	202.50	6.13	-	-	Jatol (2014)
- Mianwali	30.66	219.79	6.75		-	Jatol (2014)
- Mushki	30.75	229.60	7.10	-	-	Jatol (2014)
- Peshawari	29.90	210.60	6.45	-	-	Jatol (2014)
Ethiopia						
- Tilili	27.00	134.00	3.80	24.90	6.50	Hassen et al., 2006
- Gellilia	28.20	126.00	3.50	33.60	9.60	Hassen et al., 2006
- Debre-Ellias	27.00	127.00	3.60	34.20	9.50	Hassen et al., 2006
- Mello-Hamusit	26.00	137.00	4.00	26.20	6.60	Hassen et al., 2006
- Gassay	25.90	119.00	3.30	28.80	8.70	Hassen et al., 2006
- Guangua	29.00	142.00	4.00	24.80	6.20	Hassen et al., 2006
- Mecha	28.00	146.00	4.20	25.50	6.10	Hassen et al., 2006
- RIR	36.00	137.00	3.60	23.40	6.50	Hassen et al., 2006
Commercial chicken						
- Abor Acres	44.70	1186.39	40.77	64.72	1.56	Jaturasitha et al., 2002

BW0: hatching weight (g/bird); BW28: body weight at 28 days old (g/bird); ADG: average daily gain (g/bird/day); FCR: feed conversion ratio.

Conclusion

Study on quantitative traits in Noi chicks at the first month of age indicated a linear increase in body measurements, growth and feed conversion ratio, at which the length of wings developed very early in the first week of life, followed by the other dimensions, remarkably the development of breast and thigh diameter, in the second week. In general, the development of observed parameters is due to aging.

Kvantitativne genetske osobine pilića vijetnamske autohtone rase Noi starosti od 0 do 28 dana

Do Vo Anh Khoa, Nguyen Thi Hong Tuoi, Nguyen Thao Nguyen, Nguyen Thi Dieu Thuy, Shin Okamoto, Kataro Kawabe, Takeshi Shimogigri

Rezime

Cilj ove studije je da opiše neke kvantitativne osobine pilića rase Noi, jedne od vijetnamskih poznatih autohtonih rasa za meso i borbu, u starosti od 0 do 28 dana. Ukupno 742 pilića je korišćeno za merenje, evidenciju, analizu i procenu ovih osobina. Rezultati su pokazali da postoji značajna razlika u posmatranim osobinama među starosnim dobima (P = 0,000). Zanimljivo je da su se krila (+ 64,88%) i dužine kobilice (+ 58,83%) brzo razvila u prvoj nedelji života, a zatim i većina drugih dimenzija, posebno obim grudi (+ 31,85%), obim bataka (+ 71,17%) u drugoj nedelji starosti. Povećanje vrednosti su rezultat razvoja skeleta u različitim vremenskim tačkama posmatranja tokom eksperimenta. Međutim, u drugoj nedelji života došlo je do snažnog razvoja mišićnog tkiva, pošto je stopa obima grudi i bataka bila najveća. Noi pilići su konzumirali količinu hrane od 16,54 g/ptica/dan i dobijali su težinu od 6,98 g/ptica/dan. Tako je koeficijent konverzije hrane iznosio 2,37 u starosti 0-28 dana. Možda je direktna pozitivna veza između posmatranih osobina i starosti nasumično određena prema biološkim karakteristikama životinje. Ovaj rad je pružio početne standarde Noi pilića za dalja istraživanja.

Ključne riječi: autohtona rasa živine Noi, merenja, kvantitativne osobine

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References

AMAO S.R., OJEDAPO L.O., OSO O.E. (2015): Evaluation of two commercial broiler strains differing in efficiency of feed utilization. Journal of New Sciences, 14(1): 432-437.

FRANCESCH A., VILLALBA I., CARTAÑÀ M. (2011): Methodology for morphological characterization of chicken and its application to compare Penedesenca and Empordanesa breeds. Animal Genetic Resources, 48: 79–84.

HASSEN H., NESER F.W.C, DESSIE T., DE KOCK A., VAN MARLE-KOSTER E. (2006): Studies on the growth performance of native chicken ecotypes and RIR chicken under improved management system in Northwest Ethiopia. Livestock Research for Rural Development, 18, Article #76. Retrieved December 3, 2018, from http://www.lrrd.org/lrrd18/6/hass18076.htm

JATOI A.S., IQBAL M., SAHOTA A.W., AKRAM M., JAVED K., JASPAL M.H., MEHMOOD S., HUSSAIN J., HAMEED T., KHAN M.S., ABBAS Y., AHMAD S., ISHAQ H.M. (2014): Comparative growth performance in four varieties of native Aseel chickens maintained in Pakistan. Pakistan Journal of Zoology, 46 (6): 1565-1571.

JATURASITHA S., LEANGWUNTA V., LEOTARAGUL A., PHONGPHAEW A., APICHARTSRUNGKOON T., SIMASATHITKUL N., VEARASILP T., WORACHAI L., TER MEULEN U. (2002): A comparative study of Thai native chicken and broiler on productive performance, carcass and meat quality. Deutscher Tropentag 2002 Witzenhausen, October 9-11.

KHOA D.V.A., KHANG N.T.K., NGU N.T., MATEY Y., LOAN H.T.P., THUY N.T.D. (2013): Single Nucleotide Polymorphisms in Gh, Ghr, Ghsr and Insulin Candidate Genes in Chicken Breeds of Vietnam. Greener Journal of Agricultural Sciences, 3(10): 716-724.

KHOA D.V.A., THONG N.M. (2013): Ảnh hưởng của các loại thức ăn công nghiệp lên khả năng sinh trưởng và FCR ở gà Tàu Vàng giai đoạn 1 - 4 tuần tuổi. Tạp chí Khoa học Trường Đại học Cần Thơ, 25: 114-118 (in Vietnamese).

MOLENAAR R., REIJRINK I.A.M., MEIJERHOF R., VAN DEN BRAND H. (2008): Relationship between hatchling length and weight on later productive performance in broilers. World's Poultry Science Journal, 64: 599-603.

MUI N.B., DANG P.K. (2016) Khả năng sản suất của gà Ri và con lai (Ri-Sasso-Lương Phượng) nuôi tại An Dương, Hải Phòng. Tạp chí Khoa học Nông nghiệp Việt Nam, 14(3): 392-399 (in Vietnamese).

MUI N.B., THANH N.C., DUC L.A., HIEU N.B. (2012): Đặc điểm ngoại hình và khả năng cho thịt của gà địa phương lông cằm tại Lục Ngạn, Bắc Giang. Tạp chí Khoa học và Phát triển, 10(7): 978-985 (in Vietnamese).

NGU, N.T., NHAN N.T.H., HON N.V., QUYEN N.V., MUOI N.T., VU C.T., XUAN N.H., NGHIA H.C. (2016): Đặc điểm ngoại hình của gà Nòi nuôi tại Đồng bằng sông Cửu Long. Tạp chí KHKT Chăn nuôi 203: 7-14 (in Vietnamese).

NGU N.T., XUAN N.T.H., VU C.T., AN N.T., DUNG T.N., NHAN N.T.H. (2015): Effects of genetic polymorphisms on egg production in indigenous Noi chicken. Journal of Experimental Biology and Agricultural Sciences, 3(VI): 487-493.

OJEDAPO L.O. (2013): Evaluation of body weight and other linear parameters of marshall broiler for repeatability estimates. International Journal of Applied Agriculture and Apiculture Research, 9 (1&2): 175-181.

PHUONG, N.T., DUY N.V.,. TON V.D (2017): Khả năng sinh trưởng và chất lượng thịt của gà H'Mông nuôi theo phương thức công nghiệp. Tạp chí Khoa học Nông nghiệp Việt Nam, 15(4): 438-445 (in Vietnamese).

QUYEN N.V., SON V.V. (2008): Ảnh hưởng của các mức năng lượng trao đổi và protein thô đến khả năng sinh sản của gà Nòi nuôi thả vườn ở đồng bằng sông Cửu Long: Tạp chí KHKT Chăn nuôi, 8(2): 19-25 (in Vietnamese).

RAJKUMAR U., HAUNSHI S., PASWAN C., RAJU M.V.L.N., RAMA RAO S.V., CHATTERJEE R.N. (2017): Characterization of indigenous Aseel chicken breed for morphological, growth, production, and meat composition traits from India. Poultry Science, 96: 2120-2126.

THINH N.H., DANG P.K., HANG V.T.T., TUAN H.A., DOAN B.H. (2016): Một số đặc điểm ngoại hình, khả năng sản xuất của gà nhiều ngón nuôi tại rừng quốc gia Xuân Sơn, huyện Tân Sơn, tỉnh Phú Thọ. Tạp chí Khoa học và Phát triển, 14(1): 9-20 (in Vietnamese).

VU C.T., NGU N.T. (2016): Single nucleotide polymorphisms in candidate genes associated with egg production traits in native Noi chicken of Vietnam. International Journal of Plant, Animal and Environmental Sciences, 6(1): 162-169.

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MEATINESS OF TESTED GILTS IN THREE CONSECUTIVE YEARS

Marija Gogić[1], Čedomir Radović[1], Dragan Radojković[2], Radomir Savić[2], Maja Petričević[1], Vladimir Živković[1], Nenad Stojiljković[1]

[1]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: gogic.marija@gmail.com
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Abstract: In the present study, the aim was to determine the impact of the following factors: age, farm, and gilt genotype, as well as the regression impact of body weight at the end of the performance test on the following tested properties: age at the end of the test/final age (FA), lifetime daily gain (LDG), the backfat thickness measured in two places (according to the Main Breeding program for Central Serbia), the depth of the long back muscle (BM) and the estimated lean meat content/meatiness (M). The study included two farms of pigs (farm 1 and farm 2), for three consecutive years (year 1, year 2 and year 3). The number of tested heads per year was 974 (year 1), 1311 (year 2) and 757 (year 3). The tested gilts were of Swedish Landrace, Large White and Duroc breeds. The gilts originated from 97 sires, while the number of daughters per sires ranged from 10 to 100. The results show that the Duroc animals were the oldest (245 days), which had the highest values for both measures of backfat thickness, but the lowest values for meatiness. In the third study year, the lowest average values were determined for the properties of the LDG, BM and M. The female animals from the farm 1 showed less growth/gain and had lower values for the estimated meatiness. As a result of the study, it was established that all included factors had a very high statistically significant influence on the variation of the tested properties ($P < 0.001$), only the genotype of gilts showed ahigh statistically significant effect on the BM property ($P < 0.01$).

Key words: factor, performance test of gilts, properties, breed, estimated meatiness/lean meat content

Introduction

Swedish Landrace, Large White and Duroc are the three most numerous pure breeds of pigs in the Republic of Serbia, since they exhibit the most desired levels of production properties and provide economic gain. Pork is the most commonly consumed meat in the world (*Berton et al., 2015*). Many pig breeding programs have been based on estimation and improvement of lean traits (*Kawecka at al., 2009*) for many years, and this intensive work in breeding has led to significant progress in the meat content of pigs (*Rekiel et al., 2015). Różycki (2003)* suggests that the results of the test performance are one of the main criteria in the selection of pigs for further breeding and production. Considering that the productivity of sows in regard to the litter size is one of the key factors for economical and efficient pig production (*Kapelanski et al., 2013; Zapryanova and Malinova, 2018*), special attention should be paid to gilts (*Mijatović et al., 2009*). Also the lifetime daily gain is one of the key traits in the pig production/farming as it contributes to its effectiveness (*Nielsen et al., 2018*). The gain as well as the backfat thickness are also considered to be the most important economic characteristics of pigs (*Zebua et al., 2017*). According to *Gaughan et al. (1995*), selection aimed at increasing lean meat content leads to delaying the onset of sexual maturation. Gilts with a thinner backfat are older at first partus, and after weaning of the piglets, the signs of estrus are delayed (*Ptak et al., 2014*). Also, the increase in lean meat has led to a decrease in the backfat thickness in the growing gilts as well as the lipid reserves (*Rekiel, 2002*). For these reasons, pig breeders often face a risk factor in the sow replacement process. The use of ultrasonic devices has led to a change in the performance testing of pigs (*Kernerova et al., 2006*) and modification of the method for estimating the pig breeding value. Body weight and backfat thickness have an effect on reproduction of gilts (*Flisar et al., 2012).* According to *Gasinski (2013),* certain body weight of gilts is necessary to prevent weight loss during first lactation. Genotype, gender, and genotype and gender interaction are gaining in importance in pig production, since breeding programs depend on the selection of a good genotype and gender to concentrate on in the spreading of new generations (*Morenikeji et al., 2019*). The aim of the paper is to determine whether there is a trait variation in the gilt performance test observed under the influence of the breed, farms and year of birth, as well as how statistically significant are these effects.

Materials and Methods

The trial was carried out on two pig farms, with 1440 gilts tested on farm 1 and 1602 gilts tested on farm 2. Gilts were tested for three consecutive years (1, 2,

and 3). In the first year of testing, 974 gilts were tested, in the second year, 1311 gilts and in the third year 757 gilts. Gilts were following pure breeds: Swedish landrace (2373), Large white (455) and Duroc (214). In total 3042 gilts were tested. During the duration of the test, gilts were kept in group boxes. After reaching the final weight, which was in accordance with the *Main Breeding Program (2014)* for Central Serbia (completion of the test at a body weight of 90 to 120 kg), the backfat thickness and the depth of *Musculus longissimus dorsi* were measured, and the lean meat content evaluated with the aid of the ultrasonic apparatus PigLog 105. The backfat thickness was measured in two places: 1. backfat thickness in the lumbar zone (BFT1, between 3rd and 4th lumbar vertebrae, measured 7 cm laterally from the backline); 2. backfat thickness in the back region (BFT2, 7 cm laterally from the backline between the 3rd and 4th ribs), while the depth of the long back muscle (BM) was measured between the 3rd and the 4th ribs, 7 cm laterally from the backline. The body weight of gilts at the end of the test/final weight represents a linear regression impact and was on average of 111.53 kg. Statistical data processing was performed using the software package "LSBMMW and MIXMDL, PC-2 VERSION" *(Harvey, 1990).* The method of least squares was used to determine the significance ($P < 0.05$) of the factors on the properties of the age at the end of the test, the lifetime daily gain, the backfat thickness in the lumbar zone, the backfat thickness in the back region, the depth of *Musculus longissimus dorsi* and the estimated meatiness (M). The model used included the genotype of gilts (G), the farm (F), the year of birth (S) and the mass at the end of the (direct) test in the form of linear regression influence as factors.

Model

$$Y_{ijkl} = \mu + G_i + F_j + S_k + b_1(x_1 - \bar{x}_1) + \varepsilon_{ijkl}$$

where: Y_{ijkl} – the effect of the trait on animal *l*, of i genotype, j farm, k year of birth, μ = general population average, G_i – the effect of gilt genotype (i=1,2,3), F_j - the effect of farm (j=1,2), S_k – the effect of year of birth (i=1,2,3), ε_{ijkl} – random error (residue).

Results and Discussion

The average values of the tested properties and standard deviation values are shown in Table 1. Data in Table 1 show that the age at the end of the test/final age was 238 days, the lifetime daily gain was 468.57 g/day, the backfat thickness 1 was 12.38 mm, backfat thickness 2 was 11.24 mm, *Musculus longissimus dorsi* depth 49.18 mm, estimated meatiness 58.92%, while the final body weight was 111.53 kg.

Table 1. Average values and standard deviations for the tested properties in the performance test

Trait		$\bar{X}$ ±SD
FA	Age at the end of the test/final age, days	238.02±24.55
LDG	Lifetime daily gain, g/day	468.57±55.60
BFT1	Backfat thickness 1, mm	12.38±2.64
BFT2	Backfat thickness 2, mm	11.24±2.61
BM	Back muscle depth, mm	49.18±6.04
M	Estimated meatiness, %	58.92±2.49
FBW	Final body weight, kg	111.53±7.59

Table 2 shows the variation of the properties studied in the performance test under the influence of the year of birth, farm and genotype. Taking the year of birth as a factor, it can be seen that animals born in the third year ended the test later in relation to the first two years of the trial, and also showed lower daily gain and meatiness, while the values for backfat thickness were higher compared to the first two years. A significant variation of the investigated properties among farms was also established, and it can be concluded that better results in the performance test were achieved by animals from the second farm. Expectedly, the animals of the Duroc breed had the highest values for the backfat thickness and the lowest values for BM and meatiness. The highest values for the trait meatiness were found in the second year of testing (58.63%), on the second farm (58.84%) and in animals of lrge White breed (59.09%). It can be noticed that gilts of Swedish Landrace and Large White (as two fertile breeds) had very similar results in the performance test, unlike the Duroc gilts.

Table 2. Effect of year, farm and genotype on investigated traits (LSMean ± S.E.)

Source of variation		FA[1)], days	LDG, g/day	BFT1, mm	BFT2, mm	BM, mm	M, %
Year	1	235.49±0.83	472.08±1.42	12.71±0.10	11.58±0.09	49.28±024	58.48±0.09
	2	239.59±0.74	463.99±1.26	12.69±0.09	11.62±0.08	49.15±0.21	58.63±0.08
	3	249.00±0.93	449.91±1.59	13.27±0.11	12.32±0.11	48.16±0.26	57.89±0.10
Farm	1	254.39±0.89	437.87±1.52	13.28±0.11	12.80±0.10	50.37±0.25	57.82±0.10
	2	228.33±0.64	486.12±1.08	12.50±0.08	10.87±0.07	47.36±0.18	58.84±0.07
Genotype	SL[2)]	238.79±0.43	467.72±0.73	12.35±0.05	11.22±0.05	49.24±0.12	58.94±0.05
	LW	240.32±1.04	463.82±1.77	12.17±0.12	11.21±0.12	49.40±0.30	59.09±0.12
	D	244.98±1.46	454.45±2.48	14.15±0.17	13.10±0.17	47.95±0.41	56.97±0.16

[1)] FA- final age (age at the end of the test); LDG – lifetime daily gain; BFT1 – backfat thickness-lumbar zone; BFT2 – backfat thickness – back region; BM – deapth of *Musculus longissimus dorsi*; M – evaluated meatiness; [2)] SL – Swedish Landrace; LW – Large White, D – Duroc

Kernerevova et al. (2006) have reported the following results for Large White gilts obtained by using the PigLog105 devices: 1) gilts with the mother's line of Large White, the average values for the gain are 531 g/day, the backfat thicknesses 1 and 2 are 12.96 and 15.08 mm, BM 59.96 mm and lean meat 58.35%; 2) gilts with the father's line of Large White, the average values for gain are 511 g/day, the backfat thicknesses 1 and 2 are 9.96 and 11.16 mm, BM 59.84 mm and lean meat content 61.84%. The reason for the higher lean meat content compared to our research is that the animals tested in the Czech Republic were from the nucleus herd of the Large White breed with above average performance results, and a significantly less animals were tested compared to our research. *Radović et al. (2012)* have obtained following values for gilts of Swedish Landrace breed: gain of 483.71 g/day and backfat thicknesses 1 and 2 of 18.01 mm and 13.46 mm, respectively, with the genotype of the animal (P>0.05) having no statistically significant effect on the variation of properties, which is in contrast with our research. The year of birth had a statistically significant effect as in our study (P<0.001). *Szyndler-Nędza et al. (2016)* have reported significantly higher gains for Large White, Landrace and Duroc gilts than in our research (LW = 625.16; Landrace = 623.47; D = 632.46 g/day), but the animals completed the test significantly earlier (age at end of the test on average 163-165 days). The backfat thickness reported by the same group of authors was measured at identical points as in our research, so that the values for backfat thickness, as expected, were the highest in Duroc breed. Differences in the muscle depth are slight compared to our research, while the values for lean meat content are higher in our research.

Table 3. Statistical significance (level of significance) of the factors included in the model in the analysis of the tested traits

Source of variation	FA[1)]	LDG	BFT1	BFT2	BM	M
Year	***[2)]	***	***	***	***	***
Farm	***	***	***	***	***	***
Genotype	***	***	***	***	**	***
FBW (b)	***	***	***	***	***	***

[1)] FA- final age (age at the end of the test); LDG – lifetime daily gain; BFT1 – backfat thickness-lumbar zone; BFT2 – backfat thickness – back region; BM – deapth of *Musculus longissimus dorsi*; M – evaluated meatiness; FBW – linear regression effect of final body weight; [2)] ** = P <0.01; *** = P <0.001

Table 3 shows the statistical significance of the factors included in the model. It was found that all factors influenced statistically significantly (P<0.001) the variation of all examined gilt traits. Only the effect of genotype on BM showed slightly lower statistical significance (P<0.01).

Gogić et al. (2012) have established in their study of performance tested gilts that genotype and farm have highly significantly (P<0.001) influenced the variability of the properties, except in regard to the depth of the muscle where farm had no effect on the trait while the genotype showed slightly lower effect (P <0.01) on variation of this property. *Nevrkla et al. (2016)* have examined the local breeds in the performance test and established that the meatiness varied highly significantly (P <0.001) between the farms, which is in line with our research.

Conclusion

On the basis of the obtained results, it can be concluded that the animals in the third year of the research were the oldest so they had the lowest values for the traits of gain, meatiness and depth of the muscles; Duroc genotype animals were expected to have the highest values for the BFT1 and BFT2 properties, as well as the lowest values for the BM and M properties; The best value for BM trait was recorded in animals from farm 1, with the highest estimated meatiness in animals of Large White genotype.

Mesnatost testiranih nazimica u tri uzastopne godine

Marija Gogić, Čedomir Radović, Dragan Radojković, Radomir Savić, Maja Petričević, Vladimir Živković, Nenad Stojiljković

Rezime

U ovom istraživanju cilj je bio da se utvrdi kakav uticaj su imali sledeći faktori: godina, farma, i genotip nazimica kao i regresijski uticaj telesne mase na kraju performans testa na sledeće ispitivane osobine: uzrast na kraju testa (UKT), životni dnevni prirast (LDG), debljina slanine merena na dva mesta (u skladu sa Glavnim odgajivačkim pogramom), dubina dugog leđnog mišića (BM) i procenjena mesnatost (M). Istraživanjem su obuhvaćene dve farme svinja (farma 1 i farma 2), kroz tri uzastopne godine (godina 1, godina 2 i godina 3). Broj testiranih grla po godinama iznosio je: 974 (godina 1), 1311 (godina 2) i 757 (godina 3). Testirane nazimice su pripadale sledećim čistim rasama švedski landras, veliki jorkšir i Duroc. Nazimice potiču od 97 očeva, dok je broj kćeri po očevima iznosio od 10 do 100. Rezultati pokazuju da su najstarija grla rase Duroc (245 dana), koja imaju i najveće vrednosti za obe mere debljine slanine, ali najmanje vrednosti za mesnatost. U trećoj godini ispitivanja najmanje prosečne vrednosti su utvrđene za osobine LDG, BM i M. Ženska grla sa farme 1 su slabije prirastala i imala manje vrednosti za procenjenu mesnatost. Kao rezultat ispitivanja utvrđeno je da su svi

uključeni faktori veoma visoko statistički značajno uticali na variranje ispitivanih osobina (P <0.001), jedino genotip nazimica pokazuje visok statistički značajan uticaj na osobinu BM (P<0.01).

Ključne reči: faktor, performans test nazimica, osobine, rasa, procenjena mesnatost

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References

BERTON M.P., DE CÁSSIA DOURADO R., DE LIMA F.B.F.,RODRIGUES A.B.B., BORBA F.B. FERRARI, DO CARMO VIEIRA L.D., DE SOUZA P.A., BORBA H. (2015): Growing-finishing performance and carcass yield of pigs reared in a climate-controlled and uncontrolled environment. Int J Biometeorol. 59, 8, 955–960.

FLISAR T., MALOVRH Š., URANKAR J., KOVAČ M. (2012): Effect of gilt growth rate and back fat thickness on reproductive performance. Acta Agriculturae Slovenica, 100, 199-203.

GASINSKI M. (2013): Wybór loszki remontowej – cechy za i przeciw oraz czy pierwszy miot musi być decydujący o przydatności danej sztuki do rozrodu [Choosing replacement gilts – advantages and disadvantages and whether the first litter determine the reproductive potential of female]. Hoduj z głową świnie [Breeding pigs], 5.

GAUGHAN J.B., CAMERON R.D.A., DRYDEN G. McL. (1995): Effect of prepubertal body fat on the development of the reproductive tract in gilts. Manipulating Pig Production. No V Melbourne (Australia), 91.

GOGIĆ M., PETROVIĆ M., ŽIVKOVIĆ B., RADOVIĆ Č., RADOJKOVIĆ D., PARUNOVIĆ N., MARINKOV G. (2012): The effect of various factors on traits of performance tested gilts. Biotechnology in Animal Husbandry, 28, 2, 313-322.

HARVEY R.W. (1990): User's guide for LSBMMW and MIXMDL. Ver. PC–2, 1–91.

INSTITUTE FOR ANIMAL HUSBANDRY (2014): Glavni odgajivački program u stočarstvu, 1-224.

KAPELAŃSKI W., JANKOWIAK H., BOCIAN M., GRAJEWSKA S., DYBAŁA J., CEBULSKA A. (2013): The effect of the growth rate and meatiness of young gilts during rearing on the growth and development of their reproductive system. Acta Veterinaria Brno, 82, 019–024.

KAWECKA M., MATYSIAK B., KAMYCZEK M., DELIKATOR B. (2009): Relationships between growth, fitness and meatiness traits in gilts and their subsequent reproductive performance. Annals of Animal Science, 9, 249-258.
KERNEROVÁ N., VÁCLAVOVSKÝ J., MATOUŠEK V., HANYKOVÁ Z. (2006): The use of performance test parameters for selection of gilts before their placement into breeding. Czech J. Anim. Sci., 51, 6, 253–261.
MIJATOVIĆ M., PETROVIĆ M., RADOJKOVIĆ D., PUŠIĆ M., RADOVIĆ Č. (2009): Influence of performance test traits of gilts on variability of their reproductive performance as primiparous sows. Biotechnology in Animal Husbandry, 25, 5-6, 825-831.
MORENIKEJI O. B., OGUNSHOLA O. J., ADU O. A., ARO S. O., CHINEKE C. A. (2019): Genotype and sex effects on the performance characteristics of pigs. International Journal of Livestock Production, 10, 4, 127-134.
NEVRKLA P., VÁCLAVKOVÁ E., HADAŠ Z., HORK P. (2016): Effect of farm on productive and reproductive performance in sows of prestice black-pied pig. Acta universitatis agriculturae et silviculturae mendelianae brunensis, 64, 4, 1233-1237.
NIELSEN H.M., ASK B., MADSEN P. (2018): Social genetic efects for growth in pigs difer between boars and gilts. Genetics Selection Evolution, 50, 4.
PTAK J., MUCHA A., RÓŻYCKI M. (2014): Correlations between fattening and slaughter performance test results of gilts and their subsequent reproductive performance. Journal of Animal and Feed Sciences, 23, 149–152.
RADOVIĆ Č., PETROVIĆ M., PARUNOVIĆ N., BRKIĆ N., ŽIVKOVIĆ B., GOGIĆ M., STANIŠIĆ N. (2012): The effect of genotype and year on traits of performance tested gilts. Biotechnology in Animal Husbandry, 28, 3, 463-468.
REKIEL A. (2002): Wpływ odmiennych technik zasuszania na poziom rezerw tłuszczowych i wyniki reprodukcji loch. Praca habilitacyjna 246, Wyd. SGGW, 1-99.
REKIEL A., WIECEK J., BATORSKA M., KULISIEWICZ J., TOKARSKA G. (2015): Comparison of changes in fatness of sows in high pregnancy and at weaning and determination of their association with reproduction results and rearing of piglets. J. Central European Agric., 16, 410–422.
RÓŻYCKI M. (2003): Selected traits of polish pedigree pig-progress in the carcass meat deposition and meat quality. Animal Science Papers and Reports, 21, 1, 163-171.
SZYNDLER-NĘDZA M., ECKERT R., BLICHARSKI T. (2016): Estimation of Meat Content in the Carcasses of Young Pigs Based on Performance Testing of Live Animals and Carcass Evaluation. Annals of Animal Science, 16, 2, 551-564.
ZAPRYANOVA I., MALINOVA R. (2018): Influence of some factors on the fertility of hybrid sows. Biotechnology in Animal Husbandry, 34, 4, 387-394.

ZEBUA C. K. N., MULADNO M., SIAGIAN P. H. (2017): Comparative performance of Landrace, Yorkshire and Duroc breeds of swine. Journal of the Indonesian Tropical Animal Agriculture, 42, 3, 147-152.

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THE NUTRITIONAL QUALITY OF FEEDSTUFFS USED IN DAIRY GOAT NUTRITION IN VOJVODINA

Snežana Paskaš[1], Jelena Miočinović[2], Branislav Vejnović[3], Zsolt Becskei[3]

[1] University of Novi Sad, Faculty of Agriculture, 21000 Novi Sad, Serbia
[2] University of Belgrade, Faculty of Agriculture, 11000 Zemun-Belgrade, Serbia
[3]University of Belgrade, Faculty of Veterinary Medicine, 11000 Belgrade, Serbia
Corresponding author*:* Snežana Paskaš: snpaskas@gmail.com
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Abstract: The study was to conduct to evaluate the chemical composition and nutritive values of feedstuffs (forages and concentrate mixtures) used for dairy goats nutrition in Vojvodina. Samples were collected from six farms, including one organic farm. The results showed that the relative feed values of analyzed forages were in the range of good, medium to lower quality. Average protein content from lowest to highest for investigated forages was: corn silage (*Zea Mays*) (65.37-82.57g kg^{-1}DM), alfalfa haylage (*Medicago sativa L.)* (159.99-184.17g kg^{-1}DM), pasture (185.30g kg^{-1} DM), and alfalfa hay (*Medicago sativa L.*) (167.48-203.60g kg^{-1}DM). The non-fibre carbohydrates and protein content most varied in organic hay samples (cv: 29.25% and 19.09%, respectively). Generally, feedstuffs used in organic nutrition, including organic concentrate, were of lower nutritional quality and moreover contained higher amounts of crude fibre and lignin. Especially, a high source of variation was observed in investigated concentrate mixtures for the crude protein content ($p<0.0001$), ranged from 135.32 to 209.87g kg^{-1}DM. Corn silages also varied substantially in their chemical composition and significant difference ($p<0.05$) was observed in regard to acid detergent fibre (ADF) and lignin content (ranged: ADF: 242.20-319.24g kg^{-1}DM; ADL: 27.98-52.54g kg^{-1}DM, respectively). Furthermore, pasture contained the most soluble materials during May and June and their content was related inversely to crude fibre amount. This survey highlights that investigated farms still pay insufficient attention to the quality of the feedstuff. For the development of intensive goat farming, greater emphasis should be placed on using higher quality feedstuffs, as well, standards for feed quality must be considered and established.

Key words: feedstuffs, chemical composition, nutritional value**,** carbohydrate fractions

Introduction

Goats are often associated with vitality, inquisitiveness and high physical activity. At the same time, they are considered as easily herded animals and nowadays they are reared in various breeding systems, from extensive to highly intensive. Besides that, different feeding management systems are applied, from grazing diets to the total mixed rations (*Cannas et al., 2008*). Proper nutrition is the basis of the successful production systems and with increasing milk yield, producers require technology inputs in nutrition and feeding to improve production efficiency (*Lee et al., 2007*). Furthermore, *Rahmann (2009)* highlighted the importance of feedstuffs quality for highly productive goats and indicated that under these conditions a highly productive organic dairy milk production is possible. Similarly to other domestic ruminants, goats are usually fed *ad libitum*, whether they are reared intensively or extensively but the nutrient quality of forages, herbage from pasture and foliage from bushes fluctuate depending on geographic and climatic conditions (*Pulina et al., 2013*). Goats prefer to consume a wide variety of feedstuffs but in their diet is mostly used hay, silage, or pasture. However, forage quality varies tremendously and its nutritive value can be determined by their chemical composition (*Van Soest, 1965; Van Soest, 1996*). The chemical composition of the feedstuffs can be obtained through chemical or NIR analysis or from published tables of feed composition. The nutritive value of the feedstuffs can be calculated from the chemical composition in accordance with the feed evaluation systems (Institut National de la Recherche Agronomique, INRA; Agricultural and Food Research Council, AFRC; National Research Council, NRC; etc.) (in *Martinez-Marin et al., 2010*). Among the forages, the utilization of alfalfa as hay or pellets is very common. This forage is characterized by higher protein content (more than 16% CP on a DM basis) and lower neutral-detergent fibre (NDF) concentration than permanent pasture hay (*Rapetti and Bava, 2008*). The quality of forages is especially important in intensive systems and should be carefully evaluated (*Rapetti and Bava, 2008*). Moreover, *Oliveira et al. (2014)* added that in contrast to grains and other concentrate supplements, roughages possessed widely variable digestibility values, and thus affected the feed efficiency. The various factors can interact to influence alfalfa chemical composition and as a result, hay from the same farm and field can vary significantly within a year (*Martin et al., 2004*). If forage quality is poor, a large amount of concentrate needs to be supplied in the diet which increases not only feeding costs but also the risk of metabolic disorders (*Rapetti and Bava, 2008*). On the other hand, in order to fulfil the nutrient requirements of high yield and early lactation goats, it is required additional dietary protein sources because microbial protein can only fulfil the requirements in low production in late lactation stages (*Lee et al., 2001*).

In Vojvodina, Province of Serbia, the goat sector has increased significantly during the last two decades. Goat's milk production in Serbia exhibit a great diversity of systems: from extensive to intensive management and the milk yield of goat in our systems of production depends largely on feeding condition (*Petrović et al., 2017*). Rations containing alfalfa hay and different concentrate mixtures are the most practised feeding strategies in goat husbandry in Vojvodina. Generally, in Serbia, there is a lack of protein in herbage. Therefore, low production and high prices of milk and meat are mostly a consequence of low herbage quality and a high share of
concentrated feeds in the diet for ruminants (*Sokolović et al., 2013*). However, studies in quality of feedstuffs used in goat nutrition in Vojvodina are sparse and limited.

The aim of the paper was to determine the chemical composition and nutritional quality of the feedstuffs used in the nutrition of dairy goats in Vojvodina. The present study was therefore undertaken with the special emphasis on providing information on the nutrient components, especially those related to carbohydrate fractions.

Material and methods

Samples collection

The research was carried out in Vojvodina, Province of Serbia. Feedstuffs used in goat nutrition were sampled from 6 farms, in regards to the main breed French Alpine. The goat farms were different in the number of lactating goats (LG) and systems (Table 1).

Table 1. Characteristics of investigated farms

Farms/Parameters	Number of lactating goats (LG)	Farm systems	Feeding management
Farm A (FA)	900	organic	indoors
Farm B (FB)	420	conventional	indoors
Farm C (FC)	250	conventional	indoors
Farm D (FD)	130	conventional	outdoors
Farm E (FE)	48	conventional	indoors
Farm F (FF)	22	conventional	indoors

The feeding strategy was substantially similar in terms that alfalfa hay and water were offered *ad libitum*, and concentrate mixture twice daily at the milking time (in amount: FA: 300g-400g/day/goat, FB, FC, FD: 600-700g/day/goat and FE, FF: 500g/day/goat. Goats received both, the farm produced and commercial feedstuffs. During spring, summer and autumn the nutrition of goats on FD was based primarily on pasture (kept them indoors at night time). Samples of pasture were

collected for five months. Supplementary nutrition of concentrated diets was based mostly on cereals grains with the addition of commercial mineral-vitamin mixtures, chalk and NaCl. Furthermore, Farm B used commercial pellets concentrate mixtures. Farms A, E and D occasionally used corn silage while farms E and F alfalfa haylage (ensiling forage into big bales wrapped with plastic).

Feed chemical analyses

In total, samples of alfalfa hay (*Medicago sativa L.*) (n=18), concentrate mixtures (n=15), pasture (n=5), corn silage (*Zea Mays*) (n=9) and alfalfa silage (n=6) were analysed for chemical composition. The analysis of dry matter (DM) content, moisture, crude protein (CP), ether extract (EE) and crude ash (CA) were carried out according to the standards methods (*Official Gazette of SFRJ, 15/87*). The content of nitrogen-free extracts (NFE) was calculated. The examination of crude fibre was done as per AOCS (2005) procedure. The content of acid and neutral detergent fibre was determined using the procedures of Van Soest et al. (1991). Non-fibre carbohydrates (NFC) derived by the equations given by *Van Soest et al. (1991),* total carbohydrates (TC) were estimated from *Sniffen et al. (1992*) and relative feed values (RFV) according to the equation proposed by *Lacefield (1988).* The fractioning of carbohydrates of the feedstuffs was made according to methodologies proposed by *Sniffen et al. (1992)* being the carbohydrates divided into A and B_1 fractions (non-fibre carbohydrates, rapidly degradable), B_2 fraction (fibre carbohydrates, potentially degradable) and C fraction (fibre carbohydrates, non-degradable).

Statistical analysis

Depending on the values of coefficients of variation (cv), an appropriate method was chosen to test the difference between the groups. For homogenous datasets (cv<30%) the groups were compared using one-way ANOVA followed by Tukey`s multiple comparison test and for heterogeneous datasets (cv>30%) the groups were compared using Kruskal-Wallis ANOVA followed by Dunn`s multiple comparison test. Numerical data for homogenous datasets are presented as mean±standard deviation (Mean±SD) and for heterogeneous datasets as median values with corresponding interquartile range (IQR). Statistical analysis of the chemical feedstuff`s results obtained in the investigation was carried out using statistical software GraphPad Prism version 6 (GraphPad, San Diego, CA, USA). In addition, a significant difference between samples of alfalfa haylage was tested using the t-test.

Results and discussion

Alfalfa hay

The chemical composition of alfalfa hay samples is summarized in Table 2. The content of DM, CP and EE were similar and non-significant variation in these parameters occurs between investigated farms. Contrary, CF and CA content

showed a significant difference ($p<0.05$, $p<0.01$, respectively). Generally, alfalfa hay contains DM in approximate 820-900g kg^{-1} and our results were in this range (863.30 (FE) to 908.50g kg^{-1}DM (FC). Although the EE is an excellent source of energy, it is generally low in forages and roughages. By looking at the protein content it can be observed that our samples varied from 167.48 (FD) to 203.60g kg^{-1}DM (FB), with the highest coefficient of variation for organic hay (cv: 19.09%). *Blair (2011)* reported that value of alfalfa hay lies in its relatively high content of crude protein, which may be as high as 200g kg^{-1}DM if it is made from a crop cut in the early bloom stage. During the maturation of the alfalfa, the proportion of fibres and lignin increases while NFC and protein content decreases (*Martin et al., 2004*). The average high CF content was obtained for almost all farms with the exception of samples collected at FB. Moreover, it was significantly different ($p<0.05$) from FC, FD and FE. Feeds high in crude cellulose can furnish most of the ruminant`s maintenance energy needs. The content of fibre is needed to maintain a normal milk-fat test and a certain minimum fibre level is required for a healthy rumen function. However, too much poor quality fibre will lead to lowered levels of milk production (*Van Soest, 1994*). According to, *Morand-Fehr and Sauvant (1980)* hay of medium quality could reduce milk production by 15 to 25%. For dairy goats, the maximum and minimum fibre contents in the diet to maximize intake and production efficiency are not yet well defined (Oliveira et al., 2014). However, *Lacefield (1988)* pointed out that hay must be low in fibre and palatable for the animal to consume enough of it. Furthermore, the research of Oliveira et al. (2014) confirmed the negative effect of increasing the dietary fibre and NDF content on feed efficiency in lactating goats.

Table 2. The chemical composition of Alfalfa hay (g kg^{-1}DM)

Farms/Parametres		FA	FB	FC	FD	FE	FF	P
DM g kg $^{-1}$	M	881.30	894.43	908.50	895.47	863.30	875.60	ns
	±Sd	±13.00	±16.80	±9.68	±11.98	±53.62	±17.80	
	cv(%)	1.47	1.88	1.06	1.34	6.21	2.03	
CP	M	173.77	2036.0	168.42	167.48	179.40	201.16	ns
	±Sd	±33.17	±5.67	±22.51	±22.40	±33.55	±9.71	
	cv (%)	19.09	2.78	13.36	13.37	18.70	4.83	
EE	M	14.45	12.97	12.41	13.73	12.48	13.55	ns
	±Sd	±2.34	±0.75	±0.68	±0.62	±2.19	±0.34	
	cv (%)	16.19	5.78	5.48	4.52	17.55	2.51	
CF	M	353.84	289.05	367.24	364.84	364.86	345.49	*
	±Sd	±7.43	±29.23	±16.60	±11.76	±44.00	±13.93	
	cv (%)	2.10	10.14	4.52	3.22	12.06	4.03	
CA	M	73.41	97.76	77.94	74.25	95.51	86.77	**
	±Sd	±3.03	±6.73	±1.89	±12.18	±9.66	±2.25	
	cv (%)	4.13	6.88	2.42	16.40	10.11	2.59	
NFE	M	384.43	396.61	373.99	379.69	345.36	353.36	ns
	±Sd	±30.19	±22.12	±11.99	±24.58	±54.32	±9.27	
	cv (%)	7.85	5.58	3.21	6.47	15.73	2.62	
NDF	M	549.89	436.12	513.79	541.04	505.16	502.08	*
	±Sd	±50.95	±28.05	±23.67	±29.94	±34.91	±15.25	
	cv (%)	9.26	6.43	4.61	5.53	6.91	3.04	
ADF	M	429.15	372.24	440.14	438.35	404.78	412.90	ns
	±Sd	±17.94	±28.15	±5.88	±11.53	±52.25	±28.91	
	cv (%)	4.18	7.56	1.34	2.63	12.91	7.00	
ADL	M	89.42	76.15±	107.10	91.54	82.98	86.98	ns
	±Sd	±12.08	4.29	±5.94	±4.74	±17.67	±12.54	
	cv (%)	13.51	5.63	5.55	5.18	21.29	14.42	
NFC	M	188.19	249.56	227.44	203.49	210.79	196.44	ns
	±Sd	±55.05	±26.87	±3.30	±11.54	±31.79	±10.34	
	cv (%)	29.25	10.77	1.45	5.67	15.08	5.26	
RFV	M	114.62	150.92	115..35	111.99	131.66	123.01	*
	±Sd	±7.98	±18.40	±6.38	±7.48	±7.57	±17.30	
	cv (%)	6.96	12.19	5.53	6.68	5.75	14.06	

DM-Dry matter; CP-Crude protein; EE-Ether extract; CF-Crude fibre; CA-Crude ash; NFE-Nitrogen-free extract; NDF-Neutral detergent fibre; ADF-Acid detergent fibre; ADL-Acid detergent lignin; NFC-Non-fibre carbohydrates; RFV-Relative feed value; M±Sd: Mean±Standard deviation; cv (%)-coefficient of variation; P-Statistic probability; *: $p<0.05$; **: $p<0.01$; ns-not-significant

Our results showed that the proportion of non-degradable carbohydrates fractions were the highest in samples collected from farms C, F, D and A (Table 5.) as well indicated lower hay feeding value for dairy goats. This is confirmed by RFV which showed that hay collected from investigated farms were ranged of good (FB), medium (FE) to less quality (FA, FC, FD and FF) (Table 2).

b) Concentrate mixtures

Table 3 shows the results of the analysed concentrate mixtures. Significant interactions were observed for most investigated parameters. Especially, a highly

significant source of variation ($p<0.001$) was found for the content of dietary CP. Thus, it was varied in the range from 135.32 (FD) to 209.87g kg^{-1}DM (FE).

Table 3. The chemical composition of concentrate mixtures (g kg^{-1}DM)

Farms/Parametres		FA	FB	FC	FD	FE	P
DM	M	886.23	885.57	870.93	862.93	891.97	
g kg^{-1}	±Sd	±113.19	±20.85	±5.80	±14.37	±1.90	ns
	cv (%)	1.49	2.35	0.67	1.66	0.21	
	M	191.67	187.15	190.70	135.32	209.87	
CP	±Sd	±5.38	±4.96	±4.02	±6.38	±4.51	****
	cv (%)	2.81	2.65	2.11	4.71	2.15	
	M	41.46	62.82	29.9	43.09	44.36	
EE	±Sd	±4.81	±4.96	±2.57	±18.75	±0.45	*
	cv (%)	11.60	7.89	8.59	43.51	1.01	
	M	103.47	62.79	94.71	63.93	101.59	
CF	±Sd	±4.90	±2.20	±2.36	±3.33	±9.44	****
	cv (%)	4.74	3.50	2.49	5.21	9.29	
	M	73.16	66.33	59.24	61.31	69.56	
CA	±Sd	±3.31	±2.12	±2.62	±0.91	±10.53	*
	cv (%)	4.52	3.20	4.42	1.48	15.14	
	M	590.23	620.91	625.45	696.35	611.84	
NFE	±Sd	±6.63	±5.66	±6.35	±15.03	±44.05	**
	cv (%)	1.12	0.91	1.01	2.16	7.20	
	M	267.28	159.99	274.97	184.53	231.86	
NDF	±Sd	±27.87	±1.16	±15.46	±4.02	±11.23	****
	cv (%)	10.43	0.72	5.62	2.18	4.84	
	M	109.35	80.18	122.51	90.91	104.54	
ADF	±Sd	±9.96	±2.68	±7.50	±1.54	±13.04	***
	cv (%)	9.11	3.34	6.12	1.69	12.47	
	M	33.87	15.86	25.19	22.36	21.11	
ADL	±Sd	±1.95	±1.57	±0.87	±2.21	±5.57	***
	cv (%)	5.76	9.90	3.45	9.88	26.38	
	M	426.43	523.71	445.20	575.36	463.73	
NFC	±Sd	±18.43	±8.55	±19.24	±18.71	±57.83	***
	cv (%)	4.32	1.63	4.32	3.25	12.47	

DM-Dry Matter; CP-Crude protein; CF-Crude fibre; EE-Ether extract; CA-Crude ash; NFE-Nitrogen-free extract; ADF-Acid detergent fibre; NDF-Neutral detergent fibre; ADL-Acid detergent lignin; NFC-Non-fibre carbohydrates; M±Sd: Mean±Standard deviation; cv (%)-coefficient of variation; P- Statistic probability; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$; ns-not-significant

Concentrates mixtures in ruminant nutrition, could be either, rich in energy or protein and the occurred difference reflected different goat feeding practices in Vojvodina. The animal feed industry offers different types of concentrate, especially with different CP contents. Therefore, the current Serbian legislation does not recognize limits for quality and compositional parameters of dairy goats concentrate. The concentrates fed on dairy goat farms often determine the majority of the cost of milk production, yet little guidance is given to farmers in ration

balancing (*Delaney et al., 2008*). Most commercially prepared goat concentrates contained 12-17% of crude protein but it is of major importance that the dairy goats have a balanced diet. Different carbohydrates intake as a source of energy is important for better efficiency of nitrogen utilization. Particularly, it is important for having a balance between the amounts and availabilities of N and energy to the rumen microbial population (*Waldo, 1967*). In research done by *Schmidely et al. (1999)* goats fed with the rapidly degraded diet compared with goats fed the slowly degraded diet possessed a greater amount of N in urine and at the same, the efficiency of N use for milk output was better but N balance was lower.

Concentrate feedstuffs have a high content of rich-carbohydrate components in the form of various sugars and starch (fractions A+B1, Table 5.). NFC possessed high soluble digestibility and highest amount, as expected, was determined in low-protein concentrate (FD) but also in pelleted concentrate mixtures (Tables 3 and 5.). In addition, pelleted concentrate mixtures contained the lowest values of dietary CF, NDF, ADF and ADL, which contributes to its higher digestibility. It is advisable to use concentrates as pellets, in order to avoid feed selection and pulmonary disease caused by dust inhalation which can occur when the particle size of the feedstuff is too small (*Rapetti and Bava, 2008*).
Organic concentrate compared to other concentrate mixtures contained higher amounts of dietary CF and ADL and according to this lower nutritional quality.

Considering the fat content, it is most varied in samples collected from FD (cv: 43.51%). Fat energy density should be an advantage when formulating rations for high producing dairy goats. Higher levels of fat will limit consumption and can result in gastrointestinal discomfort, but goats, unlike other ruminants, can tolerate more than 6% of dietary fat (*Kouakou et al., 2008*). In many cases, it is possible that the nutrient composition of concentrate does not complement properly hay. As a consequence, the final ration does not fully satisfy the nutrient requirements of goats (*Rapetti and Bava, 2008). Pulina et al. (2008)* recommended that forage/concentrate ratio should be higher than 45–55 to maintain milk fat content above 3%. If the quantity of concentrate in the diet is increased milk fat content will slightly decrease (*Morand-Fehr et al., 2007; Pulina et al., 2008*) but results of *Morand-Fehr and Sauvant (1980)* showed that milk production was improved by almost 20% with the diet high in concentrate.

c) Corn silage and alfalfa haylage

The proximate composition of corn silage and alfalfa haylage has been presented in Table 4. Significant differences ($p<0.05$) were observed for the parameters of dietary CP, ADF and ADL for corn silage samples. Furthermore, analysis of this feedstuff quality showed variability in NDF and ADL cell wall content, and particularly in organic silage for EE content (cv: 34.08%).

Table 4. The chemical composition of corn silage and alfalfa haylage (g kg $^{-1}$DM)

Farms/Parameters		Corn silage			Alfalfa haylage	
		FA	FD	FE	FE	FF
DM g kg^{-1}	M	289.57	375.98	353.30	416.27	366.87
	±Sd	±34.67	±7.20	±22.93	±46.55	±45.97
	cv(%)	11.97	1.91	6.49	11.18	12.53
CP	M	74.47*	65.37*	82.50*	159.99	184.17
	±Sd	±5.81	±1.86	±5.18	±38.32	±13.30
	cv (%)	7.80	2.84	6.27	23.95	7.22
EE	M	21.54	23.82	28.30	26.14	28.78
	±Sd	±7.34	±1.10	±4.11	±8.48	±4.09
	cv (%)	34.08	4.62	14.48	32.44	14.21
CF	M	241.22	217.04	227.40	317.44*	280.51*
	±Sd	±35.59	±21.57	±67.42	±16.18	±12.93
	cv (%)	14.75	9.94	29.65	5.10	4.61
CA	M	47.23	42.17	39.70	120.41	160.95
	±Sd	±12.98	±1.08	±4.17	±27.20	±72.93
	cv (%)	27.48	2.56	10.49	22.59	45.31
NFE	M	613.13	651.60	621.80	376.90	345.59
	±Sd	±35.88	±21.72	±69.74	±25.39	±78.97
	cv (%)	5.85	3.33	11.21	6.74	22.85
NDF	M	480.08	423.44	414.00	510.32	456.65
	±Sd	±32.19	±63.54	±49.94	±93.61	±48.21
	cv (%)	6.71	15.01	12.06	18.34	10.56
ADF	M	319.24*	261.02*	242.20*	381.14	363.89
	±Sd	±23.51	±19.08	±30.66	±46.28	±21.93
	cv (%)	7.36	7.31	12.65	12.14	6.03
ADL	M	52.54*	36.24*	27.98*	75.60	82.71
	±Sd	±11.45	±0.62	±8.61	±11.50	±16.43
	cv (%)	21.79	1.71	30.77	15.21	19.86
NFC	M	376.69	445.47	435.20	183.15	190.65
	±Sd	±33.51	±63.32	±50.45	±92.85	±44.50
	cv (%)	14.68	14.21	11.59	50.70	23.34
RFV	M	121.98	153.44	159.28	110.91*	130.73*
	±Sd	±6.35	±28.52	±24.72	±24.63	±18.45
	cv (%)	5.21	18.59	15.52	22.21	14.11

DM-Dry Matter; CP-Crude protein; CF-Crude fibre; EE-Ether extract; CA-Crude ash; NFE-Nitrogen-free extract; ADF-Acid detergent fibre; NDF-Neutral detergent fibre; ADL-Acid detergent lignin; NFC-Non-fibre carbohydrates; ; M±Sd: Mean±Standard deviation; cv (%)-coefficient of variation; *:p<0.0

According to *Van Soest (1982)*, high NDF values above 480-500g kg^{-1}DM reduce the silage quality and consequently decrease consumption rates. In our study, organic corn silage possessed high levels of NDF (480.08g kg $^{-1}$DM) and lower nutrient value (RFV, 121.98). Silage production could be an alternative to haymaking but its nutritional value can be also quite variable (*Martin et al., 2004; Blair, 2011*). In comparison to alfalfa hay, corn silage contains much less protein and ash content (30-50%), but significantly more NFC. The difference in protein,

fibre and NFC between these two feedstuffs suggests that they might complement one another in dairy rations (*Martin et al., 2004*). Furthermore, *Canizares et al. (2008)* recorded that high moisture corn silage can total or partially replace corn grain without affecting milk production in Alpine goats. It must also be noted that in the presence of certain feeds, such as hay and silage goats showed selective feeding behaviour and more aggressive competition for hay than silage which confirmed their higher preference for hay (*Jorgensen et al., 2007*).

The alfalfa haylage samples showed a significant difference ($p<0.05$) in regard to the content of TC and RFV value (Tables 4 and 5). Comparing our results with values obtained by *Vranić et al. (2011)* our samples contain markedly higher values of average crude protein (141.6g kg^{-1}DM) while the content of NDF was in a similar range (447.0-527.0 g kg^{-1}DM).

Table 5. Carbohydrate fractions of the feeds in the proportion of total carbohydrates

	TC g kg^{-1}DM, M±Sd	Fraction A+B1,%	Fraction B2, %	Fraction C, %
Alfalfa hay				
FA	738.37±35.17	25.49	62.36	12.11
FB	685.68±12.33	36.39	52.50	11.11
FC	741.23±24.99	30.68	54.87	14.45
FD	744.53±34.70	27.33	60.37	12.30
FE	712.61±43.80	29.58	59.02	11.40
FF	698.52±7.42	28.13	59.42	12.45
Concentrate mixtures				
FA	693.70±9.52****	61.47	33.65	4.88
FB	683.70±7.71****	76.60	21.02	2.38
FC	720.20±3.99****	61.82	34.68	3.50
FD	760.30±15.67****	75.68	21.33	2.99
FE	676.20±14.99****	67.02	30.01	2.97
Corn silage				
FA	856.76**	43.97	49.90	6.13
FD	868.64**	51.28	44.55	4.17
FE	849.28**	51.25	45.46	3.29
Lucerne silage				
FE	693.46*	26.41	62.69	10.90
FF	626.11*	30.45	59.72	13.21
Pasture				
May	710.80	26.83	67.00	6.16
June	702.00	25.71	59.87	7.51
July	677.65	14.36	78.46	7.20
August	635.86	20.22	70.87	8.91
September	718.37	21.93	71.70	6.37

TC-total carbohydrates; fractions A+B1-rapidly degradable carbohydrates; fraction B2-potentially degradable carbohydrates; fraction C-non-degradable carbohydrate; *: $p<0.05$; **: $p<0.01$; ****: $p<0.0001$

d) Pasture

The average chemical composition of natural pasture in the five-month period is reported in Figure 1. The quality of pasture depends on many factors: grass species, nutrient composition, stage of maturity, soil and climatic conditions etc. Generally, stage of maturity and nutrient contents of these feed resources often is not correlated and protein content decreased as the season advanced and fibre fractions increased. *Leng (1990)* reported that pasture can be considered to be of low to intermediate nutritional quality if they contain less than 55% organic matter and 8% CP. According to this, investigated pasture could be categorised of lower quality forages due to the average percent of CP was below the stated value. In further, the highest content of NDF and at the same, the lowest values in RFV were registered during the summer and autumn months. The different fractions of carbohydrates vary considerably during the season. The highest values of A+B1 carbohydrate fraction were recorded in the first two months of investigation. Similar results have been published by *Tudisco et al. (2010).* They recorded the worst chemical composition of pasture in July. On the other hand, the highest protein content and low NDF was registered during May and September. This is in general means that, in summer, the marked decrease in fermentable compounds, especially sugars, is followed by an increase in fibre fractions which greatly reduce the nutritive value of the grass (*Bonanno et al., 2008*). Mature forage will have high lignin content and be of limited use to grazing animals (*Pulina et al., 2008*).

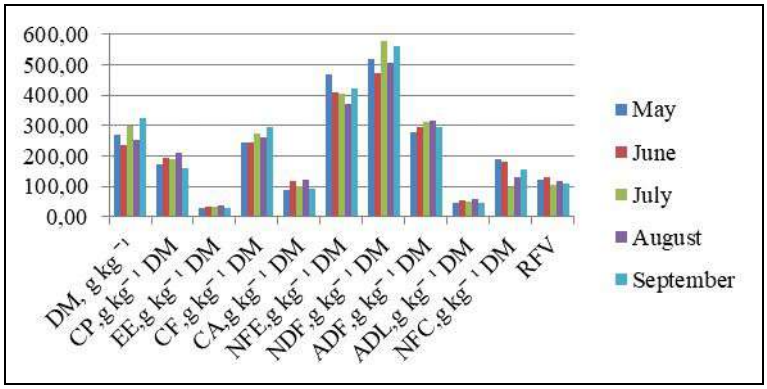

Figure1. Chemical composition of pasture in five months period

Conclusion

Investigated farms used different concentrate mixtures (energy or protein-rich concentrate) and present different supplementary feeding strategies. The obtained results showed that the nutritional quality of feedstuffs used in the goat's nutrition

in Vojvodina was widely variable. Results also indicated that in particular, organic and small conventional farms used feedstuffs of less quality and therefore of lower nutritional quality. Moreover, relative feed value for alfalfa hay, haylage and corn sillage, showed different values, in the range from the highest, medium to lowest quality forage. This was probably caused by a different productivity strategy and still insufficiently developed goat and especially, organic livestock management system. Even though considerable advances have been made in goat dairy production, and progress is being made relative to feedstuffs quality, the development of feeding systems for goats is even more challenging. It requires not only the optimization of natural and local resources but the efforts, together with government considerations should be intensified to improve and established standards for goat's feed quality. An intensive feeding system based on pelleted concentrate mixtures and quality hay showed that it could be an alternative promising feeding system to rear goats more effectively.

Nutritivni kvalitet hraniva koja se koriste u ishrani mlečnih koza u Vojvodini

Snežana Paskaš, Jelena Miočinović, Branislav Vejnović, Zsolt Becskei

Rezime

Istraživanje je bilo sprovedeno sa ciljem procene hemijskog sastava i nutritivne vrednosti kabastih hraniva i smeša koncentrata koja se koriste u ishrani mlečnih koza u Vojvodini. Ispitivano je ukupno šest farmi,uključujući i organsku farmu. Dobijeni rezultati su pokazali da se relativna hranibena vrednost analiziranih kabastih hraniva kretala od dobrog, srednjeg do slabijeg kvaliteta. Prosečan sadržaj proteina od najmanjih do najviših vrednosti se kretao u opsegu: kukuruzna silaža (*Zea Mays*) (65.37-82.50g kg^{-1}DM), senaža lucerke (*Medigao sativa L.)* (159.99-184.17g kg^{-1}DM), pašnjak (185.30g kg^{-1}DM) i seno lucerke (*Medigao sativa L.*) (167.48-203.60g kg^{-1}DM). Sadržaj nestrukturnih ugljenih hidrata i proteina pokazao je najveće varijacije u uzorcima organskog sena (cv: 29.25% и 19.09%, pojedinačno). Generalno, hraniva koja su bila ispitivana na organskoj farmi, uključujući organske smeše koncentrata, su pokazale lošiji nutritivni kvalitet usled većeg sadržaja sirovih vlakana i lignina. Posebno su utvrdjene velike varijacije u ispitivanim smešama koncentrata u pogledu sadržaja proteina ($p<0.0001$), koji je bio rangiran od 135.32 do 209.87g kg^{-1}DM. Kukuruzne silaže su takodje značajno varirale u njihovom hemijskom sastavu i signifikantna razlika ($p<0.05$) je utvrdjena u pogledu sadržaja kiselih deteržent vlakana (ADF) i lignina (u opsegu: ADF:

242.20-319.24g kg^{-1}DM; ADL: 27.98-52.54g kg^{-1}DM, pojedinačno). Osim toga, utvrdjeno je da je pašnjak posedovao najviše rastvorljivih materija tokom Maja i Juna meseca a njihov sadržaj je bio obrnuto povezan sa sadržajem sirovih vlakana. Ovo istraživanje je pokazalo da ispitivane farme još uvek ne posvećuju dovoljno pažnje kvalitetu hraniva. Za razvoj intenzivnog uzgoja koza, veći naglasak treba staviti na upotrebu kvalitetnijih hraniva i istovremeno standardi kvaliteta hrane moraju biti razmotreni i utvrđeni.

Ključne reči: hraniva, hemijski sastav, nutritivna vrednost, frakcije ugljenih hidrata

References

AOCS (2005): Approved Procedure Ba 6a-05. The AOCS-approved Ba 6a-05 procedure for crude fibre (CF) and the Filter Bag Technique (Ankom Technology Corp., Fairport, NY).

BLAIR R. (2011): Nutrition and Feeding of Organic Cattle. Faculty of Land and Food Systems.The University of British Columbia Vancouver, British Columbia Canada, 62-212.

BONANNO A., FEDELE F., DI GRIGOLI A. (2008): Grazing management of dairy goats on Mediterranean herbaceous pastures. In: Dairy goats feeding and nutrition. Edited by Antonello Cannas and Giuseppe Pulina, CAB International, Wallingford, UK, 189-220.

CANNAS A., ATZORI A. S., BOE F., TEIXEIRA I. A. M. A. (2008): Energy and protein requirements of goats. In: Dairy goats feeding and nutrition. Edited by Antonello Cannas and Giuseppe Pulina, CAB International, Wallingford, UK, 118-147.

CANIZARES I. L. G., GONCALVES C. H., RODRIGUE L., MARTINS F. M., DE ARRUDA M. M. F. G., MARLEIDE C., CANIZARES C. M. (2008): Milk production and composition of lactating goats fed with high moisture corn silage. Proceedings of the 9th International Conference on Goats "Sustainable goat production: Challenges and opportunities of small and large enterprises", August 31st to September 4th, Queretaro, Mexico, pp 362.

DELANEY C., JOHNSON D., WALDRON M. (2008): Effects of concentrate supplementation on milk yield and MUN concentration on commercial dairy goat farms. Proceedings of the 9th International Conference on Goats "Sustainable goat production: Challenges and opportunities of small and large enterprises", August 31st to September 4th, Queretaro, Mexico, pp 373.

GRAPHPAD PRISM version 6, statistical software, GraphPad, San Diego, CA, USA. https://www.graphpad.com/data-analysis-resource-center/

JORGENSEN M., MEISFJORD G. H., ANDERSEN I. L., BOE K. E. (2007): Feed intake and social interactions in dairy goats-the effects of feeding space and type of roughage. Applied Animal Behaviour Science, 107, 239-251.
KOUAKOU B., LEE H. J., KANNAN G. (2008): Effects of high dietary soybean oil for goats in late lactation on intake, milk composition and fatty acid profile. Proceedings of the 9th International Conference on Goats "Sustainable goat production: Challenges and opportunities of small and large enterprises", August 31st to September 4th, Queretaro, Mexico, pp 350.
LACEFIELD G. D. (1988): Alfalfa hay quality makes the difference. Agriculture and Natural Resources Publications, 32. https://uknowledge.uky.edu/anr_reports/32
LEE M. C., HWANG S., Y, CHIOU P. W. S. (2007): Application of rumen undegradable protein on early lactating dairy goats. Asian-Australian Journal of Animal Science, 14, 11, 1549-1554.
LENG R. A. (1990): Factors affecting the utilization of "poor quality" forages by ruminants, particularly under tropical condition. Nutrition Research Review, 3, 277-303.
MARTIN P. N., MERTENS R. D. J., WEIMER J. (2004): Alfalfa: hay, haylage, baleage, and other novel products. Presented at the Idaho Alfalfa and Forage Conference 23-24 February, Twin Falls, ID, University of Idaho Cooperative Extension, USA.
MARTINEZ-MARIN A. L., PEREZ-HERNANDEZ M., PEREZ-ALBA L. M., GOMEZ-CASTRO A. G. (2010): Choosing a feed evaluation system-NRC vs INRA- to formulate rations for growing goats using minimum cost linear programming. Journal of Animal and Feed Sciences, 19, 525-538.
MORAND-FEHR P., SAUVANT D. (1980): Composition and yield of goat milk as affected by nutritional manipulation. Journal of Dairy Science, 63, 1671-1680.
MORAND-FEHR P., FEDELE V., DECANDIA M., LE FRILEUX Y. (2007): Influence of farming and feeding systems on composition and quality of goat and sheep milk. Small Ruminant Research, 68, 1-2, 20-34.
OFFICIAL GAZETTE OF SFRJ (1987): Pravilnik o metodama uzimanja uzoraka i metodama fizičkih, hemijskih i mikrobioloških analiza stočne hrane 15/1987.
OLIVEIRA S. T., LEONEL F. P., SILVA J. C., BAFFA F. D., PEREIRA C. J., ZERVOUDAKIS T. J. (2014): Factors affecting feed efficiency in dairy goats. Revista Brasileira de Zootecnia, 43, 10, 524-529.
PETROVIĆ P. M., PETROVIĆ C. V., MUSLIĆ R. D., MAKSIMOVIĆ N., CEKIĆ B., ILIĆ Z. Z., KURČUBIĆ V. (2017): "Strategy for Sustainable Development and Utilization of Sheep and Goat Resources in Serbia" in 2nd International Conference on Sustainable Agriculture and Food Security: A Comprehensive Approach. KnE Life Sciences, 11-21. http//:doi10.18502/kls.v2i6.1015.
PULINA G., NUDDA A., BATTACONE G., FANCELLU S., FRANCESCONI A. H. D. (2008): Nutrition and Quality of Goat's Milk. In: Dairy goats feeding and

nutrition. Edited by Antonello Cannas and Giuseppe Pulina. CAB International, Wallingford, UK, 1-31.
PULINA G., AVONDO M., MOLLE G., FRANCESCONI A. H. D., ATZORI A. S., CANNAS A. (2013): Models for estimating feed intake in small ruminants. Revista Brasileira de Zootecnia, 42, 9, 675-690.
RAHMANN G. (2009): Goat milk production under organic farming standards. Tropical and Subtropical Agroecosystems, 11, 105-108.
RAPETTI L., BAVA L. (2008): Feeding Management of Dairy Goats in Intensive Systems. In: Dairy goats feeding and nutrition. Edited by Antonello Cannas and Giuseppe Pulina. CAB International, Wallingford, UK, 221-238.
SCHMIDELY, P., LLORET-PUJOL, M., BAS, P., ROUZEAU, A., SAUVANT, D. (1999): Influence of feed Intake and source of dietary carbohydrate on milk yield and composition, nitrogen balance, and plasma constituents of lactating goats. Journal of Dairy Science, 82, 747–755.
SNIFFEN C. J., O'CONNOR J. D., VAN SOEST P. J., FOX D. G., RUSSELL J. B. (1992): A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. Journal of Animal Science, 70, 11, 3562-3577.
SOKOLOVIĆ D., DINIĆ B., BABIĆ S., RADOVIĆ J., LUGIĆ Z., TOMIĆ Z., JEVTIĆ G. (2013): Forage quality, production and conservation on perennial grasses. Proceedings of the 10th International Symposium „Modern Trends in Livestock Production“, October 2-4, 364-381.
TUDISCO R., CUTRIGNELLI I. M., CALABRO S., PICCOLO G., BOVERA F., GUGLIELMELLI A., MONIELLO G., INFASCELLI F. (2010): Influence of organic systems on milk fatty acid profile and CLA in goats. Small Ruminant Research, 88, 151-155.
VAN SOEST P. J. (1965): Symposium on factors influencing voluntary intake of herbage by ruminants: chemical composition and digestibility. Journal of Animal Science, 24, 834–843.
VAN SOEST P. J. (1982): Nutritional ecology of the imminent. Comstock, ComeU Univ. Press, Ithaca, NY2. Nutritional ecology of the ruminant, 0&B Books, Corvallis, Oreg. https://trove.nla.gov.au/version/13770830
VAN SOEST P. J., ROBERTSON J. B., LEWIS B. A. (1991): Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to nutrition. In: Symposium Carbohydrate Methodology, Metabolism, and Nutritional Implications in Dairy Cattle. Journal of Dairy Science, 74, 3583-3597.
VAN SOEST P. J. (1994): Nutritional ecology of the ruminant 2e.d. NY: Cornel University Press, Ithaca, USA, pp 476.
VAN SOEST J. P. (1996): Allometry and Ecology of Feeding Behavior and Digestive Capacity in Herbivores: A Review. Zoo Biology, 15, 455-479.
VRANIĆ M., KNEŽEVIĆ M., PERČULIJA G., BOŠNJAK K., LETO J., KUTNJAK H., HORG M. (2011): Fermented high moisture maize grain as

supplement to alfalfa haylage is superior over unfermented dry maize grain in diet dry matter digestibility. Mljekarstvo, 61, 3, 244-251.
WALDO, D. R. (1967): Symposium: Nitrogen Utilization by the Ruminant Nitrogen Metabolism in the Ruminant 1. Presented at the Sixty-second Annual Meeting of the American Dairy Science Association, June, Ithaca, New York, USA, 265-275.

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STUDY OF FATTENING AND SLAUGHTER TRAITS OF CATTLE UNDER THE INFLUENCE OF FLAX SEED BASED NUTRITION

Maja Petričević[1], Dušan Živković[2], Dušica Ostojić Andrić[1], Dragan Nikšić[1], Veselin Petričević[1], Marija Gogić[1], Violeta Mandić[1]

[1]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080 Belgrade-Zemun, Republic of Serbia
[2] University of Belgrade - Faculty of Agriculture, Nemanjina 6, 11080 Belgrade-Zemun, Republic of Serbia
Corresponding author: majanovakovic@live.com
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Abstract: The trial was designed in order to examine the impact of flax seed in the nutrition of young cattle/bulls, in the final stage of the fattening. In the trial, 30 bulls of Simmental breed of uniform initial weight were selected, divided into 2 groups (control and experimental). The control animal group did not consume flax seed as a food supplement. Cattle of the experimental group consumed flax seed in an amount of 3.75% (300 g per day) of concentrated meal in the last 90 days of fattening, i.e. 300 g per day. The study included the examination of the fattening performance, slaughter traits and the composition of the bovine carcass. After slaughtering, warm carcass sides, with and without kidneys, were measured individually. Subsequent to period of cooling, the left carcass side it was cut into the main carcass parts according to the Rulebook. The results of the study showed that the addition of flax seed in the diet did not have a statistically significant effect on the body weight of bulls at the end of the trial. It was found that the addition of flax seed in the feed during the final stage of fattening did not have an impact on the differences in the average overall gain of bulls and the feed conversion ratio. Based on the data obtained by cutting of carcass sides to main parts, it was established that feeding with flax seeds had no significant effect on the share of carcass parts.

Key words: flaxseed diet, young bulls, Simmental breed

Introduction

Beef is considered to be "meat of the highest quality” in most countries because of its biological value and sensory properties. The economic effect, which is reflected in achieving the best possible results with minimal costs of production,

is important to the cattle producers. The economy of slaughtering and processing and technological quality is an important aspect for the slaughter industry, and for the consumer sensory, and increasingly nutritious quality of meat. *Pavlovski et al. (2003)* state that European consumers prefer less fatty, less caloric beef, while English consumers prefer fatty, succulent meat.

For producers, the quality of cattle depends on the properties (body weight) which have the greatest impact on the price when sold. The most important factor responsible for changes in carcass properties are the genetic traits that influence the fat deposition and structure and properties that can alter the meat quality *(Prado et al., 2008b; Prado et al., 2009; Rotta et al., 2009a).* Depending on the requirements of the final consumer, meat quality can be defined in several ways: carcass quality, nutritive, sensory and technological quality.

The amount of fatty tissue and its distribution has a significant role in the carcase value, as an excessive amount of fatty tissue can have a negative economic effect. An excess of intermuscular or subcutaneous fat tissue is eliminated in the treatment/processing of the carcass or main carcass parts and represents an economic loss for producers and processors *(Harper et al., 2001)***.**

The quality of carcasses of slaughtered animals is a matter of interest both in primary production and in the meat industry *(Petrović et al., 2016)***.** Based on the estimated value of carcasses of slaughtered animals and classification in classes, it is possible to make appropriate financial compensation to producers and in this way stimulate them to produce slaughter animals of the highest quality. The criterion for evaluating the beef carcass is mostly its weight, conformation, carcass covering with fatty tissue and the ratio of muscle and fat tissue.

The body weight of the cattle before slaughter has a significant effect on the carcass yield, the meat yield and the quality of the meat. In Serbia, Domestic Spotted beef is the most prevalent breed, with 45% in 2000 *(Petrović et al., 2001)*. *Aleksić et al. (2001)* state that the quantity and quality of meat is a phenotypic characteristic in the function of genotype and nutrition. With the increase in weight and age of the animal, the yield and the lean meat content of the carcass also increase *(Pečiulaitienė et al., 2015)*. *Petrović et al. (2016)* state that the heifers of the body weight of 450–500 kg show slaughter yields of 42–56%, and the heifers of the body weight of 550–600 kg 49–57%. The male cattle of the Domestic Simmental breed of average weight of about 554 kg achieve higher slaughter yields compared to those of the same breed of average body weight of 509 kg *(Aleksić et al., 2009)*.

One of the most important factors that significantly affects the quality of beef carcass and meat is nutrition *(Abrahão et al., 2005; Prado et al., 2008a)***.** A diet for fattening beef cattle must be balanced in terms of the contents of dry matter, energy, protein, mineral matter and vitamins. Cereals are the main source of energy in the final fattening phase, but oils and fats can also be used as alternative components *(Rotta et al., 2009b)***.** It is very important that the meal also tastes

good, so that the animals consume it better. If more concentrates are present in the meal, the gain will be higher, the duration of fattening will be shorter, but also more expensive, so the diet should include roughage. Nutrition is an important factor that affects the production of meat.

Flaxseed is used in animal nutrition because of the specific and, from the nutritional aspect, preferred fatty acid composition. This property makes flaxseed an extremely interesting raw material for the production of functional feeds that can increase the intake of essential fatty acids in the organism of animals and, consequently, change the fatty acid composition of fat and meat through which the intake of essential fatty acids can be increased in the human organism *(Larsen et al., 2012)***.** Given the high content of oil, flaxseed is used in the diet of cattle as a source of fat. Flaxseed can also be used as an alternative source of protein in ruminant nutrition, but in limited quantities due to high oil content *(Lardy and Anderson, 1999)***.** Heat treated flaxseed (by toasting, extruding) has a greater influence on the meat yield than the untreated seed *(Maddock et al., 2004)***.** In recent years, the functional feeds are being used in livestock production intensively, with the goal of improving the health and general condition of the organism of animals, improving the growth and better conversion of food.

Materials and methods

The research was carried out on the experimental farm and in the experimental slaughterhouse of the Institute for Animal Husbandry in Zemun (Serbia). In the study, male cattle of Domestic Simmental breed were used. In the trial, 30 Simmental young bulls of uniform weights were selected (431 kg), which consumed the food of the same composition until reaching the age of 390 days. The feeding of cattle prior to trial was carried out according to existing diet composition norms for these cattle used on the farm of the Institute for Animal Husbandry (whole maize silage and concentrate mixture with 12% of the total protein). The fattening of young bulls was in the free system. In order to fulfil the trial objective, it was necessary to prevent the movement of the animals when consuming a concentrated portion of the meal, so that we can reliably claim that each animal consumed the predetermined amount of concentrate. At the age of 390, two groups of 15 cattle were formed: the control group (CON) in which the cattle did not consume heat-treated flax seed and the experimental group (FXS) in which the part of the concentrate was replaced by heat-treated flax seeds, so that each animal consumed 300 g of flax seeds per day. The final pre-slaughter weights were about 570 kg. One day before slaughter, the bulls did not receive food, but they had free access to water. Slaughtering and primary processing were performed in the experimental slaughterhouse of the Institute for Animal Husbandry. Animals were measured immediately before slaughter and then slaughtered according to standard

commercial procedures. After primary processing, the carcasses were chilled at 4^0C for the next 24 hours. The weight of the warm carcass, the weight of the intestines (heart, lungs, liver, kidneys, spleen and tongue), head, tail and kidney fat was measured one hour post- slaughter and chilling. After chilling, the carcasses were measured and split along the vertebral column in two halves, and the left side was used for all measurements. The left side of each carcass was divided into twelve anatomical regions: round, beefsteak, loin, shoulder, back, neck, chest, short ribs, ribs, flank, fore shank and leg, using a standard technique.

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

Results and discussion

The results for fattening and slaughter traits of the young bulls are shown in Table 1. CON had better gain and feed conversion ratio, but there was no statistical significance between the established differences ($p > 0.05$). The addition of flax seed in the cattle feed did not have a significant effect ($p > 0.05$) on the pre- and post-slaughter weight of the carcass, as well as the yield of warm and cooled carcass. The determined values were similar among groups of cows. The larger pre- slaughter weight was recorded in CON. The weight of warm carcasses with and without fat tissue and weight of cold carcasses without fat tissue were higher in CON. The yield of warm carcasses with and without fat tissue, as well as of the cooled carcass without fat tissue were higher in FXS.

Table 1. Average values of fattening and slaughter traits of cattle/young bulls

	CON	FXS	p
ADG[1] (kg)	1.59 ± 0.43	1.48 ± 0.29	ns
Feed conversion (kg)	7.23 ± 0.73	7.74 ± 0.89	ns
Weight PS [2] (kg)	576.25 ± 25.36	561.67 ± 11.93	ns
Weight WC [3] with fat (kg)	337.52 ± 19.83	332.50 ± 9.32	ns
Yield WC [3] with fat (%)	58.56 ± 1.53	59.23 ± 2.59	ns
Weight WC [3] without fat (kg)	333.50 ± 19.33	328.33 ± 9.07	ns
Yield WC [3] without fat (%)	57.85 ± 1.32	58.49 ± 2.53	ns
Weight CC [4] without fat (kg)	326.82 ± 18.96	321.63 ± 9.17	ns
Yield CC [4] without fat (%)	56.70 ± 1.27	57.30 ± 2.53	ns
MLD[5] Cross section surface (cm^2)	100.95 ±16.35	109.94 ± 11.00	ns
Kidney fat (%)	0.70 ± 0.24	0.74 ± 0.06	ns
CL Cooling loss [6] (%)	3.17±0.44	3.27±0.05	ns
Head (%)	2.59 ± 0.09	2.65 ± 0.14	ns
Tail (%)	0.17 ± 0.02	0.21 ± 0.06	ns

[1] ADG – average daily gain; [2] PS – Pre- slaughter; [3] WC – Warm carcass; [4] CC– cooled carcass; [5] MLD – *Musculus longissimus dorsi*; [6] CL – loss of weight during cooling; ns – not significant

Maurić et al. (2016) state that the slaughter yield of the Simmental cattle ranges from 58.11–59.95% in pre-slaughter body weights from 526–588 kg. *Iwanowska and Pospiech (2010)* have established a yield of 54.96% for the Simmental breed in the body weight before slaughter of 595 kg, with a warm carcass weight of 327 kg. The results of a study by a large number of authors confirm that the use of heat-treated flax seeds in beef nutrition has no significant effect on the quality of the carcass *(Dawson et al., 2010; Barton et al., 2007; Leanne, 2008). Quinn et al. (2008), Şentürklü and Landblom (2014), Hernàndez-Calva et al. (2011)* and *Corazzin et al. (2012)* have found no significant differences in the final weight and slaughter yield between groups of cattle depending on the diet with flaxseed. *Albertía et al. (2014)* confirm that the consumption of concentrate mixture with the addition of 5% of flax seed has no effect on the carcass traits. Likewise, *Maddock et al. (2003; 2004)* and *Alvarado-Gilis et al. (2015)* find that the addition of 3–6% of flax seeds in the final nutrition of cattle does not affect the carcass traits. Results obtained by *Maddock et al. (2006)* show that the inclusion of 8% of flax seeds in the diet improves the carcass traits but it can lead to an increase in the amount of fat that negatively affects some of the properties of meat quality. *Kim et al. (2004)* have established higher slaughter yields of the cattle (57.8% and 57.7%) fed diets containing 10% and 15% of flax seeds in relation to the control group (57.1%), but these differences were not statistically significant. These authors have concluded that flax seed is an acceptable source of fat without adversely affecting the final fattening of the cattle. According to *Drouillard et al. (2002)* the inclusion of flax seed in the diet for cattle at different ages has no significant effect on the carcass traits compared to those who did not consume the flax seed. In general, the results of these studies are in accordance with the data presented in our study where it has been established that flax seed has no significant effect on the differences in the slaughter properties of beef carcasses.

The surface of M. longissimus dorsi cross section (Table 1) was not statistically significantly different ($p > 0.05$) under the influence of the examined factor. The larger surface area of the M. longissimus dorsi was recorded in young bulls of group FXS. *Rotta et al. (2009b)* and *Quinn et al. (2008) have* found a larger surface area of the M. longissimus dorsi cross section in cattle that consumed flax seed in the diet compared to cattle without flax seed in their diet. *Maddock et al. (2006)* obtained a larger surface area of the M. longissimus dorsi cross section in cattle that consumed 8% of the ground flax compared to the young bulls consuming the same amount of whole flax seed.

The loss of weight after 24 hours of cooling of the carcass sides at a temperature of -1 to +4°C did not differ significantly ($p > 0.05$) between the tested groups. *Hernàndez-Calva et al. (2011)* state that the loss of weight during cooling did not significantly change under the influence of flax seed nutrition.

The share of the main carcass parts is shown in Table 2. The shares of the most valuable parts of the carcass (beefsteak and round) were approximately the same between the groups and showed no statistically significant ($p > 0.05$) differences between the groups. In the CON and FXS group the same value for the share of beef steak was determined. The share of round varied from 28.05% in CON to 28.97% in FXS. The shares of the loin part, back, shoulders and short ribs were not significantly different ($p > 0.05$) between the groups under the influence of the examined factor. The share of loin ranged from 4.84% to 5.32%. A higher share of the back was determined in CON. FXS showed lower share of the shoulder, while the share of the short ribs was higher.

Table 2. The effect of the addition of flax seed in the feeding of cattle on the share of main carcass parts *

(%)	CON	FXS	p
Beef steak	2.41 ± 0.45	2.41 ± 0.25	ns
Round	28.05 ± 1.21	28.97 ± 0.29	ns
Loin	4.84 ± 1.15	5.32 ± 0.61	ns
Back	5.48 ± 0.69	5.32 ± 0.33	ns
Shoulder	12.60 ± 0.73	11.63 ± 0.24	ns
Leg	3.66 ± 0.52	3.91 ± 0.10	ns
Fore shank	2.78 ± 0.26	3.16 ± 0.14	ns
Neck	10.14 ± 1.01	9.96 ± 0.57	ns
Chest	5.18 ± 0.64	5.26 ± 0.38	ns
Short ribs	11.90 ± 0.43	12.26 ± 0.59	ns
Ribs	6.75 ± 1.43	6.03 ± 0.62	ns
Flank	6.16 ± 0.77	5.70 ± 0.60	ns

*Relative to the processed carcass; ns – not significant

Petričević et al. (2015) state following values for major carcass parts of young bulls not consuming flax seed in diet: round (28.36%), shoulders (12.20%), legs (3,59%) and fore shanks (2.73%).

The consumption of flax seed in the final stage of the cattle fattening did not have an effect on the share of the intestines in the pre- slaughter weight (Table 3). *Petričević et al. (2011)*, in their study, show similar values for the share of intestines in cattle fed without the addition of flax seed.

Table 3. The effect of the addition of flax seed in the cattle diet on the share of intestines**

(%)	CON	FXS	p
Kidneys	0.17 ± 0.02	0.18 ± 0.01	ns
Liver	1.01 ± 0.11	1.24 ± 0.10	ns
Lungs	0.55 ± 0.08	0.55 ± 0.03	ns
Heart	0.30 ± 0.02	0.28 ± 0.07	ns
Spleen	0.18 ± 0.03	0.22 ± 0.01	ns
Tongue	0.24 ± 0.03	0.29 ± 0.03	ns

**Relative to the pre-slaughter weight; ns – not significant

Conclusion

The addition of flax seed in the feed during the final phase of fattening of the cattle showed no statistically significant ($p>0.05$) impact on fattening (average daily gain of cattle, feed conversion ratio) and slaughter traits (weight and yield of warm and cooled carcass, share of main carcass parts, share of intestines). The results of the research confirm that the use of heat-treated flax seed in the diet of cattle does not have a negative effect on the quality of the carcass.

ISPITIVANJE TOVNIH I KLANIČNIH OSOBINA JUNADI POD UTICAJEM ISHRANE SA SEMENOM LANA

Maja Petričević, Dušan Živković, Dušica Ostojić Andrić, Dragan Nikšić, Veselin Petričević, Marija Gogić, Violeta Mandić,

Rezime

Eksperiment je postavljen sa ciljem da se ispita efekat dodavanja semena lana, u ishranu junadi, u završnoj fazi tova. Za ogled je odabrano 30 muških junadi simentalske rase ujednačenih početnih telesnih masa, koja su podeljena u 2 grupe (KON (kontrolna) i FS (ogledna)). Kontrolna grupa junadi nije konzumirala seme lana kao dodatak ishrani. Junad ogledne grupe su konzumirala seme lana u količini od 3,75% (300 g dnevno) koncentrovanog dela obroka u poslednjih 90 dana tova, tj. 300 g dnevno. Istraživanje je obuhvatilo ispitivanje rezultata tova, klanične karakteristike i sastava trupa junadi. Nakon klanja izvršeno je pojedinačno merenje toplih polutki sa i bez bubrežnog loja. Posle hlađenja leva polutka je rasecana u osnovne delove prema Pravilniku. Rezultati istraživanja su pokazali da dodatak semena lana u ishrani nije imao statistički značajan uticaj na masu junadi na kraju ogleda. Utvrđeno je da dodatak semena lana u ishranu tokom završne faze tova nije imao uticaj na razlike u prosečnom ukupnom prirastu (PUP) junadi i konverziji hrane. Na osnovu podataka dobijenih rasecanjem poluki junadi na osnovne delove utvrđeno je da ishrana sa semenom lana nema značajan uticaj na udeo delova trupa.

Ključne reči: ishrana lanom, mladi bikovi, simentalska rasa

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References

ABRAHÃO J. J. S., PRADO I. N., PEROTTO D., MOLETTA J. L. (2005): Effects of replacing corn with increasing levels of cassava starch by-products on carcass characteristics and meat for young bulls. Brazilian Journal of Animal Science, 34, 1640–1650.

ALBERTÍA P., BERIAINB M. J., RIPOLLA G., SARRIÉSB V., PANEAA B., MENDIZABALB J. A., PURROYB A., OLLETAC J.L., SAÑUDOC C. (2014): Effect of including linseed in a concentrate fed to young bulls on intramuscular fatty acids and beef color. Meat Science, 96, 3, 1258–1265.

ALEKSIĆ S., LAZAREVIĆ R., MIŠČEVIĆ B., PETROVIĆ M., JOSIPOVIĆ S., TOMAŠEVIĆ D., KOČOVSKI T. (2001): The effect of live weight prior to slaughtering on yield and weight of retal-carcass cuts. Biotechnology in Animal Husbandry, 17, 5-6, 125–131.

ALEKSIĆ S., PETROVIĆ M. M., PANTELIĆ V., NOVAKOVIĆ Ž., STANIŠIĆ N., NOVAKOVIĆ M. (2009): Investigation of the effect of mass prior to slaughtering on slaughter values of male fattening young cattle of domestic Simmental breed. Biotechnology in Animal Husbandry, 25, 93–99.

ALVARADO-GILIS C. A., APERCE C. C., MILLER K. A., VAN BIBBER-KRUEGER C .L., KLAMFOTH D., DROUILLARD J. S. (2015): Effects of flaxseed encapsulation on biohydrogenation of polyunsaturated fatty acids by ruminal microorganisms: feedlot performance, carcass quality, and tissue fatty acid composition. Journal of Animal Science, 93, 4368–4376.

BARTON L., MAROUNEK V. M., BUREŠ K. D., ZAHRÁDKOVA R. (2007): Growth performance and fatty acid profiles of intramuscular and subcutaneous fat from Limousin and Charolais heifers fed extruded linseed. Meat Science, 76, 517–523.

CORAZZIN M., BOVOLENTA S., SEPULCRI A., PIASENTIER E. (2012): Effect of whole linseed addition on meat production and quality of Italian Simmental and Holstein young bulls. Meat Science, 90, 99–105.

DAWSON L. E. R, FEARON A. M., MOSS B. W., WOODS V. B. (2010): Effects of substitution of a proportion of the concentrate in grass silage/concentrate-based diets with extruded linseed on performance and meat quality of dairy bulls. Animal Feed Science and Technology, 156, 10–18.

DROUILLARD J. S., GOOD E. J., GORDON C. M., KESSEN T. J., SULPIZIO M. J., MONTGOMERY S. P., SINDT J. J. (2002): Flaxseed and flaxseed products for cattle: Effects on health, growth performance, carcass quality, and sensory attributes. Proceedings, 59th Flax Institute, 72–87.

HARPER G. S., BARENDSEL W. J., HYGATE L., ODDY V. H., PETHICK D., TUME R. K. (2001): Biological determinants of intramuscular fat deposition in beef cattle. Current mechanistic knowledge and sources of variation. Final report to

Meat and Livestock Australia (FLOT 208). CRC, Armidale, New South Wales, Australia.
HERNÀNDEZ-CALVA L .M., HE M., JUÀREZ M., AALHUS J. L., DUGAN M. E. R., MCALLISTER T. A. (2011): Effect of flaxseed and forage type on carcass and meat quality of finishing cull cows. Canadian Journal of Animal Science, 91, 613–622.
IWANOWSKA A., POSPIECH E. (2010): Comparison of slaughter value and muscle properties of selected cattle breeds in Poland–revue. Acta Scientiarum Polonorum Technologia Alimentaria, 9, 1, 7–22.
KIM, C. M., KIM J. H., CHUNG T. Y., PARK K. K. (2004): Effects of Flaxseed Diets on Fattening Response of Hanwoo Cattle: 1. Performance and Carcass Characteristics. Asian- Australasian Journal of Animal Science, 17, 1241-1245.
LARDY G. P., ANDERSON V. L. (1999): Alternative feeds for ruminants. North Dakota State University Extension Service Bulletin AS–1182.
LARSEN M. K., HYMOLLER L., BRASK-PEDERSEN D. B., WEISBJERG M. R. (2012): Milk fatty acid composition and production performance of Danish Holstein and Danish Jersey cows fed different amounts of linseed and rapeseed. Journal of Dairy Science, 95, 7, 3569–3578.
LEANNE K. L. (2008): A thesis: Effects of flaxseed supplementation and exogenous hormones on finishing performance, carcass characteristics, and plasma and longissimus muscle fatty acid profiles I nfinishing cattle. B. S., Kansas State University, Kansas, 1–56.
MADDOCK T. D., BAUER M. L., KOCH K., ANDERSON V. L., MADDOCK R. J., LARDY G. P. (2004): The effect of processing flax in beef feedlot rations on performance, carcass characteristics, and trained sensory panel ratings. Proceedings 60th Flax Institute, 118–123.
MADDOCK, T. D., ANDERSON V. L., BERG P. T., MADDOCK R. J., MARCHELLO M. J. (2003): Influence of level of flaxseed addition and time fed flaxseed on carcass characteristics, sensory panel evaluation, and fatty acid content of fresh beef. Proc. 56th Reciprocal Meats Conference, American Meat Science Association, Columbia, Mo.
MADDOCK, T. D., BAUER, M. L., KOCH, K. B., ANDERSON, V. L., MADDOCK, R. J., BARCELÓ-COBLIJN G., MURPHY E. J., LARDY G. P. (2006): Effect of processing flax in beef feedlot diets on performance, carcass characteristics, and trained sensory panel ratings. Journal of Animal Science, 84, 1544–1551.
MAURIĆ M., STARČEVIĆ K., ŠTOKOVIĆ I., MAŠEK T. (2016): The influence of sires selection index on the intramuscular fat fatty-acid profile of their progeny. Veterinarski Arhiv, 86, 6, 753–766.
PAVLOVSKI Z., ALEKSIĆ S., CMILJANIĆ R., ŠKRBIĆ Z., LUKIĆ M. (2003): Ispitivanje odnosa potrošača prema junećem mesu kao prehrambenom proizvodu. Biotehnologija u stočarstvu, 19, 3-4, 267–283.

PEČIULAITIENĖ N., JUKNA V. MEŠKINYTĖ-KAUŠILIENĖ E., KERZIENĖ S., MOLEIKAITIENĖ S. (2015): Effects of weight and age on carcass yield and conformation of cattle. Biotechnology in Animal Husbandry, 31, 1, 73–84.
PETRIČEVIĆ M., ALEKSIĆ S., PETROVIĆ M. M., PANTELIĆ V., OSTOJIĆ-ANDRIĆ D., STANIŠIĆ N., NIKŠIĆ D. (2015): Comparative study of fattening and slaughter traits of male Simmental breed and crosses with Charolais breed. Biotechnology in Animal Husbandry, 31, 2, 265–272.
PETRIČEVIĆ M., STANIŠIĆ N., SRETENOVIĆ LJ., PETROVIĆ M. M., STAJIĆ S., NIKŠIĆ D. (2011): Properties and composition of carcass of Domestic Spotted young cattle of two pre-slaughter weights. 3rd International Congress "New Perspectives and Challenges of Sustainable Livestock Production", 5–7th October Belgrade, Biotechnology in Animal Husbandry, 27, 4, 1443–1450.
PETROVIĆ M., KARABASIL N., ĐOKOVIĆ R, CINCOVIĆ M, PETROVIĆ M, ILIĆ Z., STANIMIROVIĆ Z. (2016): Uticaj završne mase mladih bikova simentalske rase na randman. XXI Savetovanje o biotehnologiji, Čačak, Zbornik radova, 2, 21 (24), 577–581.
PETROVIĆ M., LAZAREVIĆ M., ALEKSIĆ S., MIŠČEVIĆ B., STOJIĆ P. (2001): Cattle production in Serbia at the beginning of new millenium. Biotechnology in Animal Husbandry, 17, 5-6, 87–94.
PRADO I. N., PRADO R. M., ROTTA P. P. (2008b): Carcass characteristics and chemical composition of the Longissimus muscle of crossbred bulls (Bos taurus indicus vs. Bos taurustaurus) finished in feedlot. Journal of Animal and Feed Science, 17, 295–306.
PRADO I. N., ITO R. H., PRADO J. M., PRADO I. M., ROTTA P. P., MATSUSHITA M., VISENTAINER J. V., SILVA R. R. (2008a): The influence of dietary soya bean and linseed on the chemical composition and fatty acid profile of the longissimus muscle of feedlot-finished bulls. Journal of Animal and Feed Science, 17, 307–317.
PRADO J. M., PRADO I. N., VISENTAINER J. V. (2009): The effect of breed on the chemical composition and fatty acid profile of the Longissimus dorsi muscle of razilian beef cattle. Journal of Animal and Feed Science, 18, 231–240.
Pravilnik o kvalitetu mesa stoke za klanje, peradi i divljači (1985): Sl. list SFRJ, br. 34/74, 26/75, 13/78 - dr. pravilnik, 1/81 - dr. pravilnik i 2/85 - dr. pravilnik). https://www.scribd.com/doc/183009807/Meso-i-Proizvodi-Od-Mesa
QUINN M. J., LOE E. R., DEPENBUSCH B. E., HIGGINS J. J., DROUILLARD J. S. (2008): The Effects of Flaxseed Oil and Derivatives on In Vitro Gas Production, Performance, Carcass Characteristics, and Meat Quality of Finishing Steers. The Professional Animal Scientist, 24, 161–168.
ROTTA P. P., PRADO R. M., MOLETTA J. L. (2009): Carcass Characteristics and chemical composition of the Longissimus muscle of Nellore, Caracu and Holstein Friesian bulls finished in a feedlot. Asian Australasian Journal of Animal Sciences, 22, 4, 598–604.

ROTTA P. P., PRADO R. M., PRADO I. N., VALERO M. V., VISENTAINER J. V., SILVA R.R. (2009A): The effects of Genetic Groups, Nutrition, Finishing Systems and Gender of Brazilian Cattle on Carcass Characteristics and Beef Composition and Appearance: A Review. Asianust. J. Anim. Sci., 22, (12), 1718–1734.

ŞENTÜRKLÜ S., LANDBLOM D. (2014): The Effect of Post-Weaning Steer Diets Supplemented With Field Pea, Flaxseed and a Field Pea-Flaxseed Combination on Feedlot Finishing Performance, Carcass Quality and Immune Response. Journal of the Faculty of Veterinary Medicine, Kafkas University, 20, 2, 295-300.

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RETROSPECTIVE ANALYSIS OF THE FREQUENCY AND CONTRIBUTORY CAUSES OF ASCITES SYNDROME IN BROILERS IN SOUTH BANAT

Pavle Gavrilović, Aleksandar Živulj, Igor Todorović

Veterinary Specialized Institute "Pančevo", Novoseljanski put 33, 26000 Pančevo, Serbia
Corresponding author: Pavle Gavrilović, e-mail address pavlelula@yahoo.com
Original scientific paper

Abstract: Ascites syndrome is a multi-factorial, noncontagious disease of broilers. Chickens selected for rapid growth have a high basal metabolism and consequently increased demands for oxygen. Poor environmental conditions and other unfavourable factors which reduce the amount of available oxygen cause hypoxia, pulmonary hypertension, right ventricular dilation and right heart failure, which results in generalized passive hyperaemia of organs and ascites as the most striking gross pathology manifestation. The aim of the study was to investigate, retrospectively, the frequency of the disease in a selected district with widespread poultry production and to identify main factors that contribute to the outbreak of the disease. In the period from 2011 to 2017 ascites syndrome was diagnosed by post mortem examination of chickens in 12 out of 91 flocks with health disorders, examined in the Veterinary Specialized Institute "Pančevo". Based on anamnesis, signs of disease and pathomorphological findings, poor environmental conditions and inadequate feed were identified as main contributory factors. In the majority of cases ascites syndrome occurred in small flocks, raised in unsuitable environmental conditions.

Key words: ascites syndrome, chickens, broilers, environmental conditions

Introduction

Ascites syndrome is one of the diseases that continuously affect poultry industry in South Banat over the past decade. The disease is also known as pulmonary hypertension syndrome, waterbelly, right ventricular failure and under some other descriptive terms which do not indicate the aetiology of the disease *(Palić et al., 1994; Knežević and Matejić, 1996*). Ascites syndrome is a multi-factorial, noncontagious disease of broilers, described for the first time in broilers

raised at high altitudes in Bolivia (*Hall and Machicao, 1968*). It is estimated that 4.7% of the broilers worldwide have the disease (*Maxwell and Robertson, 1997*).

The latest generation of hybrids of domestic hen (*Gallus gallus domesticus*) have been selected for a more rapid growth and more intensive protein synthesis which requires more oxygen (*Decuypere et al., 2005*). However, the capacity of the cardiovascular system, which the selection could not significantly influence, has its own physiological limits (*Lorenzoni et al., 2006*) and cannot always respond to increased oxygen demands necessary for rapid growth. Hypoxia triggers a series of events in the organism that result in the development of a metabolic disorder, characterized by hypoxaemia, increased workload of the cardiopulmonary system and central venous congestion (*Baghbanzadeh and Decuypere, 2008*). The most characteristic gross pathology lesions are ascites, dilation and hypertrophy of the right ventricle (*Knežević and Matejić, 1996*).

Fluid exchange across the walls of capillaries, according to Starling law, is regulated by the physiological values of hydrostatic and colloidal osmotic pressures in and out the blood vessels. Changes in these values, that affect the mechanism of tissue fluid exchange, lead to oedema which can occur due to increased intravascular hydrostatic pressure, decreased plasma colloidal osmotic pressure, increased vascular permeability, obstruction of lymph drainage and renal retention of salt and water (*Knežević and Jovanović, 1999*). Increased intravascular hydrostatic pressure can be caused by hepatic and cardiac diseases, and pulmonary hypertension (*Currie, 1999*). Since plasma proteins, in particular albumin, are responsible for colloidal osmotic pressure, pathological conditions with reduced synthesis or loss of albumin can cause ascites (*Baghbanzadeh and Decuypere, 2008*). Increased vascular permeability can be caused by various chemical compounds such as phenol and dioxin derivatives (*Balog, 2003*). According to the literature, primary and contributory causes of ascites include: high altitude, rapid growth rate, pulmonary disease, high energy ration, pelleted feed, cold, the presence of harmful gases and dust particles in the air, high salt concentration in feed, phosphorus deficiency, hepatotoxins, mycotoxins, furazolidone, Se and vitamin E deficiency, stress etc. (*Lister, 1997*).

Due to the lack of data related to ascites syndrome in broilers in the Republic of Serbia, we decided to investigate the frequency of the disease in a selected district with widespread poultry production and to identify main factors that contribute to the outbreak of the disease.

Material and Methods

Material for examination consisted of 595 corpses of broilers which had been delivered to the laboratory of the Veterinary Specialized Institute “Pančevo” during seven consecutive years from 2011. to 2017. The samples originated from

91 flocks with manifested health disorders from South Banat district. On the receipt of the samples detailed anamnesis was taken, including questions about the flock size, manifestation of signs of disease, morbidity and mortality rate, environmental conditions and feed mixtures used. All delivered samples were necropsied and examined pathomorphologically according to the official procedure described by *Marinković and Nešić (2013).*

Results and discussion

The necropsy revealed gross lesions typical of ascites syndrome in broilers from 12 flocks, which is 13.19% of all the examined flocks (Table 1). The lesions appeared in broilers from 3 to 6 weeks (Figure 1). The course of the disease was acute in 3 flocks and subacute in 9 flocks. Mortality ranged from 4.6 to 60.0% (Table 2). Based on anamnesis, signs of disease and gross pathology findings, diagnosed cases of ascites syndrome were classified in three groups.

Table 1. The frequency of ascites syndrome in broiler flocks examined in VSI "Pančevo"

Year	Examined flocks (N)	Flocks with ascites syndrome (N)	Flocks with ascites syndrome (%)
2011	17	2	11.76
2012	15	2	13.33
2013	20	3	15.00
2014	16	1	6.25
2015	6	1	16.67
2016	2	1	50.00
2017	15	2	13.33
Σ	91	12	13.19

Table 2. Age of chickens in which ascites syndrome was diagnosed, course of the disease and mortality rate

Flock	Year	Age (weeks)	Course	Mortality (%)
1	2011	5	acute	6.25
2	2011	6	subacute	4.60
3	2012	3	subacute	45.45
4	2012	4	subacute	60.00
5	2013	5	acute	18.00
6	2013	4	acute	15.00
7	2013	6	subacute	20.20
8	2014	6	subacute	30.00
9	2015	5	subacute	20.10
10	2016	4	subacute	5.00
11	2017	5	subacute	20.00
12	2017	3	subacute	30.00

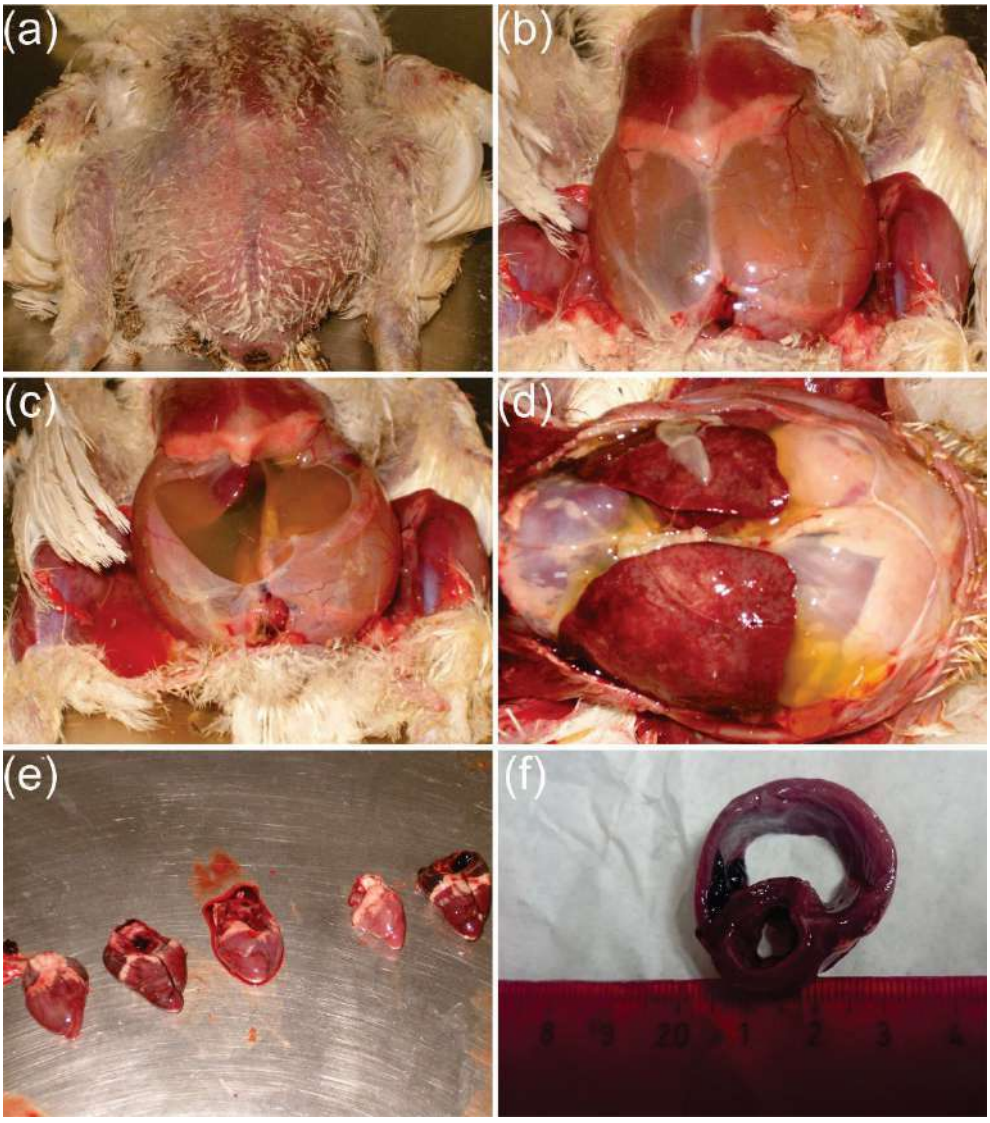

Figure 1. Gross pathology findings in broilers affected with ascites syndrome: (a) Swollen abdomen; (b, c) Accumulation of fluid in pleuroperitoneal cavity; (d) Passive hyperaemia and degenerative necrotic changes of the liver, ascites and hydropericardium; (e, f) right ventricular dilation. (Photo: P. Gavrilović)

Group 1 (flocks 1, 5, 6, 7, 8, 9, 10, 11 and 12)

Chickens from this group originated from small individual holdings and were fed pelleted feeds. Signs of disease included slower movement, blue

discolouration of the comb and wattles (cyanosis), abdominal distension and dyspnoea. The gross pathology findings were characterized by ascites, hidropericardium, right ventricular dilation, hyperaemia of the lungs, liver, spleen, kidneys and intestine. In the facilities in which these 9 flocks were raised, the zoosanitary regimes were not adequate. Low ambient temperature, inadequate ventilation and sawdust bedding with a lot of dust were identified as potential contributory causes of ascites syndrome in this group. The experimental studies have shown positive correlation between ascites incidence and low ambient temperatures. Cold temperatures influence the occurrence of ascites by increasing metabolic oxygen requirements and consequent pulmonary hypertension (*Julian et al., 1989; Stolz et al., 1993*). Poor air quality, dust and respiratory diseases can cause respiratory damage and predispose birds to ascites syndrome. Ammonia, carbon monoxide, carbon dioxide, dust and humidity are recognized air contaminants that can increase susceptibility to ascites syndrome (*Afolayan et al., 2016*). In addition to the above environmental conditions, feeding regime could have an impact on the outbreak of the disease. *Hasani et al. (2018)* showed that in broilers the occurrence of ascites syndrome under mash-fed regime was less than in pellet- and crumble-fed groups.

Group 2 (flock 2)

Chickens from flock 2 originated from a farm with high level of zoosanitary and biosecurity measures. Signs of disease were manifested as reduced growth and poor feathering. Chickens did not respond to antibiotic therapy. The necropsy revealed ascites, hepatic necrosis, gizzard erosions and enteritis. Reduced growth and poor feathering in association with gizzard erosions, enteritis and hepatic necrosis indicate inadequate feed as a potential cause of the disease in this flock. Nutrient factors exhibit their effects by different mechanisms and they can have synergistic effects. The presence of hepatotoxins in feed can cause the liver damage and ascites (*Firestone, 1973*). Excess in sodium causes the increase in blood pressure, while vitamin E deficiency, for example, predisposes tissue damage caused by free radicals and leads to degenerative changes in the myocardium (*Julian, 1987; Aftab and Khan, 2005*).

Group 3 (flock 3 and 4)

Broilers from flocks 3 and 4, in which mortality was 45.45% and 60.0%, exhibited first signs in the second week of life in the form of digestive system disorders manifested as diarrhea. The mortality was constantly increased until the third week when a sudden peak occurred. Gross pathology lesions included ascites, hydropericardium, right ventricular dilation, hyperaemia of the lungs, liver, spleen, kidneys, degenerative necrotic changes in the liver, gizzard erosions and enteritis.

Beside the same clinical manifestations and gross pathology findings, it was common for the chickens from these two flocks that they were fed complete feed mixture of a same manufacturer. Based on anamnesis, signs of disease and gross pathology findings, it was suspected intoxication as a cause of health disorders in these two flocks. Similar gross lesions were described by *Ivetić et al. (2003)* in broilers fed feed mixtures that contained poor quality fats. The literature describes intoxication in chickens accompanied with ascites due to toxic components in certain feed fats such as derivatives of dibenzo-p-dioxin *(Firestone, 1973).*

Conclusion

In broilers investigated within this retrospective study, ascites syndrome mostly appeared in small flocks raised on smallholdings under unsuitable zoosanitary regimes. The main factors contributing to the incidence of the disease were inadequate feed and environmental factors, primarily low ambient temperature and poor ventilation.

Retrospektivna analiza učestalosti i uzroka koji doprinose pojavi ascites sindroma kod brojlera u južnom Banatu

Pavle Gavrilović, Aleksandar Živulj, Igor Todorović

Rezime

Ascites sindrom je nekontagiozno oboljenje brojlera multifaktorijalne etiologije. Pilići selekcionisani na brz rast imaju visok bazalni metabolizam zbog čega su povećane potrebe organizma za kiseonikom. U nepovoljnim ambijentalnim uslovima kao i pod dejstvom drugih štetnih uticaja koji smanjuju količinu raspoloživog kiseonika dolazi do hipoksije, plućne hipertenzije, dilatacije i insuficijencije desnog srca koja ima za posledicu generalizovanu pasivnu hiperemiju organa i ascites kao najupečatljiviji patoanatomski nalaz. Cilj studije bio je da se retrospektivno istraže učestalost i glavni činioci koji doprinose pojavi oboljenja na odabranom području sa rasprostranjenom živinarskom proizvodnjom. U periodu od 2011. do 2017. godine ascites sindrom je dijagnostikovan patoanatomskim ispitivanjem kod pilića iz 12 od 91 jata sa zdravstvenim problemima ispitivanim u Veterinarskom specijalističkom institutu „Pančevo“. Na osnovu anamneze, kliničke slike i patoanatomskog nalaza kao glavni činioci koji doprinose pojavi oboljenja identifikovani su nepovoljni ambijentalni uslovi i

neadekvatna hrana. U najvećem broju slučajeva ascites sindrom se javljao u malim jatima, gajenim u neadekvatnim ambijentalnim uslovima.

Ključne reči: ascites sindrom, pilići, brojleri, ambijentalni uslovi

References

AFOLAYAN M., ABEKE F. O., ATANDA A. (2016): Ascites versus sudden death syndrome (SDS) in broiler chickens: a review. Journal of Animal Production Research, 28, 2, 76-87.

AFTAB U., KHAN A. A. (2005): Strategies to alleviate the incidence of ascites in broilers: a review. Brazilian Journal of Poultry Science, 7, 4, 199-204.

BAGHBANZADEH A., DECUYPERE E. (2008): Ascites syndrome in broilers: physiological and nutritional perspectives. Avian Pathology, 37, 2, 117-126.

BALOG J. M. (2003): Ascites syndrome (pulmonary hypertension syndrome) in broiler chickens: are we seeing the light at the end of the tunnel? Avian and Poultry Biology Reviews, 14, 3, 99-126.

CURRIE R. J. W. (1999): Ascites in poultry: recent investigations. Avian Pathology, 28, 4, 313-326.

DECUYPERE E., HASSANZADEH M., BUYS N. (2005): Further insights into the susceptibility of broilers to ascites. The Veterinary Journal, 169, 3, 319-320.

FIRESTONE D. (1973): Etiology of chick edema disease. Environmental health perspectives, 5, 59-66.

HALL S. A., MACHICAO N. (1968): Myocarditis in broiler chickens reared at high altitude. Avian Diseases, 1, 75-84.

HASANI A., BOUYEH M., RAHATI M., SEIDAVI A., MAKOVICKY P., LAUDADIO V., TUFARELLI V. (2018): Which is the best alternative for ascites syndrome prevention in broiler chickens? Effect of feed form and rearing temperature conditions. Journal of Applied Animal Research, 46, 1, 392-396.

IVETIĆ V., VALTER D., PAVLOVIĆ I., MILJKOVIĆ B., MASLIĆ-STRIŽAK D., ILIĆ Ž., SAVIĆ B., STANOJEVIĆ S., SPALEVIĆ LJ. (2003): Atlas bolesti živine. Naučni institut za veterinarstvo Srbije, Beograd. pp 156.

JULIAN R. J. (1989): Lung volume of meat-type chickens. Avian Diseases, 33, 1, 174-176

JULIAN R. J. (1987): The effect of increased sodium in the drinking water on right ventricular hypertrophy, right ventricular failure and ascites in broiler chickens. Avian Pathology, 16, 61–71.

KNEŽEVIĆ M., JOVANOVIĆ M. (1999): Opšta patologija. Makarije, Beograd, pp 306.
KNEŽEVIĆ N., MATEJIĆ M. (1996): Bolesti pernate živine. Veterinarski fakultet, Beograd, pp 185.
LORENZONI A. G., RUIZ-FERIA C. A. (2006): Effects of vitamin E and L-arginine on cardiopulmonary function and acites parameters in broiler chickens reared under subnormal temperatures. Poultry Science, 85, 12, 2241-2250.
LISTER S. (1997): Broiler ascites: a veterinary viewpoint. World's Poultry Science Journal, 53,1, 65-67.
MARINKOVIĆ D., NEŠIĆ V. (2013): Tehnika obdukcije životinja sa osnovama tanatologije. Fakultet veterinarske medicine, Univerziteta u Beogradu, Beograd, pp 177.
MAXWELL M. H., ROBERTSON G. W. (1997): World broiler ascites survey 1996. Poultry International, 36, 4, 16-30.
STOLZ, J. L. (1993): Ascites syndrome mortality and cardiological response of broiler chickens subjected to cold exposure: effects of verapamil and L-carnitine supplementation. Ph.D. Dissertation. Texas A&M University.
PALIĆ T., RAJIĆ I., NIKOLIĆ Z. (1994): Bolesti živine, Poremećaji ishrane. Savez živinara Srbije, Beograd, pp 123.

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LIVESTOCK IN RURAL PIEDMONT REGIONS OF ALGERIA

Bengouga Khalila, Lahmadi Salwa, Zeguerou Reguia, Maaoui Moufida, Halis Youcef

Center of Scientific and Technical Research on arid regions CRSTRA, BP n° 1682 R.P 07000 Biskra-Algeria
Corresponding Author: Bengouga Khalila, e-mail leila2000_11@yahoo.fr
Original scientific paper

Abstract: A livestock survey conducted during 2013/2015 as part of a CRSTRA project in 4 villages situated at North east Biskra and south Batna in Algeria. These regions are located at elevation ranging around 250-831m asl, experiencing arid and semi-arid Mediterranean climate. Respondents of 86 families demonstrated that livestock is an integral part of the region's mixed farming systems. Low livestock numbers per most households at present reflect the self-consumption breeding mode adapted in these regions. Currently, farmers focus on four main livestock types; goat, sheep, chicken and bee keeping in two regions, it is the case of Beni Souik and Branis , while Maafa includes beyond these types, turkey and pigeon whereas Ain Zaatout includes duck and swine beside the previous livestock types. In the same context; goat ranked first in the four regions, goat and sheep secondly then goat and poultry with goat combined to sheep and poultry in third place. Thus; most families use a combination of grazing, agriculture sub-products and industrial products for the nutrition of their livestock. Families keep livestock as source of milk, butter, wool or hair, leather and other products that are strongly used as nutritional, weaving supply or stocking covering resources for the family members or friends and in some cases for sell to seekers of animal products of indigenous territory origins. Most families use these products for family and friend consumption while a minority sell some of them on local markets.

Key words: Algeria, arid, breeding, family, piedmont.

Introduction

The domestication of livestock species some ten thousand years ago was a vital step in the development of human civilisation *(FABRE, 2006)*. Thus; livestock is

the second important sub sector of agriculture, it is primarily a subsistent activity to meet household food needs as well as supplement farm incomes. Almost every farm family owns some livestock .the pattern of livestock strength is mainly influenced by various factors such as farm size, cropping pattern, availability of rangelands including fodder and pasture *(Rais et al., 2013).* And even with eleven livestock production systems defined *(Seré et al., 1995a)*, the traditional breeding systems are often rather difficult to identify, because they are related to existing low input production systems and are not formally institutionalized *(Steglich and Peters, 2003)*. For example in South Africa; although dairy ranching is currently receiving little attention, it has the potential to form an integral part of resource poor cattle production systems. Such a system can contribute towards household food security in the form of milk consumption by the family from the cows as well as the cash obtained from the sale of milk. *(Grobler et al., 2008).* As well as in Mountain areas, Mediterranean grasslands, steppes, arid and semi-arid zones areas, animal production was traditionally one of the very few economic options, still contribute important natural values, including cultural landscapes and biodiversity *(Bernués, 2017)*; in Algeria, most livestock is an extensive system, thus some of it is family type intended to self-consumption in animal products (meat, milk, egg and honey) or to furnish an income consequently in good pluvial years. In addition, these animals depending on the species and regions of breeding provide manure for cropping systems that do not use chemical fertilizers mainly gardening and arboriculture and supply the rural populations' activity system in wool, hair and leather main raw materials essential for family crafts *(Feliachi, 2003).*
In this study, a sequence of survey steps was employed to assess the rural traditional livestock systems in arid and semi-arid regions of Algeria where a traditional low-input mixed crop-livestock system prevails.

Material and methods

Characteristics of the study area

The study is realised in four Aurès piedmonts villages of arid and semi-arid regions with agro-pastoral vocation; Ain Zaâtout, (altitude 831m, latitude 35°4'30.49" Nord, longitude 5°45'48.04" Est), Beni Souik (altitude 555m, latitude 35°5'15.85" Nord, longitude 5°51'43.07" Est), Branis (altitude 250 m, latitude 34°59'55.84" Nord, longitude 5°46'32.13" Est) these regions are mainly identified as hot arid eco region and Maafa (altitude 735.6 m, latitude 35°18'09.458" Nord, longitude 005°52'17.066" Est) which is known as a hot semi arid eco region. The Mediterranean climate with long summer drought period of these regions is characterized by a dry cold winter with rare morning freeze with low temperature registered in December and January that ranges from – 2 to + 4°C and a dry hot

summer with maximal temperature registered in July and august superior to 40°C. Thus seasonal temperature variation can reach 20°C while diurnal temperature variation can pass 22°C. Irregular insufficient and unequally spread annual rainfall ranges from 300 to 100 mm and mostly can pass 200 mm in these regions. The annual mean relative humidity is of 47 % *(Feliachi, 2003 and URBACO 2012).*

The study region has an agro-pastoral type vocation where palm dates are the dominant growing with two underlying growing fruit trees and low annual or perennial crop. Breeding occupies a predominant place; in fact the region of Biskra is famous by its livestock especially by the breed race Ouled Djellal the most spread in Algeria and known as resistant to arid zones *(URBACO, 2012).* This rain-fed farming systems in dry areas with mixed crop-livestock and pastoral systems merging into systems with very low current productivity or potential because of extreme aridity. Where each individual farm has its own specific characteristics, which arise from variations in resource endowments and family circumstances *(Dixon et al., 2001).*

The studied areas are especially traditional extensive breeding system maintained in rural areas employing self or family labour (women and children) based on free fodder offer and grazing animals on cropped land after harvest with small herd size. Animals are used as a multi-purpose breed providing milk and some of its sub-products, egg, meat, wool or hair, leather and sometimes manure.

Data collection

The study was conducted among 86 family farmers owning a livestock. The data were collected by questioning a men or women per household that accept to participate in this study. The interviewed person was inquired to generate information on breeding practices on the basis of four main questions: Types of livestock owned by the farmer family, the head number of livestock owned by the farmer family, Food source of livestock and Breeding sub-products and their consumption (whether the products are intended to be commercialised or for self-family consumption and friend consumption). Thus in our inquiry we investigate 19 women from Ain Zaatout, 19 women from Béni Souik, 20 women from Branis, and 28 women from Maafa.

Data have been processed by the Microsoft Office Excel 2007 package.

Results and discussion

Types and head number of livestock

The data regard types of livestock are presented in Table 1.

Table 1. Type of livestock

Livestock type	Ain Zaatout=19		Beni Souik=19		Branis=20		Maafa=28		Total 86
	nbr	%	nbr	%	nbr	%	nbr	%	
Goat	182	38.47	97	20.50	88	18.60	106	22.41	473
Sheep	48	21.52	43	19.28	68	30.49	64	28.69	223
Chicken	70	37.63	14	7.52	36	19.35	66	35.48	186
Beekeeping	18	48.64	15	40.54	2	5.40	2	5.405	37
Turkey	10	90.90	0	0	0	0	1	9,09	11
Pigeon	0	0	0	0	0	0	6	100	6
Duck	15	100	0	0	0	0	0	0	15
Swine	10	100	0	0	0	0	0	0	10
Total	318	34.60	169	18.38	194	21.10	238	25.89	919

Four main types of livestock are registered thus goat breeding is the most dominant one followed respectively by sheep breeding, poultry breeding and, beekeeping. Meanwhile big livestock like cattle does not exist in these regions this is related to climatic as well as socio-economic characteristics of these regions thus *Seré et al. (1995b)* revealed Africa to have vast livestock resources in semi-arid and arid regions where small ruminants play an important role while *Kwaku (2003)* reported that, under certain socio-economic conditions only small stock (e.g. sheep and goats) or micro stock (rabbits, grass cutters, etc.) may be suitable in a given agriculture-livestock system.

Families are maintaining a number of animals of different types: goat, sheep, chicken, bee hives, turkey, pigeon, duck and swine. Goat and sheep are good source of milk and meat while poultry are mostly source for eggs and bees for honey.

Data given in Table 1 show that total population of livestock raised by the respondents was 919. Total number of heads of goat, sheep, chicken, turkey, pigeon, duck and swine are 473, 223, 186, 11, 6, 15 and 10, respectively while bee hives total number is 37one. In general, these small folk sizes are intended for family consumption in contrast what is reported by *El Aich (2018),* who mentioned that Moroccan Atlas Mountains livestock farming systems are characterized by large folk sizes.

Thus the number of each category differs from region to another these differences are attributed to characteristics of each region. Livestock number of heads maintained by a family indicates their interest in animals. Thus the mean number per family of goats (5. 50) is similar to that registered in Esera District, of Dawro Zone, Southern Ethiopia by *Beyene et al. (2018)* with 5.69 goats per household.

Distribution of families according to livestock type

Table 2 presents data concerning the number of families per region according the type of livestock owned.

Table2. Number of families per type of livestock

	Ain Zaatout		Beni Souik		Branis		Maafa		Total	
Livestock type	nbr	%	nbr	%	nbr	%	nbr	%	nbr	%
Goat	7	36.84	4	21.05	8	40	5	17.86	24	27.90
Sheep	0	0	0	0	0	0	1	3,57	1	1,16
Poultry	0	0	0	0	0	0	1	3,57	1	1,16
Goat + Sheep	1	5.26	10	52.63	5	25	3	10.71	19	22.09
Goat + Poultry	2	10.53	2	10.52	1	5	8	82.57	13	15.11
Goat +Beekeeping	0	0	0	0	1	5	1	3 ,57	2	2.32
Goat + Sheep +Beekeeping + Poultry	5	26.32	1	5.26	0	0	1	3.57	7	8.13
Goat + Sheep + Poultry	3	15.79	2	10.52	3	15	5	17.86	13	15.11
Beekeeping + Poultry	1	5.26	0	0	0	0	0	0	1	1.16
Sheep + Poultry	0	0	0	0	0	0	1	3.57	1	1.16
Goat + Sheep +Beekeeping	0	0	0	0	1	5	1	3.57	2	2.32
Goat +Beekeeping + Poultry	0	0	0	0	1	5	1	3.57	2	2.32

Goat livestock ranked first among the four regions (27. 90%); followed by Goat and sheep livestock together (22. 09) while goat with sheep with poultry and goat with poultry presents 15.11% each of the total inquired families. Thus every region has its specificity; Ain Zaatout families are mainly breeders of goats only (36.84%) or associated with sheep, poultry and bees (26. 32%). Beni Souik families are mainly breeders of goats associated with sheep (52. 63%) or goats lonely (21.05%). Branis families are mainly breeders of goats solely (40%) or associated with sheep (25%). Meanwhile Maafa families are mainly breeders of goats associated with poultry (82. 57%) or goats only or associated with sheep and poultry (17.86%) for each category. The dominance of goat breeding is a traditional rural asset not intended for regular consumption in these regions this result is similar to what *Maass et al. (2012)* found about cattle livestock in the Rusizi plains of Congo. Meanwhile abundance of goat and sheep breeding in

combination is attributed that they are main small body perfect resources of red meat and milk for the rural population in these regions. This consolidates *Masiga (1995)* who reported that Sheep and goats account for almost 30% of the meat consumed and 16% of the total milk produced in Africa. Because of their small body size, high reproductive capacity and ability to rapidly multiply their numbers, small ruminants are ideally suited to smallholder production systems. The capital requirement for starting and keeping or expanding small ruminant production is low. They are a moving bank because they can easily be sold. Also production investment risks are low.

Generally rural population consume less animal proteins than urban population thus *Ranganathan et al. (2016)* reported that within developing countries and emerging economies, per capita consumption of animal-based foods tends to be highest in urban areas. We suppose that regular consumption of eggs, milk, or meat from small animals, such as poultry and goat would, consequently, impact on family nutritional status in these rural piedmont regions. Similarly *Maass et al. (2012)* reported about cavies.

Food source of livestock

The source of food used in the four villages is summarised in Table 3.

Table 3. Food type used in the four sites for animal's nutrition

Food type	Ain Zaatout	Beni Souik	Branis	Maafa	Total
Grazing	0	1	1	0	2
Sub-product	0	1	0	0	1
Industrial	1	0	0	0	1
Grazing and sub-product	3	0	0	0	3
Grazing and industrial	3	2	3	1	9
Sub-product and industrial	1	3	3	1	8
Grazing and sub-product and industrial	11	12	13	26	62

In the four villages the combination of grazing, agriculture sub-product and industrial products for feeding animals is the most used with a total percentage of 72.09 compared to depending on grazing only 2.32%, agriculture sub-products (1.16%), industrial products (1.16%) or a combination of two of them; 3.48% for a combination of grazing and agriculture sub-products, 10.46% for the combination between grazing and industrial products and 9.30% for the combination between

agriculture sub-products and industrial products. Thus, many Mediterranean farming systems have traditionally been based on the extensive use of natural pasture *(Casasûs et al., 2014)*.

Breeding products and their consumption

Different sub-products are listed according to number of families exploiting them in Table 4.

Table 4. Number of families per region engendering breeding sub-products

Sub-product	Ain Zaatout	Beni Souik	Branis	Maafa	Total
Milk	18	19	20	26	83
Butter	18	18	20	26	82
Klila	10	10	15	8	43
Wool/hair	18	17	17	24	76
leather	14	18	17	19	68
manure	7	15	15	14	51
commercial	7	0	0	2	9
Self-family and friends consumption	12	19	20	24	75

Most families produce milk (96. 51%) thus milk is the most important product. Similarly *Steglich and Peters (2003)* reported that milk production is important in Gambia. Furthermore milk is used for the preparation of *Klila* a form of dried cheese used locally by families in traditional dishes (50%). While butter, wool or hair and leather are produced by 95.34%, 88.37% and 79.06% respectively of interviewed families. These livestock products are mainly used as nutritional, weaving supply or stocking and covering resources for the family members or friends and in some case for sell to seekers of animal products of indigenous territory origins. Most of the families (87.20%) use these products for family members or friends consumption while (10.46%) only announced that they sell some of these products on local markets. Manure is another product used as fertiliser by 59.30% of interviewed families; thus animal manure is often essential for maintaining soil fertility reported *Steinfeld et al. (2006)*. Likewise *Cox (2011)* mentioned that in the traditional mixed farming of the region, soil fertility of main fields near the homestead used to be maintained by available cattle manure.

Conclusion

Livestock system in rural piedmont regions of Algeria is based on a variety of types with a dominance of goat followed by a combination of goat and sheep breeding, while goat combined to poultry or with sheep and poultry came in third rank. With the low numbers per household it is typically grazing, agriculture sub-

product and industrial products based-livestock system resulting in provision of healthy protein in the human diet ensures sustainability of the soil fertility. This kind of livestock system must be consolidated by programs for adequate forage grain reproduction involving smallholder farmers. It is also important that institutions work in cooperation with the farmers to provide forage and there must be focus on animal products delivered by these systems as being products of bio and healthy origin to raise farm income.

Stočarstvo u ruralnim pijemontskim regijama Alžira

Bengouga Khalila, Lahmadi Salwa, Zeguerou Reguia, Maaoui Moufida, Halis Youcef

Rezime

Istraživanje populacija stoke sprovedeno je tokom 2013/2015. godine u okviru projekta CRSTRA u 4 sela na severoistoku Biskre i južnoj Batni u Alžiru. Ovi regioni se nalaze na nadmorskoj visini od oko 250-831m, i izloženi su sušnoj i polusušnoj mediteranskoj klimi. Ispitanici iz 86 porodica pokazali su da je stoka sastavni deo mešovitih poljoprivrednih sistema u regionu. Nizak broj stoke u većini domaćinstava trenutno odražava način uzgoja za zadovoljenje sopstvenih potreba u ovim regijama. Trenutno, poljoprivrednici se fokusiraju na četiri glavne vrste stoke; koze, ovce, živinu i pčelarstvo u dva regiona, što je slučaj u Beni Souik i Branis regijama, dok Maafa regija, pored ovih vrsta, uključuje uzgoj ćurki i golubova, dok Ain Zaatout regija uključuje i uzgoj patki i svinja pored prethodno navedenih vrsta stoke. U istom kontekstu, koze su na prvom mestu u sva četiri regiona, koze i ovce na drugom mestu, zatim koze i živina sa kozama u kombinaciji sa ovcama i živinom na trećem mestu. Tako; većina porodica koristi kombinaciju ispaše, poljoprivrednih podproizvoda i industrijskih proizvoda za ishranu svoje stoke. Porodice drže stoku kao izvor mleka, maslaca, vune ili dlake, kože i drugih proizvoda koji se koriste u ishrani, ili kao sirovina za tkanje ili skladištenje resursa za članove porodice ili prijatelje, a u nekim slučajevima za prodaju zainteresovanima za životinjske proizvode autohtonih vrsta na određenoj teritoriji. Većina porodica koristi ove proizvode za porodičnu potrošnju i prijatelje, dok manji broj prodaje neke od proizvoda na lokalnim tržištima.

Ključne reči: Alžir, suva klima, uzgoj, porodica, pijemont.

References

EL AICH A. (2018): Changes in livestock farming systems in the Moroccan Atlas Mountains. Open Agriculture. 3, 131–137. https://doi.org/10.1515/opag-2018-0013.

BERNUÉS A. (2017): Animals on the land: ecosystem services and disservices of grazing livestock systems. In Project Marcas de Calidad de Carne y Productos Carnicos Iberoamericanos (MARCARNE) 31 p. file:///C:/Documents%20and%20Settings/Administrateur/Mes%20documents/Downloads/AnimalsontheLand_v3%20.pdf.

BEYENE A., ALILO A.A., MOLA M. (2018): Assessment of Sheep and Goat (Small Ruminants) Production System in Esera District, of Dawro Zone, Southern Ethiopia. Journal of Advanced Dairy Research, 6, 215. doi:10.4172/2329-888X.1000215

CASASÛS I., RIEDEL J. L., BLANCO M., BERNUÉS A. (2014): Extensive livestock production systems and the environment. Chapter in Animal farming and environmental interactions in the Mediterranean region. EAAP Scientific Series, January 2012. Ed Wageningen Academic publishers, n° 131, 81-88. DOI: 10.3920/978-90-8686-741-7_9.

COX T.P. (2008): The land as a casualty: soil, cattle, and the future in South Kivu, DRC. Unpublished MSc thesis, Department of Anthropology, University College London, UK, pp 55. http://citeseerx.ist.psu.edu/viewdoc/download;jsessionid=76D8D96A195450AC837B4654943A768E?doi=10.1.1.551.1943&rep=rep1&type=pdf.

DIXON J., GULLIVER A., GIBBON D. (2001): Summary farming systems and poverty improving farmers' livelihoods in a changing world. Ed FAO and World Bank. Rome and Washington DC.P 41p.

FABRE. (2006): Sustainable Farm Animal Breeding and Reproduction A Vision for 2025. Working Group "FABRE Technology Platform", pp 30.

FELIACHI K. (2003): Rapport National sur les Ressources Génétiques Animales: Algérie. Commission nationale AnGR, pp 46.

GROBLER S.M., SCHOLTZ M.M., BESTER J., MAMABOLO J.M., RAMSAY K.A. (2008): Dairy production systems in the emerging and communal sectors of South Africa: Results from a structured survey. Applied Animal Husbandry & Rural Development 1: 25-30: www.sasas.co.za/aahrd/

KWAKU A. (2003): Agriculture and Livestock Integration in the context of the developing world with emphasis on sub-Saharan Africa. (DRAFT). An invited Key Presentation at the Global Forum for Agricultural Research (GFAR) 2003 Conference, Meridien President Hotel, Dakar, Senegal May 22-24, 2003. International Trypanotolerance CentreP.M.B.14Banjul.59p.

http://www.fao.org/fileadmin/templates/agphome/images/iclsd/documents/wk4_c1_agyemang.pdf.
MAASS B. L., MUSALE D. K., CHIURI W. L., GASSNER A., PETERS M. (2012): Challenges and opportunities for smallholder livestock production in post-conflict South Kivu, eastern DR Congo. Tropical Animal Health Production, 44, 1221–1232. DOI 10.1007/s11250-011-0061-5.
MASIGA W.N. (1995): Livestock research requirements in sub-Saharan Africa. In Global Agenda for Livestock Research Proceedings of a Consultation ILRI, Nairobi, Kenya, 18–20 January 1995. ILRI (International Livestock Research Institute), Nairobi, Kenya, 13-38.
RAIS M. U.l.N., SOLANGI A.W., SAHITO H.A. (2013): Economic assessment of rural women involved in agriculture and livestock farming activities. Wudpecker Journal of Agricultural Research. 2(4), 115 – 121.
RANGANATHAN J., VENNARD D., WAITE R., DUMAS P., LIPINSKI B., SEARCHINGER T., GLOBAGRI-Wrr Model Authors. (2016): Shifting Diets for a Sustainable Food Future." Working Paper, Instalment 11 of Creating a Sustainable Food Future. Washington, DC: World Resources Institute. Accessible at http://www.worldresourcesreport.org.
SERÉ C., STEINFELD H., GROENEWOLD J. (1995a): World livestock production systems: Current status, issues and trends. FAO animal production and health paper no. 127, pp 51.
SERÉ C., STEINFELD H., GROENEWOLD J. (1995b): World livestock production systems: Current status, issues and trends. In Global Agenda for Livestock Research Proceedings of a Consultation ILRI, Nairobi, Kenya, 18–20 January 1995. ILRI (International Livestock Research Institute), Nairobi, Kenya, pp 13-38.
STEINFELD H., WASSENAAR T., JUTZI S. (2006): Livestock production systems in developing countries: status, drivers, trends. Revue scientifique et technique (International Office of Epizootics). 25 (2), 505-516.
STEGLICH M., PETERS K. J. (2003): Participatory Methods to Assess Traditional Breeding Systems: The Case of Cattle Breeding in the Gambia. Conservation and Sustainable Use of Agricultural Biodiversity. Sourcebook produced by CIP-UPWARD, in partnership with GTZ GmbH, IDRC of Canada, IPGRI and SEARICE, 123-132.

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

[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, **e-mail address**
Review paper

Example 2

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

Zdenka Škrbić, Zlatica Pavlovski, Miloš Lukić, Veselin Petričević

Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia
Corresponding author: Zdenka Škrbić, **e-mail address**
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Milan M. Petrović, Stevica Aleksić, Milan P.Petrović, Milica Petrović, Vlada Pantelić, Željko Novaković, Dragana Ružić-Muslić

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In scientific journals:

PETROVIĆ M. M., SRETENOVIĆ LJ., BOGDANOVIĆ V., PERIŠIĆ P., ALEKSIĆ S., PANTELIĆ V., PETROVIĆ D. M., NOVAKOVIĆ Ž. (2009): Quantitative analysis of genetic improvement of milk production phenotypes in Simmental cows. Biotechnology in Animal Husbandry, 25,1-2, 45-51.

ŠKRBIĆ Z., PAVLOVSKI Z., LUKIĆ M. (2007): Uticaj dužine tova u različitim sistemima gajenja na klanične osobine brojlerskih pilića genotipa Redbro. Biotechnology in Animal Husbandry 23, 3-4, 67-74.

WEBB E., O'NEILL H. (2008): The animal fat paradox and meat quality. Meat Science, 80, 28-36.

PhD Thesis:

RUŽIĆ-MUSLIĆ D. (2006): Uticaj različitih izvora proteina u obroku na proizvodne rezultate jagnjadi u tovu. Doktorska disertacija. Univerzitet u Beogradu, Poljoprivredni fakultet.

CAETANO A.R. (1999): Comparative mapping of the horse (*Equss caballus*) genome by synteny assignment of type-I genes with a horse-mouse somatic cell hybrid panel. Ph.D. Dissertation, University of California, Davis.

In Scientific Books:

PETROVIĆ P.M (2000): Genetika i oplemenjivanje ovaca. Naučna knjiga, Beograd, pp365.

FITZGERALD M. (1994): Neurobiology of Fetal and Neonatal Pain. In: Textbook of Pain. 3rd edition. Eds Wall P. and Melzack R. Churchchill Livingstone, London, UK, 153-163.

At Scientific Meetings:

ŠKRBIĆ Z., LUKIĆ M., BOGOSAVLJEVIĆ-BOŠKOVIĆ S., RAKONJAC S., PETRIČEVIĆ V., DOSKOVIĆ V., STANOJKOVIĆ A. (2015): Importance of farm management in reducing broilers skin lesions. Proceedings of the 4th International Congress "New Perspectives and Challenges of Sustainable Livestock Production", October 7 – 9, Belgrade, 145-158.

Citations in the text are presented in italic form, examples: …results of *Petrović (2009)*; *Petrović et al. (2009)*; *Webb and O'Neill (2008)….*; *(Škrbić et al., 2015); (Ružić-Muslić, 2006); (Webb and O'Neill, 2008)*

Editor's office

12th International Symposium
"Modern Trends in Livestock Production"
9th – 11th October 2019, Belgrade, Serbia

Organizer

INSTITUTE FOR ANIMAL HUSBANDRY, BELGRADE-ZEMUN

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

SECOND ANNOUNCEMENT

On behalf of the International Scientific and Organizing Committee, it is our pleasure to invite you to participate at the **12th International Symposium on Modern Trends in Livestock Production**, which will be held **from 9th to 11th October 2019 in Belgrade**.

We invite you to take part with an oral or poster presentation. You also have the opportunity to present your institution or company at the Symposium.

At the Symposium, the experts from Serbia and abroad will present the results of their research in order to enable a better transfer of scientific achievements in all fields of animal husbandry and science and making them available to the scientists, researchers and practitioners in livestock production, as well as students, in the private sector and to the general public.

The aim of the scientific meeting is to establish better cooperation between researchers in the field of animal science from different institutions, and experts from the industry, trade and other related fields, as well as producers from Serbia, Western Balkans, EU and other parts of the world in the field of science, education and good livestock production practice.

MAIN TOPICS OF THE SYMPOSIUM

1. Genetics and Selection of Farm Animals
2. Breeding and Reproduction of Farm Animals
3. Physiology and Nutrition of Farm Animals
4. Organic Livestock Production
5. Livestock Feed and Ecology
6. Animal Welfare and Health Care
7. Technology and Quality of Animal Products
8. Protection of the Environment and Biodiversity in Animal Production
9. Alternative Production Methods in Livestock Production

OFFICIAL LANGUAGE

The official language of the Symposium is English.

REGISTRATION AND PAYMENTS

Registration and submission of full papers to the e-mail address: biotechnology.izs@gmail.com The authors shall submit full papers prepared acording to the **Instruction for Authors** for scientific journal "Biotechnology in Animal Husbandry" (www.istocar.bg.ac.rs). All submitted papers will be peer reviewed. Accepted papers will be published in the Proceedings.

REGISTRATION FEE	**Before 30th June 2019 (Early registration)**	**After 30th June 2019 (Late registration)**
Registration Fee, covers publishing of paper, Symposium material, participation in all sessions of the Symposium, coffee/tea break	**80** €	**100** €
Registration Fee, covers publishing of paper, Symposium material, participation in all sessions of the Symposium, coffee/tea break, tourist program and gala dinner	**120** €	**150** €

IMPORTANT DATES

Deadline for full paper submission **Aprile, 30th 2019**

Request for Proforma invoice for Registration fee to the e-mail address: biotechnology.izs@gmail.com

Symposium participants from Serbia can make the payment (in RSD value on the day of payment according to the exchange rate), on the following account:

Institut za stočarstvo, Beograd-Zemun
11080 Zemun, Autoput 16
Tekući račun br. 205-65958-94
Komercijalna banka

INSTRUCTION FOR EUR PAYMENTS
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ACCOMMODATION AND SYMPOSIUM LOCATION

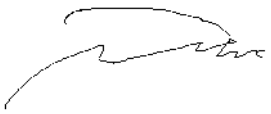
The Symposium will be held in Hotel Park, Belgrade Njegoševa street 2, 11000, Belgrade, Serbia (www.hotelparkbeograd.rs)

Single room at special rate of 50 € daily per room
Double room at special rate of 70 € daily per room
City tax is not included and is approximately 1.2 € per person daily.

Accommodation at SPECIAL RATES is possible for reservations before **August, 31st 2019.**

Hotel reservation telephone: +381114146800
Hotel reservation e-mail: reception@hotelparkbeograd.rs

On behalf of Organizing Committee



Dr. Milan P. Petrović,
Principal Research Fellow
Serbia

HOTEL RESERVATION FORM

Hotel Park Beograd welcomes the guests of
INSTITUTE FOR ANIMAL HUSBANDRY SYMPOSIUM
9-11th October 2019
We are pleased to advise the special rates and conditions available for this event:
(Please check appropriate box)

	Single room at special rate of 50 € daily per room
	Double room at special rate of 70 € daily per room

All the above mentioned rates are INCLUSIVE of full buffet breakfast in our Continental restaurant, VAT, complimentary internet access.
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